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The endocannabinoid system, a novel and key participant in acupuncture's multiple beneficial effects

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Highlights

- Endocannabinoid system (ECS) activation and acupuncture induce similar effects.
- ECS mediates multiple acupuncture's effects, such as analgesia and neuroprotection.
- ECS is a novel and key participant in acupuncture's multiple beneficial effects.
- Acupuncture may produce different beneficial effects via similar key pathways.
- Findings may enable new techniques which increase the efficacy of acupuncture.

Abstract

Acupuncture and its modified forms have been used to treat multiple medical conditions, but whether the diverse effects of acupuncture are intrinsically linked at the cellular and molecular level and how they might be connected have yet to be determined. Recently, an emerging role for the

endocannabinoid system (ECS) in the regulation of a variety of physiological/pathological conditions has been identified. Overlap between the biological and therapeutic effects induced by ECS activation and acupuncture has facilitated investigations into the participation of ECS in the acupuncture-induced beneficial effects, which have shed light on the idea that the ECS may be a primary mediator and regulatory factor of acupuncture's beneficial effects. This review seeks to provide a comprehensive summary of the existing literature concerning the role of endocannabinoid signaling in the various effects of acupuncture, and suggests a novel notion that acupuncture may restore homeostasis under different pathological conditions by regulating similar networks of signaling pathways, resulting in the activation of different reaction cascades in specific tissues in response to pathological insults.

Keywords: Acupuncture

Endocannabinoid system

Analgesia

Neuroprotection

1. Introduction

Acupuncture is an important component of traditional Chinese medicine (TCM). It has been established as an effective treatment and used therapeutically in China and other Asian countries for centuries. As a modified form of traditional acupuncture, electroacupuncture (EA) uses electrical currents with standard parameters, and EA is believed to be more accurate, reliable and without harmful side-effects. EA is now widely used in clinical

practice as a complementary therapy to treat a variety of conditions, such as pain and cerebral/cardiac ischemia-reperfusion injury. In 1997, the National Institutes of Health acknowledged the efficacy and potential therapeutic effects of acupuncture for treating multiple pathological conditions, such as chronic pain and stroke. Although basic research findings have begun to clarify the mechanisms underlying the action of acupuncture, these mechanisms have yet to be firmly established (NIH, 1997). Acupuncture signals that originate at acupoints activate peripheral nerves, and the input signals are transmitted to the spinal cord and brain via various neuronal pathways. This induces a series of neurological effects at peripheral, spinal and supraspinal levels, which in turn, leads to various physiological and functional effects, such as neuroprotection and analgesia. To date, the most well-studied endogenous system known to be involved in regulating these pathways both centrally and peripherally is the endogenous opioid system (Zhao, 2008). To better understand the underlying mechanisms and scientific basis of acupuncture as well as to explore the roles and contributions of other endogenous systems in its biological effectiveness, a growing number of researchers have begun to focus their efforts on acupuncture research (Cheng, 2014; Li et al., 2012).

In 2009, Wang and colleagues firstly found that EA conferred neuroprotection against cerebral ischemia by stimulating the mobilization of endocannabinoids in the brain and activating CB1 receptors (Wang et al., 2009). Furthermore, Tjen-A-Looi and colleagues demonstrated that EA attenuated sympathoexcitatory reflex responses by causing the release of endocannabinoids and the activation of presynaptic CB1 receptors in the

ventrolateral periaqueductal gray (vlPAG) (Tjen et al., 2009). Chen and colleagues also showed that EA increased levels of the endocannabinoid anandamide (N-arachidonoylethanolamine, AEA) in inflamed skin tissues and induced analgesia by activating CB2 receptors (Chen et al., 2009). These interesting findings indicated a potential link between EA and the endocannabinoid system (ECS), both centrally and peripherally, and suggested that this endogenous system might play a key role in the therapeutic effects of EA.

Because acupuncture and ECS activation are associated with many of the same biological effects, such as neuronal and cardiovascular protection, analgesic and antiemetic effects, and the maintenance of energy balance (Centonze et al., 2007; Chang, 2013; Pacher and Kunos, 2013), it is not surprising that recent studies have repeatedly described the involvement of ECS activation in EA-induced analgesic, neuroprotective and cardiovascular regulatory effects under various pathological conditions (Chen et al., 2009; Tjen et al., 2009; Wang et al., 2009). These interesting findings strongly suggest that ECS might be one of the primary mediators as well as a regulatory factor of all of the beneficial effects of acupuncture. Thus, to highlight the existence and importance of this newly discovered role, we have specifically reviewed the analgesic, neuroprotective and cardiovascular regulatory effects of both EA and the ECS in the following sections. We also focus on the evidence that supports the role of the ECS as a novel and key regulator of the multiple biological and therapeutic effects of EA.

2. Acupuncture induces biological effects related to ECS activation

2.1. Overview of acupuncture

Acupuncture is an important therapeutic technique that has been used for thousands of years in ancient China. It involves the insertion and manipulation of fine needles into specific acupoints. The TCM concepts of “meridian” and the vital energy “Qi” are part of the theoretical basis of acupuncture (Yang et al., 2011). Currently, a variety of acupuncture-related techniques, such as manual acupuncture (MA), EA, transcutaneous electrical acupoint stimulation (TEAS), and transcutaneous electrical nerve stimulation (TENS) are being used in clinical practice treating various disorders (Han, 2011; Kasat et al., 2014; Liu et al., 2016). EA is the most frequently used strategy. EA is similar to regular acupuncture in that it incorporates the physiological effects of traditional acupuncture needling and integrates them with the benefits of electrotherapy. It is performed by inserting acupuncture needles that are connected to an electrical stimulator into acupoints. The stimulation frequency, current intensity, pulse width, and pulse interval are adjusted for optimal effect (Yu et al., 2014). Compared to traditional acupuncture, there are several advantages to using EA. For example, the electrical stimulation can be easily regulated and repeated, and the frequency and intensity of the stimulation can be precisely adjusted. Different sets of parameters may produce different physiological effects. For instance, EA at different frequencies (e.g., 2, 15, and 200 Hz) may lead to the release of different opioid peptides (Han, 2003). Alternatives to EA, such as TENS, use

electrodes that are applied to the skin instead of needle insertion. TENS is applied under specific conditions, such as in patients contraindicated for needle insertion (Kasat et al., 2014). Several transmitters and modulators have been hypothesized to be responsible for the effects of acupuncture, such as opioid peptides (Han, 2003), cholecystokinin octapeptide (CCK-8) (Han et al., 1986) and 5-hydroxytryptamine (5-HT) (Kim et al., 2005). Nonetheless, our understanding of how acupuncture works remains incomplete.

2.2. Acupuncture-induced analgesia

Acupuncture has been used in China and other Asian regions for nearly 3000 years to alleviate pain. Its increasing popularity among patients and physicians in the Western world, where it is incorporated as part of TCM, in recent years is remarkable, and it is especially valued for its effectiveness and persistence of the effects in treating chronic pain (Eshkevari and Heath, 2005; Han and Ho, 2011; MacPherson et al., 2016). Since the first study of acupuncture's analgesic effects was published in the early 1970s (Andersson et al., 1973), acupuncture and related therapies have been shown to serve as effective treatments for various types of pain (Wang et al., 2008) by triggering a series of biological effects in the nervous system (Zhang et al., 2017; Zhao, 2008). For example, the opioid system has been shown to be involved in acupuncture-induced analgesia, both centrally and peripherally, due to the release of opioids and the activation of μ-, δ-and κ-opioid receptors (Han, 2003; Zhang et al., 2005a). Moreover, other endogenous substances in the central nervous system (CNS), including CCK-8 (Han et al., 1986), 5-HT (Kim

et al., 2005) and adenosine (Goldman et al., 2010), also contribute to acupuncture-induced analgesia.

Because it is a safe, cost-effective and user-friendly therapy (White et al., 2001; Zhu et al., 2013a), acupuncture has become a common pain treatment option in the clinic, especially for chronic pain (Eshkevari and Heath, 2005). Consistent with laboratory findings, increasing evidence from randomized controlled trials (RCTs) and meta-analyses shows that acupuncture is clinically effective in treating painful conditions, such as chronic back pain (Manheimer et al., 2005), chronic neck pain (Blossfeldt, 2004; Vas et al., 2006), osteoarthritis of the knee (Witt et al., 2005), headache (Schiapparelli et al., 2011) and postoperative pain (Lu et al., 2015b). For example, an RCT reported by Dang and Yang investigated the efficacy of acupuncture in alleviating stomach carcinoma pain and showed that patients undergoing acupuncture for two months experienced less pain than patients who underwent sham acupuncture or those in the control group (Dang and Yang, 1998). Similarly, a systematic review of RCTs also suggested that acupuncture and related techniques were effective in reducing postoperative pain scores and opioid consumption (Sun et al., 2008). Furthermore, results from other clinical studies support the idea that acupuncture is superior to typical care in treating chronic back pain (Trigkilidas, 2010). Such clinical investigations have enabled the identification of optimal acupuncture parameters for different pain conditions and shed light on the mechanisms that underlie acupuncture-induced analgesia in humans.

2.3. Acupuncture-induced neuroprotection against stroke

Studies have demonstrated the neuroprotective effects of acupuncture against CNS disorders, especially cerebral ischemic stroke. For example, in 2003, Xiong and colleagues reported that pretreatment of rats with repeated EA stimulation at the Baihui acupoint (GV20) before cerebral ischemia significantly reduced the volume of infarcts caused by transient middle cerebral artery occlusion (MCAO) and improved subsequent neurological outcomes (Xiong et al., 2003). A single 30-minute session of EA stimulation at the Baihui acupoint induced biphasic tolerance against focal cerebral ischemia; the acute phase was observed 2 hours after EA pretreatment, while the delayed ischemic tolerance was observed 24 hours after the stimulus (Wang et al., 2005). In addition to the protection provided against ischemic cerebral injury, EA pretreatment also protected rats that were exposed to high-sustained positive acceleration (+Gz) by reducing pathological injury to hippocampal neurons and the number of apoptotic neurons in area CA1 as well as by improving learning and memory caused by +Gz exposure (Feng et al., 2010).

Acupuncture-induced neuroprotection against stroke has also been consistently reported in clinical trials. Hu and colleagues investigated the feasibility of the use of acupuncture in combination with conventional supportive treatments for acute stroke patients in an RCT and found that acupuncture more effectively improved neurological outcomes than the standard rehabilitation procedure (Hu et al., 1993). This finding was later verified by Magnusson and colleagues, who found that in stroke patients, electrostimulation resulted in better functional recovery than the control treatments (Magnusson et al., 1994). Similarly, in a meta-analysis of 8 RCTs,

Wang and colleagues found that compared to conventional western medicines, scalp acupuncture was associated with greater improvement in neurological deficit scores and higher clinical efficacy rates in patients after acute ischemic stroke (Wang et al., 2012). Wu and colleagues comprehensively assessed the efficacy of acupuncture in post-stroke rehabilitation and found that acupuncture was associated with significantly better outcomes than sham treatment or no acupuncture in the treatment of post-stroke rehabilitation (Wu et al., 2010). Consistently, acupuncture is also considered to be an effective rehabilitation tool for increasing quality of life and improving mobility and activities of daily living in post-stroke patients in a recent review (Farmer, 2015). Furthermore, an overview of systematic reviews and meta-analyses related to stroke and stroke-related disorders that was recently published suggested that although further rigorous RCTs are required, acupuncture may be effective for treating post-stroke neurological impairment and dysfunction (Zhang et al., 2014a). Potential protective effects of acupuncture against surgical brain damage were also observed in patients receiving selective craniocerebral tumor resection (Lu et al., 2010). Acupuncture was also found to offer beneficial effects to patients suffering from vascular dementia, Alzheimer's disease and Parkinson's disease by improving their neurological function and quality of life (Lee and Lim, 2017; Zeng et al., 2014; Zhou et al., 2015).

2.4. Protective effect of acupuncture against cardiovascular disorders

2.4.1. Acupuncture treatment for hypertension

Acupuncture has been demonstrated to provide protection against renovascular hypertension, hypoxia-induced pulmonary hypertension and spontaneous hypertension in rodents (Lu et al., 2015a). The mechanisms have been reported to include the activation of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) (Kim et al., 2006a), regulation of the balance between endothelium-derived vasoconstrictors and vasodilators (Pan et al., 2010), and effects on microcirculation and hemorheology (Zhou et al., 1993). Longhurst's team showed that EA modulated the elevation in blood pressure that was evoked by gastric distension in rats (Li et al., 2002) and by gallbladder stimulation in cats (Tjen et al., 2006). They repeatedly inflated a balloon that was implanted in the stomach to produce 20 mmHg of tension on the gastric wall to induce a consistent rise in mean arterial pressure. This pressor response was significantly decreased by the application of EA (Li et al., 2002) through an opioid mechanism (Li et al., 2013). Similarly, the increase in blood pressure that was caused by cardiovascular reflex responses induced by gallbladder chemosensitive afferent stimulation was also reduced by EA (Tjen et al., 2006). These results suggest that acupuncture may modulate central sympathoexcitatory cardiovascular responses, resulting in a decrease in blood pressure, and the authors suggested that this modulation may mediated by an opioid mechanism.

Emerging evidence from published case reports and clinical trials also supports the idea that acupuncture induces antihypertensive effects in patients with essential hypertension (Painovich and Longhurst, 2015; Severcan et al., 2012). For example, in 1975, Tam and Yiu reported that

acupuncture significantly restored normal blood pressure in patients with essential hypertension (Tam and Yiu, 1975). Yin and colleagues also assessed the efficacy of acupuncture as an adjunctive therapy for essential hypertension and found that it significantly decreased mean blood pressure (Yin et al., 2007). Similarly, another randomized trial found that acupuncture lowered mean 24-hour ambulatory blood pressure in patients with uncomplicated arterial hypertension (Flachskampf et al., 2007). A recently published systematic review and meta-analysis also provided evidence of the efficacy of acupuncture as an adjunctive therapy for treating hypertension (Zhao et al., 2015).

2.4.2. Cardioprotection

Acupuncture-induced cardioprotection against myocardial ischemia, as well as the underlying mechanisms, have been investigated by several laboratories around the world. In animal models of myocardial ischemia and ischemia-reperfusion, acupuncture exerted cardioprotective effects by decreasing myocardial infarct size, reducing cardiac myocyte apoptosis, and improving cardiac function (Cao et al., 1998; Cheng et al., 2013; Zhang et al., 2009a). Several possible mechanisms were proposed, including the suppression of cardiac norepinephrine release, the activation of opioid receptors and protein kinase C (PKC)-dependent pathways in the myocardium (Zhou et al., 2012), the upregulation of cardiovascular endothelial growth factor expression (Fu et al., 2014), the differential regulation of nitric oxide synthase (NOS)/nitric oxide (NO) in the rostral ventrolateral medulla (RVM) (Xia et al., 2008), and inhibition of the activation of the myocardial β1-

adrenoceptor-Gs-protein–cAMP pathway by myocardial ischemia and reperfusion (Gao et al., 2007a).

Clinical observations have also suggested that acupuncture may have beneficial effects in myocardial ischemia (Painovich and Longhurst, 2015). Early work by Ballegaard and colleagues on patients with stable angina pectoris found that acupuncture significantly increased cardiac work capacity, expressed as dPRP (difference in the pressure-rate-product between rest and maximum exercise) and maximal PRP (pressure-rate-product), during exercise (Ballegaard et al., 1986). In patients with moderate stable angina pectoris, acupuncture was also effective in increasing exercise tolerance and delaying the onset of pain (Ballegaard et al., 1990). Richter and colleagues also showed that applying acupuncture three times per week for four weeks in patients with angina pectoris reduced anginal attacks, increased the performance before the onset of pain during exercise test, decreased the intensity of pain at maximal workload and ST-segment depressions at maximal comparable load, and improved quality of life (Richter et al., 1991). Similar improvements were also observed in patients with severe angina pectoris using TENS (Mannheimer et al., 1985). In an RCT, Yang and colleagues revealed that EA had a cardioprotective effect against myocardial injury in patients undergoing heart valve replacement surgery by reducing serum levels of cardiac troponin I, inotrope use and intensive care unit stay time (Yang et al., 2010). This finding was then confirmed in subsequent studies that showed acupuncture was effective at attenuating myocardial injury in children undergoing cardiac surgery (Ni et al., 2012) and in patients undergoing percutaneous coronary intervention (Wang et al., 2015).

3. ECS activation mediates similar biological effects as acupuncture

3.1. Overview of the ECS

The ECS is composed of cannabinoid receptors, their endogenous ligands and enzymes that mediate the synthesis, uptake and degradation of endocannabinoids. As a pivotal endogenous signaling system that is involved in multiple physiological and pathological processes, the ECS has been thoroughly studied since the discovery and cloning of the type-1 (Matsuda et al., 1990) and type-2 (Munro et al., 1993) cannabinoid (CB1 and CB2) receptors in the early 1990s.

The two best-studied cannabinoid receptors are the CB1 and CB2 receptors, which are 7-transmembrane G protein-coupled receptors with differential expression patterns (Howlett, 1995; Pertwee, 1997). CB1 receptors are found primarily at the presynaptic terminals of neurons in the central and peripheral nervous systems (Pertwee, 1997) as well as in some other peripheral tissues, such as the heart, liver (Maccarrone et al., 2001; Nong et al., 2001) and adipose tissue (Maccarrone et al., 2001). CB2 receptors are concentrated primarily in the immune system (Munro et al., 1993) and, to a lesser extent, in cells of the CNS, such as microglia (Cabral and Griffin-Thomas, 2009), astrocytes (Stella, 2010), and some neurons (Brusco et al., 2008; Van Sickle et al., 2005). Endocannabinoids are endogenous lipid agonists of cannabinoid receptors, and the most widely investigated endocannabinoids are AEA (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Sugiura et al., 1995), both of which are

generated postsynaptically “on demand” through different biosynthetic routes (Marsicano et al., 2003). For example, the synthesis of AEA is catalyzed by N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), whereas diacylglycerol lipase (DAGL) catalyzes the synthesis of 2-AG. Endocannabinoids exert their effects briefly and locally by acting as retrograde messengers via activation of their presynaptic receptors. After rapid cellular re-uptake, AEA is metabolized by fatty acid amide hydrolase (FAAH), and 2-AG is metabolized by monoacylglycerol lipase (MGL) (Piomelli, 2003). Furthermore, it has been demonstrated that endocannabinoids could inhibit GABAergic synaptic transmission in a tonic manner via CB1 receptor under basal conditions in specific neurons. It is considered that this tonic endocannabinoid signaling may be involved in regulation of basal synaptic transmission (Kano et al., 2009; Katona and Freund, 2012; Morena et al., 2016).

3.2. ECS-mediated antinociceptive effects

Cannabis has been used around the world to alleviate pain for centuries (Mechoulam and Hanus, 2000). In the past decades, numerous studies have verified the analgesic effects of cannabinoids in different pain conditions. It has been suggested that endogenous, plant-derived and synthetic cannabinoids exhibit analgesic effects in multiple pain models, including acute pain caused by thermal, mechanical, and chemical pain stimuli, and chronic pain, such as inflammatory and neuropathic pain (Maldonado et al., 2016; Walker and Huang, 2002; Whiting et al., 2015; Woodhams et al., 2015; Zogopoulos et al., 2013). Cannabinoids exert their antinociceptive effects mainly by activating CB1 and CB2 receptors. CB1 receptors are located

throughout the pain pathways in the nervous system, and their analgesic activity has been verified in regions ranging from supraspinal areas, such as the periaqueductal grey matter (PAG) and RVM, to the spinal cord and peripheral terminals (Burston and Woodhams, 2014). As an emerging therapeutic target alternative to the CB1 receptor, the CB2 receptor has also been found to play a functional role in suppressing chronic pain through its effects on immune and immune-derived cells in peripheral areas and on microglial cells in the CNS (Anand et al., 2009; Zhang et al., 2014b).

3.3. ECS-mediated neuroprotection

The ECS is known to play an important role in neuroprotection against ischemic stroke (England et al., 2015). It has been demonstrated that during cerebral ischemic injury *in vivo*, endocannabinoids, such as AEA and 2-AG, are released on demand and bind to both CB1 and CB2 receptors to elicit a robust neuroprotective effect. Consistent with these findings, the beneficial effects induced by the administration of endogenous and exogenous cannabinoids have also been demonstrated in various *in vitro* and *in vivo* models of ischemic injury (Capettini et al., 2012). The activation of CB1 receptors on neurons is associated with Gi/o-mediated modulation of various intracellular pathways in the neurons, such as the PI3K/Akt/GSK-3 pathway (Ozaita et al., 2007) and the MEK1/2-ERK1/2 cascade (Derkinderen et al., 2003), which may lead to decreased glutamate release, calcium influx and excitotoxicity; enhanced anti-oxidant activity and neurotrophin expression; and inhibited neuronal apoptosis. All of these downstream effects result in reduced infarct volume and improved neurological function. Moreover, it has also been reported that CB1 receptors expressed in neuronal mitochondrial membranes

(mtCB1R) are involved in the protective effects of ACEA, a selective cell-permeable CB1 receptor agonist, on neurons and mitochondrial function, suggesting that the mtCB1R may be a potential novel target for treatments for ischemic brain injury (Ma et al., 2015b). The CB2 receptor has recently been shown to contribute to ECS-mediated neuroprotection (van der Stelt and Di Marzo, 2005). Similar to the CB1 receptor, the CB2 receptor also exerts its protective effects via the activation of Gi/o signaling, which in turn inhibits the cAMP/PKA and PI3K/Akt pathways and activates MAPK cascades (Fernandez-Ruiz et al., 2008). These downstream pathways subsequently activate excitatory amino acid transporter 2 (EAAT2) in astrocytes, reduce microglial activation and neutrophil invasion (Murikinati et al., 2010), suppress pro-inflammatory cytokines, and attenuate leukocyte/endothelial cell interactions and adhesion molecule expression after ischemic injury (Zhang et al., 2009b; Zhang et al., 2007a; Zhao et al., 2010). ECS-mediated effects contribute to the prevention of excitotoxicity, inhibition of post-stroke inflammation and neuroprotection against ischemic neuronal damage. Moreover, the ECS is considered to be a promising therapeutic target in acute neuronal injury, including traumatic brain injury (TBI) (Schurman and Lichtman, 2017), epilepsy (Friedman and Devinsky, 2015), and chronic neurodegenerative disorders such as Parkinson’s disease, Huntington’s disease, and Alzheimer’s disease (Cassano et al., 2017; Fernandez-Ruiz et al., 2015; Glass, 2001).

3.4. Regulatory effect of the ECS on the cardiovascular system

Activation of the ECS induces pronounced cardiovascular effects in different pathological conditions (O'Sullivan, 2015; Zubrzycki et al., 2014); for

example, both endogenous and exogenous cannabinoids, such as AEA and Δ9-tetrahydrocannabinol (THC), exert powerful hypotensive effects in different models of experimental hypertension, such as the spontaneously hypertensive and angiotensin II–induced hypertensive rats. The underlying mechanisms of these cardiovascular effects are complex. It has been reported that CB1 receptor activation mediates the hypotensive, bradycardic and cardiodepressive effects induced by AEA, THC, and synthetic cannabinoids (Batkai et al., 2004; Lake et al., 1997; Varga et al., 1995). Moreover, preventing the degradation of AEA exhibited increased sensitivity to the CB1 receptor-mediated hypotensive effects of AEA without affecting normal blood pressure and cardiac contractility, which may lead to new strategies for pharmacotherapies for hypertension that target endocannabinoid signaling without the addictive psychological effects produced by direct cannabinoid administrations (Batkai et al., 2004; Pacher et al., 2005). The beneficial cardioprotective role of the ECS in myocardial ischemic injury has also been confirmed in isolated heart models and *in vivo* ischemia/reperfusion models. Cannabinoids, including WIN 55,212-2 (Di Filippo et al., 2004) and 2-AG (Lepicier et al., 2003), significantly reduced infarct size, an effect that was primarily mediated by CB2 receptors via its anti-inflammatory properties, such as the inhibition of proinflammatory cytokine release, leukocyte migration to the ischemic area, neutrophil infiltration and oxidative stress (Di Filippo et al., 2004; Montecucco et al., 2009), and downstream targets of the CB2 receptor, including PI3K, ERK1/2, PKC and STAT-3, were found to be involved (Lepicier et al., 2003; Montecucco et al., 2009).

4. The role of the ECS in determining the effectiveness of acupuncture's benefits

4.1. ECS in acupuncture-induced analgesia

EA has been used as an alternative therapy for pain relief around the world, and its analgesic effects have been demonstrated experimentally in both animal studies and clinical trials (Han, 2003; Wang et al., 2008). To date, different theories about its mechanisms have been suggested and the most thoroughly described one is the endogenous opioid system (Han, 2003; Zhang et al., 2005a). As previously mentioned, 2 Hz EA was shown to accelerate the release of enkephalins and endorphins, whereas 100 Hz EA stimulated the release of dynorphin to produce analgesic effects (Han, 2003). Interestingly, the ECS has been shown to interact with the endogenous opioid system, for example, CB1 and opioid receptors are co-expressed in several regions of the CNS (Rodriguez et al., 2001; Salio et al., 2001), they are both primarily located presynaptically, and their activation inhibits synaptic neurotransmitter release (Mansour et al., 1995; Schlicker and Kathmann, 2001). Thus, it is highly likely that EA activates both the ECS and the endogenous opioid system to produce analgesia. Indeed, the ECS was recently reported to contribute to EA-induced analgesia both centrally and peripherally.

Shou and colleagues reported that EA at the Zusanli (ST36) and Kunlun (BL60) acupoints significantly increased CB1 receptor expression in the striatum and also increased paw withdrawal latency (PWL) in a complete Freund's adjuvant (CFA)-induced arthritis rat model. These effects were

attenuated by the CB1 receptor antagonist AM251, suggesting that the CB1 receptor plays a key role in the analgesic effects induced by EA. They further found that EA administration also increased the levels of dopamine D1 and D2 receptor mRNA in the corpus striatum, an effect that was also blocked by AM251 (Shou et al., 2013). This finding demonstrated cross-regulation between the ECS and the dopamine system when EA was administered to treat inflammatory pain. Similarly, Gondim and colleagues also found that EA treatment significantly reduced mechanical hypernociception in a rat model of zymosan-induced arthritis of the temporomandibular joint (TMJ); the CB1 receptor was also centrally involved in this process. Interestingly, they further discovered that AM251 only reversed the antinociceptive effect of EA and not its anti-inflammatory effect in the TMJ. In contrast, the CB2 receptor antagonist AM630 fully blocked this anti-inflammatory effect but appeared to have no influence on the antinociceptive effect. Based on these findings, the authors suggested that the antinociceptive effect of EA might be central, direct, and independent of its anti-inflammatory effect, which suggests that the central regulatory effects of EA via CB1 receptors might overcome a possible peripheral effect that occurs via CB2 receptors in the TMJ. Furthermore, in the spinal trigeminal tract, CB1 receptor gene expression was significantly increased by EA at 6 hours after the induction of arthritis and was even higher at 24 hours. In contrast, CB2 receptor gene expression peaked at 6 hours and was downregulated at 24 hours after the induction of arthritis. The authors suggested that upregulation of the CB2 receptor gene expression at 6 hours might have inhibitory effect in microglial cells, and that its downregulation at 24 hours was consistent with the finding that the nociceptive thresholds

returned to baseline at 24 hours. These results suggest that by this time point, the quantity of inflammatory mediators released had already been controlled and may have no longer been sufficient to stimulate CB2 receptor gene expression (Gondim et al., 2012). Consistent with these results, it has been recently reported that the orofacial antinociception produced by EA at acupoint ST36 in rats was prolonged and intensified by pretreatment of endocannabinoid metabolizing enzyme inhibitor and anandamide reuptake inhibitor. More importantly, this EA-induced orofacial antinociceptive effect was abolished by antagonist of CB1 receptor, not CB2 receptor; the authors suggested that CB2 receptors might participate selectively in the anti-inflammatory effects of EA stimulation (Almeida et al., 2016).

However, the involvement of CB1 receptor in the anti-inflammatory effects of EA was observed in a rat migraine model (Zhang et al., 2016), and the participation of peripheral CB2 receptor in the antinociceptive effects of EA against inflammatory pain has been emphasized by a series of research studies, suggesting that the CB1 and CB2 receptors might play different roles under different pain conditions. Chen and colleagues found that the application of EA to the Huantiao (GB30) and Yanglingquan (GB34) acupoints significantly reduced the thermal withdrawal latency and the mechanical threshold in a rat model of inflammatory pain induced by subcutaneous injection of CFA into the dorsal surface of the left hind paw in rats. These analgesic effects were attenuated by the injection of AM630, but not AM251, into the paw (Chen et al., 2009). EA also potentiated the release of the endocannabinoid AEA (Chen et al., 2009) and increased CB2 receptor mRNA and protein levels in the inflamed skin tissue (Zhang et al., 2010b). The

increase in CB2 receptor expression occurred specifically in T-lymphocytes, macrophages and keratinocytes in the epidermis and dermis of the inflamed skin tissues (Zhang et al., 2010b). These CB2 receptors were activated by the EA-induced release of AEA, and resulted in inhibition of the release of proinflammatory cytokines, including IL-1β, IL-6 and TNF-α, in the inflamed skin tissue; and produced analgesia (Su et al., 2012). Interestingly, the ECS was not the only system involved in the peripheral area by the administration of EA. Interaction between CB2 receptors and the endogenous opioid system at the inflammatory site was also confirmed by findings that showed EA stimulated endogenous opioid β-endorphin expression on T-lymphocytes, macrophages and keratinocytes in the inflamed skin tissues via CB2 receptor activation (Su et al., 2011). This finding suggested the possibility that EA peripherally activated both the endocannabinoid and the opioid system simultaneously and that these two systems in turn synergistically produced antinociceptive effects (Fig. 1).

4.2. The ECS in acupuncture-induced neuroprotection

In 2003, Xiong and colleagues observed the neuroprotective effect of EA by showing that administering EA at the GV20 acupoint before focal cerebral ischemia significantly reduced cerebral infarct size and improved neurological outcomes (Xiong et al., 2003). Based on these findings, they introduced the concept of “EA pretreatment” for the first time. This discovery opened a new chapter in the search for the physiological functions of EA and piqued the interest of the scientific community in seeking the contributors to this fascinating EA effect. The finding that cannabinoid receptor activation resulted in a neuroprotective effect (Capettini et al., 2012) suggested that the ECS

might mediate the neuroprotective effects of EA. This idea was supported by studies that showed that EA pretreatment significantly increased the amount of AEA and 2-AG in the brain, indicating that EA administration activated the ECS of the CNS (Wang et al., 2009). And CB1 and CB2 receptors were both showed to mediate the EA-induced neuroprotection through different mechanisms (Ma et al., 2011).

Consistent with its high expression in the brain and its contribution to the physiological effects of cannabinoids (Pertwee, 1997), the CB1 receptor was found to be centrally involved in the beneficial effects of EA pretreatment. CB1 receptor mRNA and protein levels were significantly increased after EA pretreatment, and blockade of its activation using AM251 or CB1 receptor siRNA abolished the neuroprotective effects of EA (Wang et al., 2009). The existing evidence suggests that CB1 receptors are primarily responsible for EA-induced alleviation of excitotoxicity, oxidative damage and apoptosis after cerebral ischemia. Liu and colleagues revealed that endocannabinoid signaling rapidly contributes to the regulatory effect of EA pretreatment on the excitotoxic cascades that result from excessive glutamate release after severe brain ischemia. Their data showed that EA enhanced neuronal expression of GluR2 in the hippocampus after cerebral ischemia and effectively reversed the GluR2-lacking a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-mediated excitotoxicity, whereas this protective elevation of GluR2 expression was fully blocked by CB1 receptor siRNA and the CB1 receptor antagonists AM251 and SR141716A (Liu et al., 2015). The CB1 receptor is also known for its anti-oxidant effects against cerebral ischemia. For example, an *in vitro* study of HT22 cells showed that the endocannabinoid

AEA protected neurons from oxidative injury via CB1 receptor-mediated inhibition of neuronal NADPH oxidase 2 (Jia et al., 2014). In order to verify whether this anti-oxidant activity of CB1 receptor contributes to the neuroprotective effect of EA, Sun and colleagues recently demonstrated that EA upregulated manganese superoxide dismutase (Mn-SOD) protein expression and the number of Mn-SOD-positive neuronal cells in the brain after cerebral ischemia. In turn, Mn-SOD knockdown blocked the protective effects of EA against ischemic oxidative damage. Interestingly, in the brain, the EA-induced Mn-SOD upregulation and STAT3 phosphorylation were both abolished by the CB1 receptor antagonists AM251 and SR141716 (Sun et al., 2016). Consistent with these findings, Zhou and colleagues also showed that the EA-induced activation of STAT3 (Ser-727) and the resulting effects against cerebral ischemia were mediated by the CB1 receptor. Similarly, this STAT3 activation was decreased by AM251 and CB1 receptor siRNA and increased by WIN 55,212-2 and ACEA (Zhou et al., 2013). Because STAT3 has already been shown to be a transcription factor that regulates the Mn-SOD gene and provides crucial regulation of the levels of reactive oxygen species in the brain after cerebral ischemia (Jung et al., 2009), Sun suggested that activation of the CB1 receptor-STAT3 pathway by EA is likely to attenuate the oxidative stress during cerebral ischemia/reperfusion injury. Furthermore, CB1 receptor activation induced by EA was also shown to reduce neuronal apoptosis in the peri-ischemic penumbra via multiple pathways. Wei and colleagues showed that the activation of CB1 receptors by EA was followed by promoted phosphorylation of GSK-3β at Ser-9 after focal cerebral ischemia. This phosphorylation was inhibited by AM251 and CB1 receptor

siRNA and increased by the CB1 receptor agonists WIN 55,212-2 and ACEA (Wei et al., 2014). Similarly, Wang and colleagues reported that activation of epsilon protein kinase C (εPKC) signaling was also one of the downstream pathways involved in CB1 receptor-mediated, EA-induced neuroprotection. They observed that EA pretreatment enhanced εPKC activation in the brain and that its protective effect was blocked by the εPKC-selective peptide inhibitor TAT-εV1–2. Furthermore, EA-induced enhancement of εPKC levels was reversed by the CB1 receptor antagonist AM251 but not by the CB2 receptor antagonist AM630 (Wang et al., 2011). Notably, these EA-CB1-GSK-3β and EA-CB1-εPKC pathways, as well as the EA-STAT3 pathway, all reduced the Bcl-2/Bax ratio, which switched off the cellular apoptotic machinery and resulted in the inhibition of neuronal apoptosis in the ischemic penumbra. In addition, based on results showing that inhibiting ERK1/2 activity abolished the protective effect of EA and that EA-mediated upregulation of p-ERK1/2 expression in the brain was reversed by the CB1 receptor antagonist AM251, the ERK1/2 pathway appears to be involved in CB1 receptor-mediated EA pretreatment-induced cerebral ischemic tolerance (Du et al., 2010).

Like the ischemic preconditioning induced ischemic tolerance, EA pretreatment-induced neuroprotection is also known to be biphasic (with rapid and delayed phases), and according to the studies described above, the CB1 receptor is involved primarily in the rapid phase of EA pretreatment-induced neuroprotection. To further explore the role of the ECS during the delayed phase, Ma and colleagues investigated the activation of cannabinoid receptors that was induced by EA at different time points (Ma et al., 2011).

Their results reinforced the evidence showing that CB1 receptors, but not CB2 receptors, were required for the rapid phase of EA-induced neuroprotection (2 hours after the end of EA) and further illustrated a novel mechanism wherein the CB2 receptor, instead of the CB1 receptor, contributed to the delayed phase (24 hours after the end of EA). Because CB2 receptor expression was detected primarily in microglia (Cabral and Griffin-Thomas, 2009) and astrocytes (Stella, 2010) in the CNS, it was presumed that this CB2 receptor upregulation was probably associated with the decreased inflammatory responses that were also observed. These responses included the attenuation of microglial activation by a shift from the M1 to the M2 state that was mediated by the CB2 receptor-PKC pathway (Ma et al., 2015a), the reduction of leukocyte/endothelial cell interactions and the inhibition of neutrophil recruitment to the brain (Zhang et al., 2009b; Zhang et al., 2007a; Zhao et al., 2010) during the delayed phase of EA pretreatment-induced neuroprotection. The excitatory amino acid transporters (EAATs) are a group of proteins that transport excitatory amino acid neurotransmitters, such as glutamate, from the extracellular space into neurons and glial cells to terminate neurotransmission (Danbolt, 2001). EAATs have been reported to be neuroprotective (Li and Zuo, 2011; Rao et al., 2001). A recently published experimental study found that in the cerebral cortex, only EAAT2, instead of EAAT1 and EAAT3, played a key role in the beneficial effect of EA (Zhu et al., 2013b). In light of this finding, our group conducted follow-up experiments to further elucidate the role of EAAT2 signaling in the neuroprotective effects of EA. Our preliminary data suggested that the upregulation of EAAT2 that was induced by EA depended on the activation of CB2 receptors on astrocytes,

suggesting that EAAT2 is a downstream effector of the CB2 receptor in EA-induced neuroprotection.

All of these findings indicate that the ECS significantly contributes to the neuroprotective effects of EA, and more interestingly, that both CB1 and CB2 receptors are involved in a time-dependent manner, and act through different mechanisms (Fig. 2).

4.3. The role of the ECS in acupuncture-induced cardiovascular regulation, cardioprotection and other biological effects

EA-induced inhibition of sympathoexcitatory cardiovascular reflexes has been demonstrated in animals, and this effect was mediated by CB1 receptors in the brain. Tjen-A-Looi and colleagues showed that microinjection of AM251 into the vlPAG reversed the reduction of gastric distention-evoked reflexive responses in blood pressure induced by EA; and this AM251-associated reversal of EA inhibition was inhibited by preblockade of GABAa vlPAG receptors with gabazine (Tjen et al., 2009). Consistently, the extracellular vlPAG GABA concentration was also found to be decreased by EA, while the glutamate concentration was not affected, and this reduction in GABA release was also blocked by AM251 (Fu and Longhurst, 2009). These results indicate that EA activated presynaptic CB1 receptors in the vlPAG, which, in turn, decreased GABA release in the vlPAG and may have disinhibited the vlPAG cells and attenuated the sympathoexcitatory reflex responses.

EA-induced cardioprotection against ischemic injury has been demonstrated experimentally and in clinical trials. For example, EA at the

Neiguan (PC6) acupoint significantly attenuated myocardial infarct size, myocardial apoptosis, the duration of arrhythmia, left ventricular remodeling and the mortality rate in animals with myocardial ischemic injury. Consistent with these results, an RCT of 60 patients undergoing heart valve replacement surgery also indicated that EA at the PC6, Lieque (LU7), and Yunmen (LU2) acupoints significantly decreased the serum level of cardiac troponin I and inotrope use after arrival into the intensive care unit as well as shortened intensive care unit stays (Yang et al., 2010). Some of the studies also explored the underlying mechanisms of these protective effects of EA and found that they were mediated by opioid and PKC-dependent pathways (Zhou et al., 2012) and reduced the Bcl-2/Bax ratio in myocardial tissue. The activity of glutathione peroxidase (GSH-PX), a main endogenous anti-oxidant enzyme, was also enhanced by EA in a myocardial ischemia model (Zhang et al., 2009a). Overlap between these pathways and the downstream signaling of CB2 and CB1 receptors mediated EA-induced neuroprotection, indicating a potential role for the ECS in EA-induced cardioprotection. This might be a promising new area in which to further explore the participation of CB2 and CB1 receptors in EA's many biological effects.

In addition, EA at the ST36 and SP6 acupoints induced conditioned place preference via the activation of CB1 receptors (Xia et al., 2011). In contrast, Escosteguy-Neto and colleagues showed that 2 Hz EA at the Zusanli and Neiguan acupoints and 100 Hz EA at the Dazhui and Baihui acupoints inhibited ethanol withdrawal-induced CB1 receptor upregulation in several encephalic areas. This inhibition may have increased GABA release, inhibiting

neuronal function in these encephalic areas in ethanol withdrawn animals (Escosteguy-Neto et al., 2012).

5. Endogenous signaling pathways that may participate in the ECS-mediated beneficial effects of acupuncture

Based on the evidence we list above, ECS appears to play the role of a primary mediator to different downstream signaling pathways in acupuncture's effects under different pathological conditions. Here, we list some potential downstream neuronal networks of the ECS-mediated beneficial effects of acupuncture to provide a more comprehensive view of ECS's key role. (Fig. 3)

5.1. Glutamatergic signaling

Glutamate is the principal excitatory neurotransmitter in the CNS (Nicholls and Attwell, 1990), and excitotoxicity resulting from dysfunctional glutamatergic signaling may lead to neuronal damage and neurological disorders, such as pain hypersensitivity (Aanonsen and Wilcox, 1987). Moreover, this excitotoxicity also contributes to the pathophysiology of a variety of neuronal diseases, including stroke, Parkinson's disease, inflammatory and neuropathic pain, and sympathetic hyperactivity and hypertension. Thus, the modulation of glutamatergic transmission in the CNS has been recognized as a promising strategy for treating excitotoxicity-related pathological conditions. At the synaptic level, glutamate signaling is mediated via two types of glutamate receptors: the ligand-gated ionotropic glutamate receptors (iGluRs) and the G protein-coupled metabotropic glutamate

receptors (mGluRs). The iGluRs include N-methyl-D-aspartate (NMDA) receptors, AMPA receptors, and kainic acid (KA) receptors, and can produce long-lasting changes in synaptic excitability. A total of 8 members of the mGluR family have been cloned to date (mGluR1–mGluR8) and were divided into three groups (I–III). The neuroprotective, analgesic and hypotensive effects of both acupuncture and activation of the ECS are known to involve the regulation of glutamatergic signaling and the restoration of glutamate homeostasis in the CNS. Therefore, it is highly likely that the glutamatergic signaling may participate as downstream pathway in ECS-mediated acupuncture effects.

5.1.1. Glutamatergic signaling contributes to both acupuncture- and cannabinoid-induced neuroprotection

In a transient ischemia model in gerbils, EA significantly attenuated the ischemia-induced release of extracellular glutamate (Pang et al., 2003), and in a rat model of MCAO, EA also markedly blocked the increase of glutamate levels in the topical cerebral infarct tissue (Zhang et al., 2007b). Similarly, the increase in cerebral extracellular glutamate release that was induced by global ischemic stroke in normal and diabetes mellitus rats was also suppressed by the administration of acupuncture (Choi et al., 2010; Lee et al., 2010). Moreover, Guo and colleagues further demonstrated that upregulation of glutamate transporter-1 (GLT-1, EAAT2) contributed to the EA-induced inhibition of excessive glutamate release after cerebral ischemia (Guo et al.,

2015). Consistent with these findings, acupuncture has also been shown to protect the brain against ischemic injury through the modulation of glutamate receptors. For example, in a rat model of MCAO, the upregulation of *NMDAR1*mRNA expression observed in the hippocampus following cerebral ischemia was inhibited by acupuncture (Shi, 1999), and the high expression of NMDA receptor NR1 mRNA and protein that was induced by ischemia was reversed by EA via the PI3K pathway (Sun et al., 2005). Consistent with these results, in clinical trials, the glutamate level in blood serum was found to be significantly reduced by both acupuncture and EA in stroke patients who displayed improved recovery (Yue et al., 2012).

The inhibitory effect of CB1 receptor agonists on neuronal excitability in the CNS is well-documented (Marsicano et al., 2003; Piomelli, 2003; Schlicker and Kathmann, 2001). One physiological role of the ECS is to restrain glutamatergic activity in the CNS to maintain it within safe limits, thereby protecting neurons from glutamate neurotoxicity (Auclair et al., 2000; Hampson and Grimaldi, 2001; Levenes et al., 1998; Shen et al., 1996). For instance, Marsicano and colleagues reported that in conditional mutant mice that lack CB1 receptor expression in principal forebrain neurons, the excitotoxin KA induced more excessive seizures. The threshold to KA-induced neuronal excitation was also severely reduced in the hippocampal pyramidal neurons of the mutants *in vitro* (Marsicano et al., 2003). Furthermore, this cannabinoid receptor-mediated inhibitory effect on glutamate release is also

thought to be involved in the neuroprotective effects of cannabinoids against hypoxic-ischemic brain injury, possibly via a subsequent decrease in Ca^{2+} entry and excitotoxic damage, in the ischemic tissue (Fowler, 2003). In an *in vitro* study, incubation of forebrain slices with WIN 55,212-2 reduced oxygen-glucose deprivation-induced glutamate release and provided robust neuroprotection via CB1 and CB2 receptors (Fernandez-Lopez et al., 2006). Similarly, activation of the CB2 receptor by COR167 was also found to potently protect rat brain cortical slices against oxygen-glucose deprivation and reperfusion injury by reducing glutamate release (Contartese et al., 2012).

5.1.2. Glutamatergic signaling contributes to both acupuncture- and cannabinoid-induced analgesia

In addition to neuroprotection, glutamatergic transmission also plays a pivotal role in the transmission of nociceptive information and central sensitization to pain (Aanonsen et al., 1990). Previous studies have shown that glutamatergic transmission also mediates the analgesic effects of acupuncture and cannabinoids. For example, Zheng and colleagues found that administration of the glutamate antagonist glutamate diethylester (GDEE) in the motor cortex (MCtx) reduced the inhibition of nociceptive responses normally induced by EA or by stimulation of cortical somatosensory area II (S II), suggesting that glutamate released from S II to MCtx might be involved in corticofugal modulation of intralaminar nuclei from S II via MCtx in

acupuncture-induced analgesia (Zheng et al., 1994). Similarly, in the lumbar spinal cord (L4-6), glutamate level was also found to be reduced by EA and helped to alleviate neuropathic pain in chronic constrictive injury (CCI) rats (Yan et al., 2011). Further studies revealed that both iGluRs and mGluRs are involved. For example, EA has been shown to reverse the chronic constrictive injury-induced increase in RNA levels of the NMDA receptor subunits NR2A and NR2B in the amygdala, resulting in analgesia (Feng et al., 2014). Similarly, EA also attenuated inflammatory and neuropathic pain, the expression of NR1, NR2 and GluR1 in L4−5 segments of the spinal cord (Choi et al., 2005; Sun et al., 2004; Zhang et al., 2012) and of NR2B in C1-3 segments of the spinal cord (Gao et al., 2009), and the number of IB4 and NR1 double-labeled neurons in the lumbar DRG (Wang et al., 2006). Pharmacological studies provided additional evidence that blocking NMDA receptors has a synergistic analgesic effect with acupuncture, which strengthened the idea that the glutamatergic system plays an important role in acupuncture-induced analgesia. Moreover, intraperitoneal injection of ketamine, a non-competitive NMDA receptor antagonist, enhanced EA-induced antinociception, prevented or delayed the development of chronic tolerance to EA under normal conditions (Huang et al., 2005), and potentiated EA-induced anti-allodynic effects in a neuropathic pain model (Huang et al., 2004). A similar phenomenon was demonstrated in a rat model of inflammatory pain, which showed that i.t. injection of a low dose of the NMDA

receptor antagonist AP5, the AMPA/KA receptor antagonist DNQX or the wide-spectrum glutamate receptor antagonist KYNA significantly potentiated EA-induced analgesia and inhibited the increase in Fos expression in the L4-5 spinal cord that is normally induced by carrageenan injection (Zhang et al., 2002, 2003). In addition, synergistic analgesic effects of EA and the NMDA receptor antagonist MK-801 were also observed in models of carrageenan- or CFA-induced inflammatory pain (Zhang et al., 2005b) and diabetic neuropathic pain, and these effects were hypothesized to be related to p35/p25 expression in the spinal cord (Hwang et al., 2011). For mGluRs, an analysis of hypothalamus gene expression profiles after EA revealed that the expression level of glutamate receptor, metabotropic 6 was significantly increased in the EA responder groups, which exhibited a significant increase in tail flick latency (Gao et al., 2007b). These results indicate that the glutamatergic system is involved in EA-induced analgesia in the CNS.

Similarly, cannabinoid-induced analgesia has also been demonstrated to involve presynaptic inhibition of glutamatergic transmission in descending inhibitory pain pathways (Manzanares et al., 2006; Richardson, 2000). The expression of monoacylglycerol lipase (MGL), the main degradation enzyme of 2-AG, on glutamatergic axon terminals in the spinal dorsal horn has been described, suggesting a role for 2-AG-regulated glutamatergic signaling in the pain circuitry of the dorsal horn (Docs et al., 2014; Horvath et al., 2014). Consistent with these results, presynaptic CB1 receptors have also been

shown to inhibit glutamate release in the spinal dorsal horn, a process that may contribute to the modulation of spinal excitatory transmission and thereby result in analgesia (Bishay et al., 2010; Morisset and Urban, 2001). Moreover, Vaughan and colleagues demonstrated that in the PAG, which is a major site of cannabinoid-mediated analgesia, the amplitude of glutamatergic postsynaptic currents was reduced by cannabinoid receptor agonists, while these effects were blocked by rimonabant, indicating that cannabinoids might inhibit glutamatergic synaptic transmission and induce analgesia by acting through CB1 receptors (Vaughan et al., 2000). The pivotal role of the CB1 receptor in maintaining NMDA receptor activity within appropriate limits to maintain basal nociceptive thresholds has also been demonstrated. For instance, Richardson and colleagues reported that thermal hyperalgesia was evoked when CB1 receptors in the spinal cord were reduced in density or antagonized and was blocked by NMDA receptor antagonists (Richardson et al., 1998). Consistent with these results, at the supraspinal level, cannabinoid-mediated activation of CB1 receptors was also shown to selectively inhibit presynaptic N-type Ca^{2+} channels and therefore the exocytotic release machinery, attenuating transmitter release at trigeminal nociceptive synapses (Liang et al., 2004). Ghalandari-Shamami and colleagues further demonstrated that intra-accumbal infusion of the NMDA receptor antagonist AP5 prevented the antinociceptive effect induced by intra-basolateral amygdala (BLA) administration of WIN 55,212-2, indicating that glutamatergic

projections from the BLA to the nucleus accumbens may be necessary for the potent analgesic effects of cannabinoids (Ghalandari-Shamami et al., 2011). Moreover, interactions between mGluRs and the ECS during pain processing have also been verified by a large body of evidence (Hu et al., 2014; Palazzos et al., 2006).

5.1.3. Glutamatergic signaling contributes to both acupuncture- and cannabinoid-induced cardiovascular effects

Glutamatergic signaling has been reported to participate in the cardiovascular effects of both acupuncture and cannabinoids. Evidence demonstrates that glutamate contributes to visceral sympathoexcitatory cardiovascular reflexes and is involved in EA-induced modulation of visceral cardiovascular responses. EA significantly reduced the increased blood pressure and RVLM extracellular glutamate concentration that were evoked by bradykinin-induced gallbladder stimulation (Zhou et al., 2007). Similarly, Tjen-A-Looi and colleagues found that glutamatergic nucleus ambiguus-projecting nucleus tractus solitarius neurons were likely involved in the inhibitory effects of EA on phenylbiguanide-evoked bradycardia (Tjen et al., 2014). Consistent with these results, electrophysiological and neurochemical experiments demonstrated that endocannabinoids and cannabinoid agonists could presynaptically inhibit glutamatergic synaptic transmission throughout the PAG (Vaughan et al., 2000) and that FAAH inhibition could unmask CB1 receptor-mediated presynaptic inhibition in the PAG (Kawahara et al., 2011).

Subsequent investigations revealed that the increase of local anandamide levels by the administration of an FAAH inhibitor prevented the cardiovascular responses that were evoked by local injection of NMDA in the dlPAG, including the increase in mean arterial pressure and the decrease in heart rate (Viana et al., 2015).

5.2. GABAergic signaling

GABA (γ-Aminobutyric acid) is the primary inhibitory neurotransmitter within the CNS, and together with glutamate, GABA maintains the balance between excitation and inhibition. Its inhibitory effects are mediated through the activation of ionotropic (GABAa and GABAc) and metabotropic (GABAb) receptors and have been reported to be involved in multiple physiological and pathological functions (Malcangio and Bowery, 1996). As we summarized in the previous section, ECS-induced inhibition of GABAergic signaling has already been shown to participate in acupuncture-induced cardiovascular regulation (Tjen et al., 2009); therefore, we only outline the evidence supporting the involvement of the GABAergic system in acupuncture and in activation of the ECS-induced neuroprotective and analgesic effects.

5.2.1. GABAergic signaling contributes to both acupuncture- and cannabinoid-induced neuroprotection

The activation of GABA receptors has been shown to decrease glutamatergic activity and excitotoxicity during cerebral ischemic stroke.

Moreover, it can facilitate local cerebral blood flow and induce hypothermia, which provide neuroprotection against ischemic injury (Green et al., 2000; Liu and Wang, 2013). There is also increasing evidence supporting the participation of GABAergic signaling in acupuncture-induced neuroprotective effects. Findings from basic medical research showed that acupuncture stimulation increased the intensity of GABAergic signaling in the CNS, resulting in protective effects (Lu and Lu, 2013). For example, in rats, EA was reported to upregulate GABA immunoexpression after it was reduced by MCAO and to protect neurons in the ipsilateral cerebral cortex and striatum from ischemic damage; this neuroprotective effect of EA was blocked by picrotoxin (PTX), a GABA receptor antagonist (Gan et al., 2005). In a recent study, the level of GABAaγ2 and GABAbR2 expression, in addition to GABA levels, were found to be decreased in the striatum and spinal cord after MCAO in rats. Furthermore, although acupuncture did not significantly affect the levels of GABA or the trafficking protein, kinesin binding 1 (TRAK1) in these regions, it restored the expression of GABAaγ2 and GABAbR2 receptors (Xu et al., 2015). These data suggest that GABAergic inhibitory neurotransmission plays a vital role in acupuncture-induced neuroprotection. This idea was also supported by a clinical study of the effects of acupuncture and EA on spastic paralysis in stroke patients. The results of this study showed that both acupuncture and EA increased blood serum levels of GABA

and decreased blood serum levels of glutamate as well as the Glu/GABA ratio (Yue et al., 2012).

The precise functional role of cannabinoid signaling in GABAergic transmission and neuroprotection has yet to be established (Chiarlone et al., 2014), and the existing evidence is highly controversial. A recently published study shed light on this issue at the level of layer-specific changes in CA1-evoked responses following lateral fluid percussion injury (lFPI)-induced TBI in mice (Johnson et al., 2014). Johnson and colleagues identified an increase in stratum radiatum (SR)-evoked stratum oriens (SO) hyperpolarization and a decrease in hippocampal CA1 output after lFPI, and this decrease in CA1 output was suggested to contribute to the cognitive impairments associated with TBI. They then found that the SR-evoked SO hyperpolarization increase persisted when glutamatergic transmission was blocked, indicating that it was primarily due to the direct electrical activation of interneurons. Furthermore, a low concentration WIN 55,212-2 restored CA1 output in slices obtained from injured animals. These findings support the hypothesis that increased GABAergic signaling by cannabinoid-sensitive interneurons contributes to the reduction in CA1 output that follows TBI. They are also consistent with the hypothesis that in slices from injured animals, WIN 55,212-2 restored the AP firing of injured cells to normal levels by over-suppressing GABA release to compensate for a possible diminished excitatory responses in injured animals (Johnson et al., 2014). However, Chiarlone and colleagues investigated the

neuronal population specificity of the neuroprotective effects of CB1 receptors and demonstrated that a restricted population of CB1 receptors located on glutamatergic, not GABAergic, terminals plays a key role in protecting against excitotoxic damage and neurodegeneration in the mouse brain (Chiarlone et al., 2014). These findings may support the idea that in contrast to the key role of glutamatergic CB1 receptors, GABAergic CB1 receptors were postulated to provide a compensatory mechanism to maintain the equilibrium between excitatory and inhibitory transmission in the CNS. For example, stressful experiences, which are characterized by enhanced glutamatergic tone, have been proposed to lead to an imbalance between excitatory and inhibitory transmissions and that this imbalance subsequently causes the downregulation of CB1 receptors exclusively on GABAergic terminals, resulting in an increase in the strength of GABAergic inhibition of glutamatergic transmission (Ruehle et al., 2012). Based on these notions, we postulate that the effect of acupuncture on CB1 receptors could also be neuronal population-specific, that is, the increase in GABA signaling induced by acupuncture in neuroprotection might be directly or indirectly mediated by the downregulation of GABAergic CB1 receptors. This would result in enhanced extracellular GABA availability, attenuation of the sharp increase in excitatory transmission that occurs during cerebral ischemic stroke and restoration of the equilibrium between GABAergic and glutamatergic neurotransmission in the brain.

5.2.2. GABAergic signaling contributes to both acupuncture- and cannabinoid-induced analgesia

As the major inhibitory system in the CNS, GABA is widely distributed throughout peripheral and central nociceptive pathways, where it contributes to pain sensation (Jasmin et al., 2004; McCarson and Enna, 2014). The roles of GABAergic activity in pain modulation are region-specific. For example, the facilitation of GABAergic transmission in the spinal cord generally raises nociceptive thresholds, whereas the activation of supraspinal GABAa receptors generally lowers nociceptive thresholds, an effect that is primarily due to tonic inhibition of descending antinociceptive pathways by the GABAergic system (McCarson and Enna, 2014). Consistent with these results, the role of GABAergic signaling in acupuncture-mediated analgesia is also region-specific and displays similar patterns, and the seemingly contradictory results could be explained by differences in CNS regions. For example, GABA levels in the hippocampus and brainstem were decreased by EA-mediated analgesia (Zheng et al., 1995). EA might also suppress the release of GABA and consequently remove tonic GABAergic inhibition of serotonergic neurons in the RVM to produce analgesia (Zhang et al., 2011). Moreover, microinjection of muscimol, a GABAa receptor agonist, or 3-MP, a GABA synthesis inhibitor, into the PAG markedly suppressed and potentiated, respectively, acupuncture-induced analgesia (Zhao, 2008). Similarly, EA-induced analgesia was also found to be mediated by the inhibition of

GABAergic activity in the thalamus and pons-medulla, and this analgesic effect was blocked by diazepam via disinhibition of the EA-induced inhibition of GABAergic transmission (Chakrabarti and Poddar, 1989). In contrast, at the spinal level, acupuncture-induced analgesia was found to be mediated by increased GABAergic transmission. Yan and colleagues reported that in the lumbar spinal cord (L4-6), EA significantly upregulated GABA levels, which helped alleviate neuropathic pain in CCI rats (Yan et al., 2011). Similarly, TENS also bilaterally elevated synaptosomal GABA levels in the dorsal horn of L4-5 segments and reduced mechanical allodynia in CCI rats (Somers and Clemente, 2009). For the GABA receptors, Gao and colleagues demonstrated that EA significantly increased the expression levels of GABAa R and GABAb R1 and R2 mRNA in the cervical spinal cord (C1-3) and produced analgesia against regional thyroid inflammatory pain (Gao et al., 2012). Consistent with these results, intrathecal administration of the GABAa receptor antagonist gabazine or the GABAb receptor antagonist saclofen into the spinal subarachnoid space blocked the relieving effects of EA stimulation on cold allodynia (Park et al., 2010). This phenomenon was replicated by another study that showed that intrathecal injection of the GABAa receptor antagonist bicuculline and the GABAb receptor antagonist phaclofen into the subarachnoid space at the L5-6 level reduced the intensity and/or duration of the effects of the administration of EA with different parameters (Silva et al., 2011). In support of these behavioral results, an electrophysiological study

demonstrated that the application of bicuculline to the surface of the spinal cord significantly inhibited EA-enhanced depolarization of the presynaptic terminals of primary C-afferents, suggesting that GABAergic signaling is involved in EA-induced regulation of presynaptic inhibition in the spinal cord (Li et al., 1993).

To date, studies of GABAergic signaling-mediated cannabinoid-induced analgesia have focused on effects at the supraspinal level in the midbrain PAG. The PAG is a crucial site for the initial activation of descending antinociceptive pathways, and the PAG-RVM-spinal cord pathway is known to be an essential neural circuit for cannabinoid-produced, centrally-mediated analgesia. To date, the most well-studied mechanism is the activation of CB1 receptors, which are expressed on the presynaptic terminals of GABAergic interneurons in the PAG and reduces GABA release to disinhibit the PAG-RVM-dorsal horn antinociceptive pathway (Vaughan et al., 2000). A large body of evidence indicates that endocannabinoid signaling-induced disinhibition of the PAG–RVM system is involved in the analgesic effects of different endogenous and exogenous agents, such as orexin A, cholecystokinin, substance P, neurotensin and capsaicin. For example, orexin A has been reported to depress GABAergic-evoked IPSCs, to increase the paired-pulse ratio of paired IPSCs and to decrease the frequency of miniature IPSCs in vlPAG slices. This depression was mimicked by WIN 55,212-2, enhanced by an inhibitor of monoacylglycerol lipase (MGL), and blocked by

AM251 and inhibitors of phospholipase C (PLC) and diacylglycerol lipase (DAGLα). Moreover, orexin A was shown to have an overall excitatory effect on evoked postsynaptic potentials and to thereby increase vlPAG neuronal activity. Consistent with these effects, which were observed in PAG slices, intra-vlPAG microinjection of orexin A *in vivo* was also found to reduce hot-plate nociceptive responses in a CB1 receptor-dependent manner. These data suggest that orexin A may stimulate the synthesis of 2-AG, which would, in turn, induce retrograde inhibition of GABA release (disinhibition) in the vlPAG and result in antinociception (Ho et al., 2011). Through the use of similar approaches, neurotensin was also shown to inhibit GABAergic synaptic transmission within the PAG through activation of presynaptic CB1 receptors by endocannabinoids (Mitchell et al., 2009). Furthermore, substance P was shown to facilitate the descending analgesic effects in part by enhancing the endocannabinoid-mediated disinhibition of PAG-RVM projection neurons (Drew et al., 2009). Cholecystokinin was demonstrated to induce analgesia through direct neuronal depolarization, which also occurred via the inhibition of GABAergic synaptic transmission by endocannabinoid signaling in the PAG (Mitchell et al., 2011). Moreover, capsaicin was found to stimulate the synthesis of 2-AG to retrogradely inhibit GABA release via presynaptic CB1 receptors in the vlPAG, which resulted in the disinhibition of descending pain inhibitory pathways (Liao et al., 2011). In addition to this line of research, it has also been demonstrated that the cyclooxygenase 2 (COX2)

inhibitors parecoxib (par) and valdecoxib (val) may exert their analgesic effects against neuropathic pain at least partially through a direct interaction with CB1 receptors as well. And the K^+-stimulated GABA release in rat hippocampal slices was found to be reduced by val and WIN 55,212-2, suggesting the possible involvement of cannabinoid-mediated inhibition of GABA release (Schroder et al., 2011). Furthermore, microinjection of the GABAa receptor agonist muscimol into the RVM reportedly blocked the antinociceptive effect of systemic WIN55,212-2 administration, as measured using the tail-flick test in rats (Meng et al., 1998). Similarly, bilateral local microinjections of muscimol into the central nucleus of the amygdala (CeA), but not BLA, also significantly reduced the antinociceptive effect of WIN55,212-2 in both the tail-flick and formalin tests in rats (Manning et al., 2003). These data indicate that cannabinoid-induced antinociceptive effects are mediated by the inhibition of GABAergic transmission in the RVM and CeA.

5.3. Adenosinergic signaling

Like endocannabinoids, adenosine has also been accepted as a modulator of neurotransmission in the CNS, and four different G protein-coupled adenosine receptors have been cloned to date, including the A1, A2A, A2B, and A3 receptors. It is commonly stated that A1 and A3 are inhibitory receptors and that A2A and A2B are stimulatory receptors (Fredholm et al., 2005a; Jacobson and Gao, 2006). Among these, the A1 and A2A receptors

are the most well-studied (Cunha, 2001). A1 receptors are expressed ubiquitously throughout the CNS, and they inhibit neuronal activity. A2A receptors are expressed primarily in the striatum and a few other brain regions, and they excite neurons. Activation of the adenosine system provides neuroprotection against cerebral ischemia that is mediated through A1 receptors and regulates pain transmission at spinal and peripheral levels via A1 and A2A receptors (Boison, 2008; Cunha, 2001; Fredholm et al., 2005b; Ribeiro et al., 2002).

5.3.1. Regulatory effects of endocannabinoids on adenosinergic signaling in the CNS

Endocannabinoids have been shown to regulate adenosine in the brain. By analyzing microdialysis samples collected from the basal forebrain of rats, Murillo-Rodriguez and colleagues reported that anandamide increased extracellular adenosine levels in the basal forebrain and that this effect was mediated by the CB1 receptor (Murillo-Rodriguez et al., 2003). THC and WIN 55,212-2 were then found to inhibit the uptake of adenosine in microglia and macrophage cultures, suggesting that the enhancement of adenosine signaling contributes to the anti-inflammatory properties of cannabinoids (Carrier et al., 2006). Consistent with these results, in the striatum of rats and mice, both endocannabinoids, such as anandamide and 2-AG, and exogenous cannabinoids, such as WIN 55,212-2, were capable of inhibiting the uptake of adenosine, possibly via direct interaction with the membrane

transporters. The authors further suggested that in addition to the direct effects that are mediated by the CB1 receptor, cannabinoids may also indirectly affect the release of glutamate by modifying extracellular levels of adenosine (Pandolfo et al., 2011). Moreover, they also proposed a novel plausible negative feedback system via which anandamide and 2-AG may augment the synaptic half-life of adenosine, which in turn would inhibit endocannabinoid generation (Pandolfo et al., 2011). This idea is supported by demonstrations that the degree of cannabinoid signaling and the inhibition of glutamatergic and GABAergic synaptic transmission were regulated by A1 receptors in the hippocampus and, more importantly, that this regulation depended on extracellular levels of endogenous adenosine (Hoffman et al., 2010; Serpa et al., 2009; Sousa et al., 2011). In addition, because CB1 and A1 receptors are both expressed at high levels in the hippocampus (Fastbom et al., 1987; Herkenham et al., 1991) and exert protective effects through the inhibition of glutamatergic synaptic transmission (Serpa et al., 2009), it has been recently suggested that combined application of A1 and CB1 receptor agonists could cumulatively dampen NMDA-mediated excitotoxicity with an additive combined effect higher than the one obtained activating each receptor alone (Serpa et al., 2015). Interaction between the ECS and other adenosine receptors has also been demonstrated (Lane et al., 2010; Tebano et al., 2012). For example, 2-AG has been reported to be a negative allosteric modulator of the human A3 adenosine receptor (hA(3)R) and to decrease the

basal signaling of this receptor. Since the hA(3)R is expressed in astrocytes and microglia, it was suggested that these findings may be relevant in certain pathological conditions, such as cerebral ischemia, in which levels of endocannabinoids are raised (Lane et al., 2010).

5.3.2. Adenosinergic signaling is involved in the beneficial effects of acupuncture

Wang and colleagues reported that EA preconditioning-induced rapid tolerance to focal cerebral ischemia in rats was reversed by DPCPX, a selective A1 receptor antagonist, suggesting that this beneficial effect of EA may have been mediated through an adenosine A1 receptor-related mechanism (Wang et al., 2005). These findings were replicated by another group who further revealed that the concentration of adenosine deaminase (ADA), a metabolic enzyme that breaks down adenosine, in the cortex was decreased at 60 minutes after EA preconditioning and restored back to basal levels at 120 minutes. In contrast, there was an increase in adenosine concentration at 120 minutes after EA preconditioning, suggesting that the neuroprotective effects of EA might be achieved through a reduction in the ADA concentration and elevation of the adenosine level to activate A1 receptors in the cortex (Wang et al., 2013). Moreover, local A1 receptors at the Baihui acupoint play an important role in EA-induced cerebral ischemia tolerance, as demonstrated by evidence that local injection of CCPA, an A1 receptor agonist, significantly decreased cerebral infarct volume and improved

neurological outcomes in focal cerebral ischemia, similar to the effects of EA. These results indicate that local A1 receptors at the Baihui acupoint might mediate EA-induced tolerance to cerebral ischemia (Liang et al., 2013).

The involvement of A1 receptors in EA-induced analgesia has also been emphasized. Liu and colleagues reported that intraperitoneal administration of the adenosine receptor antagonists theophylline and caffeine blocked EA-induced elevation of nociceptive thresholds in a dose-dependent manner, whereas dipyridamole, an inhibitor of adenosine release, shortened the after-effects of EA, also in a dose-dependent manner (Liu et al., 1994b). Moreover, the authors also found that theophylline and caffeine were capable of blocking EA-induced depression of the nociceptive discharges of wide-dynamic-range neurons in lumbar segments of the spinal cord in rats (Liu et al., 1994a). Furthermore, Goldman and colleagues subsequently confirmed that the acupuncture-induced antinociceptive effects were mediated by peripheral A1 receptors. They demonstrated that acupuncture triggered a sharp increase in local extracellular concentration of purines, including adenosine and ATP metabolites, at the Zusanli acupoint and that injection of the A1 receptor agonist CCPA into this acupoint induced a significant antinociceptive effect against inflammatory pain and neuropathic pain in wild type mice but not in A1 knockout mice. Similarly, EA also failed to produce any antinociceptive effect against inflammatory pain or neuropathic pain in the A1 receptor knockout mice. Moreover, inhibition of the enzymes involved in adenosine degradation

by intraperitoneal injection of deoxycoformycin potentiated the acupuncture-elicited increase in adenosine and prolonged its antinociceptive effects as well. These observations indicate that local activation of adenosine A1 receptors contributes to the antinociceptive effects of acupuncture and that interfering with adenosine metabolism may prolong the clinical benefits of acupuncture (Goldman et al., 2010). Based on these data, Hurt and Zylka introduced a novel therapeutic approach, which they termed “PAPupuncture”. They injected prostatic acid phosphatase (PAP), an ectonucleotidase that dephosphorylates extracellular AMP to adenosine, into acupuncture points and showed that this treatment produced dose- and A1 receptor-dependent antinociceptive effects in mouse models of acute and chronic pain (Hurt and Zylka, 2012). In support of the key role of the A1 receptor, it was later reported that intraperitoneal or oral preadministration of caffeine reversed acupuncture-induced analgesia in a mouse model of postoperative pain (More et al., 2013). These findings were further augmented by a study that used microdialysis to sample interstitial fluids in human subjects who received acupuncture. The results showed that the interstitial adenosine concentration increased significantly during acupuncture and remained elevated for 30 minutes after the acupuncture (Takano et al., 2012).

In addition to the evidence cited above, which focused on the involvement of the A1 receptor in acupuncture-induced neuroprotection and analgesia, a recently published study also suggested that the A2A receptor may be

involved in EA-induced anti-inflammatory effects by showing that in mice with collagen-induced arthritis, the anti-inflammatory and tissue-protective effects of EA treatment were reversed by intraperitoneal coadministration of SCH58261, an A2AR antagonist (Li et al., 2015).

6. Conclusion

In line with its multiple sites of action and regulatory roles under both healthy and pathological conditions, the ECS has been shown to be involved in many of the various therapeutic effects induced by acupuncture. To identify and better understand the role of the ECS in acupuncture, we initially reviewed the biological effects shared by both acupuncture and the ECS, including analgesia, neuroprotection and cardiovascular regulation. We then emphasized the evidence that supports the role of the ECS in the effects of acupuncture and concluded that the ECS is a novel and key participant in many of the beneficial effects induced by acupuncture by serving as a primary mediator and as a regulatory factor of multiple acupuncture-initiated signaling pathways under different pathological conditions. Moreover, to provide a more comprehensive view of the ECS's key role in the multiple effects of acupuncture, we used existing evidence to augment the potential downstream neuronal network of the ECS by listing several common endogenous signaling systems (glutamatergic, GABAergic and adenosinergic) that might also mediate the beneficial effects of acupuncture under regulation of the ECS within the CNS.

ECS-mediated regulation of acupuncture's biological effects occurs at different levels of the CNS and multiple peripheral organs at different time

points and under different conditions. In most cases, increased endocannabinoid mobilization and CB receptor activity were observed, except for one study that observed downregulation of the CB1 receptor (Escosteguy-Neto et al., 2012), which raises the interesting possibility that under different pathological conditions, EA may exert completely opposite effects on the activity of the ECS to maintain homeostasis. Interestingly, although the analgesic properties of cannabinoid receptor ligands have been well documented (Whiting et al., 2015; Woodhams et al., 2015), an unexpected pronociceptive role for endocannabinoids and CB1 receptors in the spinal cord has been reported (Pernia-Andrade et al., 2009; Zhang et al., 2010a), indicating that the endocannabinoid signaling at different synapses may contribute to opposite effects in distinct physiological and pathological contexts. It would be interesting to explore if acupuncture has different regulatory effects upon endocannabinoid signaling in different neuronal circuits under different conditions.

Although recently published studies have presented solid evidence showing that the ECS plays a critical role in the effects of acupuncture, the available evidence is still limited and is mainly focused on analgesia, neuroprotection and cardiovascular regulation. Acupuncture administration and ECS activation produce several of the same biological effects, including the maintenance of energy balance and the regulation of immune, respiratory and gastrointestinal functions. For example, the ECS has been found to regulate the stimulated food intake in a bimodal manner. In particular, the orexigenic effects of THC depend on CB1 receptors on glutamatergic neurons, while CB1 receptors on GABAergic neurons contribute the

hypophagic effects of THC (Bellocchio et al., 2010). Acupuncture-induced reduction of food intake and body weight has been reported (Ji et al., 2013; Kim et al., 2006b), it would be interesting to investigate if this effect is mediated by cell type-specific CB1 receptors. Moreover, acupuncture is known to have specific antiemetic effects, and its clinical effectiveness has been documented (Anders et al., 2012; Liodden et al., 2011; Vickers, 1996). Similarly, cannabinoids, such as THC, are also recommended as efficacious therapeutic agents against nausea and vomiting triggered by many causes, such as chemotherapy or radiotherapy (Abalo et al., 2012; Martin and Wiley, 2004; Rock and Parker, 2016). Furthermore, it has been reported that some of the humoral factors that participated in acupuncture-induced beneficial effects (Long et al., 2015; Zhu, 2014) were also involved in similar cannabinoid-induced effects. For example, vasoactive substances, such as endothelin, were related to both acupuncture (Tian et al., 2013; Yang et al., 2007) and cannabinoid-induced neuroprotective effects (Chen et al., 2000; Li et al., 2017; Mechoulam et al., 2002; Schmidt et al., 2012) to maintain the vascular homeostasis under pathological conditions. All of these interesting correlations are indications of potential links between acupuncture and the ECS, and they also suggest new directions to further explore the role of the ECS in other key acupuncture-related regulatory effects. A better understanding of the intrinsic links between acupuncture and the ECS should enable the development of new techniques that combine acupuncture treatment with therapeutic agents that target endocannabinoid signaling. Such an approach should increase the clinical efficacy of acupuncture, lower the

dosages and side effects of cannabinoid drugs and even produce much stronger therapeutic effects.

Conflict of interest

None of the authors have any actual or potential conflicts of interest, including financial, personal or other relationships with people or organizations within three years of the submitted work.

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Figure;1; Captions

Fig. **1. Schematic diagram illustrating the mechanisms of endocannabinoid system in EA-induced analgesia.** (A) CB1 and CB2 receptors are both centrally activated by EA and contribute to its analgesic and anti-inflammatory effects via unique downstream pathways. (B) CB2 receptor plays a key role in EA-induced analgesia at the peripheral level by activating opioid system and inhibiting pro-inflammatory cytokine release in inflamed skin tissues.

Fig. **2. Schematic diagram summarizing the reported central downstream pathways of endocannabinoid system mediating EA-induced neuroprotection.** Endocannabinoid AEA and 2-AG are mobilized by EA stimulation and activate CB1 receptors on presynaptic neurons and CB2 receptors on astrocytes. Endocannabinoid system activation results in alleviation of excitotoxicity, oxidative damage and apoptosis after cerebral ischemia, via multiple signaling pathways resulting in neuroprotection.

Fig. **3. Possible downstream signaling pathways for endocannabinoid-mediated acupuncture's beneficial effects.** The endocannabinoid system mobilized by acupuncture might subsequently decrease the intensity of central glutamatergic signaling and supraspinal GABAergic signaling, increase the intensity of central and peripheral adenosinergic signaling to produce neuroprotection, analgesia and other beneficial effects.