

Neural mechanism underlying acupuncture analgesia

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ABSTRACT

Acupuncture has been accepted to effectively treat chronic pain by inserting needles into the specific “acupuncture points” (acupoints) on the patient’s body. During the last decades, our understanding of how the brain processes acupuncture analgesia has undergone considerable development. Acupuncture analgesia is manifested only when the intricate feeling (soreness, numbness, heaviness and distension) of acupuncture in patients occurs following acupuncture manipulation. Manual acupuncture (MA) is the insertion of an acupuncture needle into acupoint followed by the twisting of the needle up and down by hand. In MA, all types of afferent fibers (A β , A δ and C) are activated. In electrical acupuncture (EA), a stimulating current via the inserted needle is delivered to acupoints. Electrical current intense enough to excite A β - and part of A δ -fibers can induce an analgesic effect. Acupuncture signals ascend mainly through the spinal ventrolateral funiculus to the brain. Many brain nuclei composing a complicated network are involved in processing acupuncture analgesia, including the nucleus raphe magnus (NRM), periaqueductal grey (PAG), locus coeruleus, arcuate nucleus (Arc), preoptic area, nucleus submedius, habenular nucleus, accumbens nucleus, caudate nucleus, septal area, amygdale, etc. Acupuncture analgesia is essentially a manifestation of integrative processes at different levels in the CNS between afferent impulses from pain regions and impulses from acupoints. In the last decade, profound studies on neural mechanisms underlying acupuncture analgesia predominately focus on cellular and molecular substrate and functional brain imaging and have developed rapidly. Diverse signal molecules contribute to mediating acupuncture analgesia, such as opioid peptides (μ -, δ - and κ -receptors), glutamate (NMDA and AMPA/KA receptors), 5-hydroxytryptamine, and cholecystokinin octapeptide. Among these, the opioid peptides and their receptors in Arc-PAG-NRM-spinal dorsal horn pathway play a pivotal role in mediating acupuncture analgesia. The release of opioid peptides evoked by electroacupuncture is frequency-dependent. EA at 2 and 100 Hz produces release of enkephalin and dynorphin in the spinal cord, respectively. CCK-8 antagonizes acupuncture analgesia. The individual differences of acupuncture analgesia are associated with inherited genetic factors and the density of CCK receptors. The brain regions associated with acupuncture analgesia identified in animal experiments were confirmed and further explored in the human brain by means of functional imaging. EA analgesia is likely associated with its counter-regulation to spinal glial activation. PTX-sensitive Gi/o protein- and MAP kinase-mediated signal pathways as well as the downstream events NF- κ B, *c-fos* and *c-jun* play important roles in EA analgesia.

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Abbreviations: Acupoint, acupuncture point; MA, manual acupuncture; EA, electrical acupuncture; TENS, transcutaneous electrical nerve stimulation; fMRI, functional magnetic resonance imaging; PET, positron emission tomography; RF, receptive field; VLF, ventrolateral funiculus; RVM, rostral ventromedial medulla; NRM, nucleus raphe magnus; PAG, periaqueductal gray; LC, locus coeruleus; Arc, arcuate nucleus; Po, preoptic area; CM, centromedian nucleus; Sm, nucleus submedius; APTn, anterior pretectal nucleus; Hab, habenular nucleus; LHb, lateral habenula; Ac, nucleus accumbens; Cd, caudate nucleus; Sp, septal area; Amyg, amygdale; PVH, hypothalamic paraventricular nucleus; ACC, anterior cingulate cortex; OFQ, orphanin; CCK, cholecystokinin octapeptide; 5-HT, 5-hydroxytryptamine; NA, noradrenalin; GABA, γ -amino-butyric acid; AII, angiotensin II; SOM, somatostatin; NT, neurotensin; DA, dopamine; SP, substance P; PPE, preproenkephalin; PPD, preprodynorphin; POMC, proopiomelanocortin; NF- κ B, nuclear factor-kappa B; ERK, extracellular signal-regulated protein kinase.

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1. Introduction

Acupuncture has been a healing art in traditional Chinese medicine for more than 2000 years. Various disorders can effectively be cured by inserting long, fine needles into specific “acupuncture points” (acupoints) on the skin of the patient’s body. Besides China, acupuncture has spread to over 160 countries and regions. The World Health Organization recommends the use of acupuncture treatment for 43 diseases. Since acupuncture was proposed by NIH consensus as a therapeutic intervention of complementary medicine (NIH, 1997), acupuncture efficacy has become more accepted in the Western world.

Among acupuncture therapies, the acupuncture-induced analgesic effect has been used widely to alleviate diverse pains, particularly chronic pain, and is termed “acupuncture analgesia.” Considering the clinical therapy of acupuncture, it is inevitable that psychological factors are involved in the analgesia. Whether acupuncture analgesia has a physiological basis or is simply attributable to hypnosis or other psychological effects has long been a focus of argument. Consequently, increasing attention has been paid to exploring the physiological and biochemical mechanisms underlying acupuncture analgesia, particularly the brain mechanisms. In the past decades, our understanding of how the brain processes signals induced by acupuncture has developed rapidly (Cao, 2002; Carlsson, 2001; Chang, 1973, 1980; Chung, 1989; Han and Terenius, 1982; Han, 1986, 1989, 2003; Le Bars and Willer, 2002; Mayer, 2000;

Pomeranz, 2001; Sims, 1997; Staud and Price, 2006; Takeshige, 1989; Vincent and Richardson, 1986; Ulett, 1989; Ulett et al., 1998; Wang et al., 2008). This review focuses on the neuronal mechanisms of acupuncture analgesia. On the basis of the data obtained in the last decades and the use of multidisciplinary new techniques, more studies on neural mechanisms underlying acupuncture analgesia are predominately interested in cellular and molecular substrate and functional brain imaging during the last 10 years. The main advancements are: (1) individual differences of acupuncture analgesia are associated with inherited genetic factors and the density of CCK receptors (Chae et al., 2006; Lee et al., 2002; Wan et al., 2001). (2) The brain regions associated with acupuncture analgesia identified in animal experiments were confirmed and further explored in the human brain by means of functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) (see Section 3.5). Some brain regions were activated and de-activated, when acupuncture treatment evoked acupuncture feeling “De-Qi” associated with the efficiency of acupuncture analgesia (see Section 1.1). (3) Frequency-dependent EA analgesia is mediated by the different opioid receptor subtypes (see Section 4.1.2). (4) Both CCK release and the density of CCK receptors are closely associated with individual sensitivity to acupuncture. (5) EA and NMDA or AMPA/KA receptor antagonists have a synergic anti-nociceptive action against inflammatory pain. (6) EA and disrupting glial function synergistically suppress inflammatory pain. EA analgesia is likely associated with its counter-regulation to spinal

glial activation (see Section 5.1). (7) PTX-sensitive Gi/o protein-, opioid receptor- and MAP kinase-mediated signal pathways as well as the downstream events NF- κ B, *c-fos* and *c-jun* play important roles in EA analgesia (see Section 5.2).

1.1. Acupoint: trigger of acupuncture analgesia

The acupoints used by acupuncturists are based on the ancient meridian theory, in which the meridians are referred to as channels “Jing” and their branches “Luo,” where 361 acupoints are located. The meridians are considered as a network system to link acupoints via so-called “Qi” (energy) streaming in the meridians. On the basis of this theory, traditional acupuncturists deem that pain is attributed to a disease-induced blockade of meridians. Therefore, when the blockade is purged by acupuncture, this elicits the smooth streaming of “Qi” in the meridians, and pain is alleviated. However, no convincing evidence shows the existence of novel structures serving as the anatomical foundations of meridians, although related studies have been carried out. Given that the meridian theory has been effectively used for treatment in traditional Chinese medicine, it is conceivable that the meridians might be a functional, but not an anatomical, concept that includes a summation of multiple physiological functions, including the nervous, circulatory, endocrine and immune systems. It is well known that the concept of the constellation has played an important role in astronomy and navigation for a long time. The meridian system might resemble the concept of the constellation in which fictive lines (channels) link various stars (acupoints).

Traditional acupuncturists remarkably emphasize “needling feeling” in clinical practice. It seems that acupuncture analgesia is manifest only when an intricate feeling occurs in patients following manipulation of acupuncture (Hui et al., 2005; Haker and Lundberg, 1990; Pomeranz, 1989). This special feeling is described as soreness, numbness, heaviness and distension in the deep tissue beneath the acupuncture point. In parallel, there is a local feeling in the acupuncturist’s fingers, the so-called “De-Qi.” The acupuncturist feels pulling and increased resistance to further movement of the inserted needle, which is similar to that of a fisherman when a fish takes the bait (Kong et al., 2005; Langevin et al., 2001; MacPherson and Asghar, 2006). A clinical observation showed that acupuncture needles inserted into the lower limbs fail to produce this “De-Qi” feeling or have any analgesic effect on the upper part of the body in paraplegic patients (Cao, 2002). Consequently, great attention was first paid to the involvement of somatic sensory functions of the nervous system in acupuncture analgesia and the innervation of acupoints. There are a total of 361 acupoints on the skin of the human body. In the 1970s, an elegant morphological study showed the topographical relationships between 324 acupoints of 12 meridians as well as the Ren meridian (Zhou et al., 1979, in press). By means of opography, they observed the innervation of the different tissues underneath acupoints, including epidermis, dermis, subcutaneous tissue, muscle and tendon organs in 8 adult cadavers, 49 detached extremities and 24 lower extremities. It was found that out of 324 acupoints located on the meridians, 323 exhibited rich innervation mainly in the deep tissues, clearly indicating that the acupoints on all of the meridians were innervated by peripheral nerves. A recent study indicated a significantly decreased number and density of subcutaneous nerve structures compared with non-acupoints in human (Wick et al., 2007). Unfortunately, their observation was limited only to the acupoints on the skin. It has demonstrated that afferent fibers innervating the skin are not important in mediating acupuncture signals (Chiang et al., 1973; Han et al., 1983; Shen et al., 1973).

To further trace the innervation of acupoints, a recent study explored the distribution of afferent nerve endings with acupoints in the rat hindlimb, in light of the fact that the location of these acupoints are anatomically identical to those of humans (Li et al., 2004). By combining single fiber recordings with Evans blue extravasation, the location of the receptive fields (RFs) for each identified unit recorded was marked on scaled diagrams of the hindlimb. Noxious antidromic stimulation-induced Evans blue extravasation was used to map the RFs of C-fibers in the skin or muscles. The RFs were concentrated either at the sites of acupoints or along the orbit of meridian channels, indicating that the distribution of RFs for both A- and C-fibers is closely associated with acupoints. Similarly, the majority of deep sensory receptors are located at acupoints in muscle. Therefore, authors assumed that acupoints in humans may be excitable muscle/skin-nerve complexes with a high density of nerve endings (Li et al., 2004).

1.2. Characteristics of acupuncture analgesia

- (1) Two acupuncture manipulations are clinically used: manual manipulation (MA) and electrical acupuncture (EA). In MA, the acupuncture needle is inserted into the acupoint and twisted up and down by hand; this is commonly used by traditional acupuncturists. In EA, stimulating current is delivered to acupoints via the needles connected to an electrical stimulator. Instead of insertion of acupuncture needles, a surface electrode on the skin over the acupoint is also described as EA. But it is different from transcutaneous electrical nerve stimulation (TENS). The surface electrodes of TENS are delivered on the skin of pain region rather than acupoints.
- (2) The acupuncture-induced intricate feeling (soreness, numbness, heaviness and distension) in the deep tissue beneath the acupoint is essential to acupuncture analgesia.
- (3) Following the application of acupuncture, the pain threshold gradually increases in both humans and animals, indicating a delayed development of acupuncture analgesia. Moreover, there is a long-lasting analgesic effect after acupuncture stimulation is terminated (Cui et al., 2005; Chiang et al., 1973; Han et al., 1983; Mayer et al., 1977; Pomeranz and Chiu, 1976; Research Group, 1973). The pain threshold to potassium iontophoresis at eight points distributed on the head, thorax, back, abdomen and leg was fairly stable during a period of over 100 min in volunteers. Acupuncture manipulation at the “Hegu” acupoint (LI-4) gradually produced an increase in pain threshold with a peak increase occurring 20–40 min after needle insertion, and persisted over 30 min after withdrawal of the needle. Injection of 2% procaine into LI-4 just prior to acupuncture produced neither local sensation nor an analgesic effect (Fig. 1) (Chiang et al., 1973; Farber et al., 1997; Han et al., 1983; Research Group, 1973). In a recent study on healthy subjects, baseline thermal thresholds (cold and warm sensations and cold and hot pain) were measured at the medial aspects of both lower extremities. Five seconds of hot pain (HP) was delivered to the testing sites and the corresponding pain visual analog scale (VAS) scores were recorded. Thirty seconds of EA (5 Hz, 6 V) was delivered via “Yinbai” (SP1) and “Dadun” (LR1) on the left lower extremities. The warm thresholds of both medial calves significantly increased ($p < 0.01$), whereas the VAS scores of the acute thermal pain threshold were reduced significantly at the ipsilateral calf during electrical acupuncture in comparison to pre-acupuncture and post-acupuncture ($p < 0.01$) measurements, suggesting that EA has an inhibitory effect on C-fiber afferents and the analgesic benefit observed is most likely A δ afferent-mediated (Leung et al., 2005).

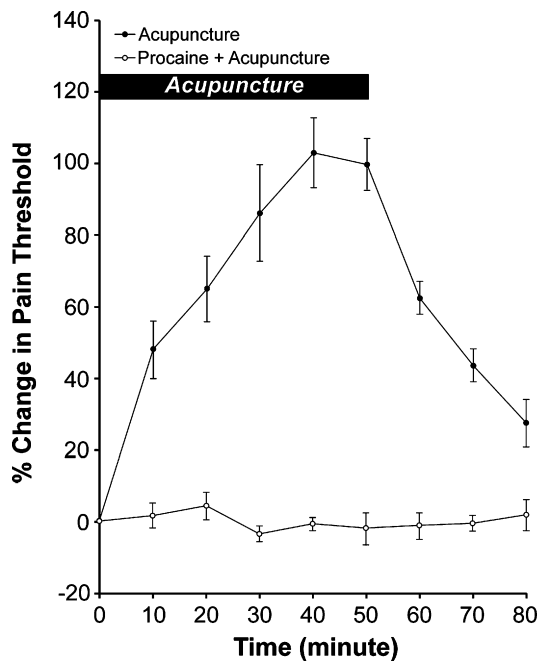


Fig. 1. Elevation of pain threshold by acupuncture at the “Hegu” (LI-4) acupoint in volunteers (solid dot). Blockade of acupuncture analgesia by injection of 2% procaine into LI-4 just prior to acupuncture (open dot) (modified with permission, from Research Group, 1973).

(4) The analgesic effect of acupuncture is characterized by clear individual differences. In a study comparing the analgesic effects of three acupuncture modes (manual, electroacupuncture, and placebo) in healthy subjects, acupuncture treatment, but not placebo, lowered pain ratings in response to calibrated noxious thermal stimuli. Highly significant analgesia was found in 5 of the 11 subjects. Of the five responders, two responded only to electroacupuncture and three only to manual acupuncture, suggesting that acupuncture’s analgesic effects on experimental pain may depend on both subject and mode (Kong et al., 2005). In addition, a role of inherited genetic factors in the individual differences of acupuncture analgesia was reported (Chae et al., 2006; Lee et al., 2002).

1.3. Psychological factors in acupuncture analgesia

The existence of psychological factors in acupuncture analgesia when treating a patient’s chronic pain is not unexpected, as with many medical treatments (Price et al., 1984). But increasing evidence demonstrates that acupuncture analgesia is predominantly attributable to its physiological rather than psychological action despite a high skepticism (Ezzo et al., 2000; Lee and Ernst, 2005; Lundeberg and Stener-Victorin, 2002). Clinical observations have shown that acupuncture analgesia is very effective in treating chronic pain, helping from 50% to 85% of patients (compared to morphine which helps only 30%). Furthermore, acupuncture analgesia is better than placebo in patients (Lewith and Machin, 1983; Richardson and Vincent, 1986) and in normal volunteers (Lee and Ernst, 1989; Ulett, 1989). In support of these early results, recent studies provide new evidence for physiological actions of acupuncture (Bausell et al., 2005; Pariente et al., 2005). In 14 patients suffering from painful osteoarthritis scanned with PET, it was found that the insula ipsilateral to the site of acupuncture needling was activated to a greater extent during acupuncture than during the placebo intervention. Acupuncture and placebo (with the same expectation of effect as acupuncture) caused

greater activation than skin prick (no expectation of a therapeutic effect) in the right dorsolateral prefrontal cortex, anterior cingulate cortex, and midbrain (Pariente et al., 2005). Stimulation of the hypothalamo–pituitary axis (HPA) resulting in adrenocorticotrophic hormone (ACTH) secretion occurs in response to a great variety of psychological or physical stressors. In deeply anaesthetized rats, EA enhanced ATCH plasma release and up-regulated expression of Fos in the hypothalamic–pituitary corticotrope axis without usual autonomic responses to psychological stress, such as tachycardia or blood pressure elevation, which was blocked by deprivation of nociceptive primary afferent input using neonatal capsaicin. (Pan et al., 1996, 1997). In the awaking rat, immobilization stress predominantly is a psychological stressor. Further results showed that immobilization stress-induced ATCH release and Fos expression were not changed by capsaicin treatment. These findings suggest that EA depends on the physiological afferent signal elicited in the somatosensory pathway. Taken together, it is reasonable that acupuncture has specific physiological effects and that patients’ expectation and belief regarding a potentially beneficial treatment modulate activity in the hypothalamic–pituitary corticotrope system.

2. Peripheral mechanisms underlying acupuncture analgesia

2.1. Acupuncture-induced the “De-Qi” feeling

In clinical practice, traditional acupuncturists remarkably emphasize “De-Qi” feeling, including a characteristic needling sensation in the patient and the finger feeling of the acupuncturist, suggesting that efficacy of acupuncture analgesia closely depends upon the “De-Qi” (Hui et al., 2005; Haker and Lundeberg, 1990; Pomeranz, 1989; Wang et al., 1985). To address these reactions to acupuncture needling may be a stepping-stone to understanding the mechanism of acupuncture analgesia.

2.1.1. Origin from muscle contraction

An elegant study was performed on 32 normal volunteers (Shen et al., 1973). An insulated acupuncture needle (except for the tip) was inserted into LI-4 or Zusanli (ST-36) for stimulation and EMG recording. To exclude suggestion, communication between the subject and acupuncturist was forbidden during the period of recording. The results clearly showed that the EMG magnitude recorded from the insulated acupuncture needles in the muscle beneath the acupoint is positively related to the intensity of the subjective sensation derived from acupuncture manipulation and with the local “De-Qi” feeling in the acupuncturist’s fingers. Both sensations are blocked after injection of procaine into the muscle beneath the acupoints. In 11 patients, following lumbar anesthesia, both acupuncture feeling and electromyograph produced by stimulation of ST-36 were completely abolished (Shen et al., 1973). It is suggested that the acupuncture feeling mainly originates from acupuncture-induced impulses from muscle, although other deep tissues are not ruled out (Lin et al., 1979). The activity of polymodal-type receptors in deep tissues may play a key role (Kawakita et al., 2002). This notion is supported by a rare observation. In two patients with congenital insensitivity to pain, one had no skin pain when the needle was inserted into the acupoint, but had some needling feeling (De-Qi) and autonomic reactions during needle twirling, compared with normal subjects. Another patient had neither skin pain sensation nor the needling feeling (Xu et al., 1984). In the former, it was likely that the function of afferent terminals innervating deep tissues might be normal and the deep sensation was partially retained. Therefore, acupuncture-activated polymodal-type receptors in deep tissues (mainly muscle) may be involved in induction of needling feeling (De-Qi).

2.1.2. Origin from connective tissue

Recently, a challenging hypothesis has been raised to explain the peripheral mechanism underlying “De-Qi” (Langevin et al., 2001, 2002). It is reported that the needle grasp is due to mechanical coupling between the needle and connective tissue with winding of tissue around the needle during rotation. Winding may allow needle movements to deliver a mechanical signal into the tissue and may be a key to acupuncture’s therapeutic mechanism. In support of this notion, a recent study found that acupuncture at ST-36 induced significant analgesia and enhanced the degranulation of mast cells. After the mast cells were pharmacologically destroyed by injection of disodium chromoglycate in the acupoint area, the analgesia was weakened, suggesting an important role of mast cells in connective tissue in acupuncture analgesia (Zhang et al., 2007a).

2.2. Afferent nerve fibers activated by acupuncture

Given that the acupuncture needle is a physical sensory stimulus, the intensity, frequency, duration and interval between stimuli directly influence the kind of receptors activated. Increasing evidence has revealed that the types of afferent nerve fibers activated by acupuncture are diverse, depending upon the different manipulation methods of acupuncture and individual differences in acupuncture sensitization.

2.2.1. Electroacupuncture (EA)

The stimulating current at various parameters applied to acupoints through acupuncture needles can produce bilateral analgesic effects in human subjects and experimental animals (Han et al., 1983; Kim et al., 2000; Lao et al., 2004; Takakura et al., 1995). It is commonly accepted that persistent analgesia can be elicited only when relatively high intensities with longer pulse durations are used (Romita et al., 1997). Since the 1970s, it has been controversial which kinds of afferent fibers mediate EA analgesia. The bone of contention is whether C-type afferents are involved (Hu, 1979; Liu et al., 1986, 1990). In electrophysiological, animal behavioral and human experimental studies, lines of evidence demonstrate that electrical current via acupuncture needles at intensities sufficient to excite A β -type afferents (group II) is capable of producing analgesia (Chung et al., 1984; Levine et al., 1976; Lu et al., 1979; Toda and Ichioka, 1978; Toda, 2002; Pomeranz and Paley, 1979; Wu et al., 1974). But excitation of some A δ -type afferents (group III) induces a more potent analgesia (Kawakita and Funakoshi, 1982; Leung et al., 2005; Wu et al., 1974). The early electrophysiological studies showed that EA activating the whole spectrum of A-type afferents induced stronger inhibition of nociceptive responses in cat spinal dorsal horn neurons than exciting only A β fibers (Pomeranz and Paley, 1979; Wu et al., 1974). Similarly, there was a significant correlation between the amplitude of A β fibers in the compound action potentials elicited by EA stimulation and the degree of suppression of the jaw opening reflex induced by noxious stimulation in the rat (Toda, 2002). When the EA current intensity partially excites A δ afferent fibers, the EA-induced feeling in humans is acceptable, even comfortable for some (Shao et al., 1979).

However, using the electrophysiological method of collision, when ST-36 was stimulated by EA, researchers found that the amplitude of the antidromic C wave of the compound action potential in the peroneus nerve (innervating ST-36) was clearly decreased due to collision with the orthodromic EA stimulation, suggesting excitation of some C fibers (Liu et al., 1990). If C afferents play an important role in EA analgesia, degeneration of primary afferent C fibers would prevent or reduce it. Given that about 90% of unmyelinated fibers are destroyed by neonatal

capsaicin treatment (50 mg/kg) in rats (Nagy, 1982), the same treatment was used to test the effect on EA analgesia in the rat (Zhu et al., 1990b). In the capsaicin-treated rats, EA analgesia was significantly reduced compared to the control group. In addition, when conduction in A β - and A δ -type fibers was blocked, EA analgesia still remained in the rat, suggesting the involvement of C-type afferents in EA analgesia. However, conflicting results have been reported (Pan et al., 1997; Uchida et al., 2003). In normal rats, 45–50% of DRG neurons display TRPV1-immunoreactivity. After degeneration of C afferent fibers by neonatal capsaicin treatment, all these neurons disappear from DRGs. Fos expression in the dorsal horn after injection of formalin into the hindpaw was severely attenuated by neonatal capsaicin treatment. However, Fos expression after EA to the pads of the hindpaw was unaffected by the same treatment. These results suggest that EA induces the expression of Fos in dorsal horn neurons via capsaicin-insensitive afferents, presumably A δ rather than C afferents, regardless of blockade of Fos expression in the paraventricular, arcuate and other hypothalamic nuclei by neonatal capsaicin treatment (Pan et al., 1997).

The clinical observations seem to support the notion of C fiber involvement in EA analgesia in animal experiments (Bing et al., 1990; Chung et al., 1984). In patients with syringomyelia, when the anterior commissure of the spinal cord is damaged, the pain and temperature sensation deficits are accompanied by reduced or abolished acupuncture effects and acupuncture feeling (Cao, 2002). But then, it must be pointed out, excitation of C fibers by synchronous strong electrical pulses will inevitably elicit insufferable pain in clinical practice. Strong intensity of EA is, therefore, not suitable for analgesia in patients.

Surface electrodes on the skin over an acupoint instead of insertion of acupuncture needles (EA-like) is similar to transcutaneous electrical nerve stimulation (TENS), but the differences lie in the location of surface electrodes and stimulation intensity and frequency. When EA-like surface stimulation is delivered to the skin over acupoints at low-frequency and high-intensity at five to eight times the sensory threshold, strong muscular contractions are evoked, and experimental tooth pain threshold increases (Andersson and Holmgren, 1975). Furthermore, TENS is delivered at the site of pain with high-frequency and low-intensity (Chan and Tsang, 1987; Lundeberg, 1984). The types of afferent fibers activated by surface electrodes on acupoints correspond to those of EA. Excitation of A β fibers (group II) and some A δ fibers (group III) is involved in analgesia (Han, 2003).

The conflicting results may stem from the variety of stimulation parameters used, such as biphasic square pulses of variable duration, brief monophasic pulses, and different frequencies and intensities.

2.2.2. Manual acupuncture (MA)

An early experimental study revealed that MA at the Hegu (LI-4) acupoint increased the pain threshold in volunteers (Chiang et al., 1973). Blockade of cutaneous branches of the radial nerve innervating the skin at LI-4 by procaine failed to alter the acupuncture-induced increase in pain threshold, whereas blockade of the muscular nerves, deep branches of the ulnar nerve and the median nerve innervating muscles at LI-4 completely abolished acupuncture analgesia, suggesting activation of afferent fibers predominantly from muscle.

As mentioned above, the “De-Qi” feeling is essential to induce analgesia. To obtain the “De-Qi” feeling, acupuncture needles are repetitively penetrated up and down in different directions, so that the muscle fibers beneath acupoints are exposed to strong mechanical stimulation and even injury. Consequently, the types of afferents activated by MA depend upon stimulating intensity (mild or strong) and duration of manipulation. A-type fibers are

mainly activated when gentle stimulation induces the “De-Qi” feeling. However, when the needles are twisted up and down repetitively, the deep tissues, particularly muscle, are locally injured; proinflammatory mediators, such as histamine, bradykinin, PGE₂, 5-HT and ATP, are released and excite nociceptors directly or indirectly (Boucher et al., 2000; Meyer et al., 2005). It is, therefore, conceivable that C-type fibers are involved in MA-induced analgesia. Experimental studies in the cat provide compelling support for this view (Wei et al., 1973, 1976, 1978). By means of single-fiber recording from deep peroneal nerve innervating the anterior tibial muscle, where ST-36 is located in the cat, following needle penetration into the anterior tibial muscle by hand, firing of a C-type afferent persisted for a prolonged period. Using selective blockade of conduction in A δ - and C-type afferents by applying capsaicin to the bilateral sciatic nerves, researchers found that MA-induced analgesia was completely abolished in the rat. The C-type fiber mediation of analgesia in MA seems to be similar to that evoked by so-called diffuse noxious inhibitory control (DNIC), which is mediated by A δ - and C-type afferents (Bing et al., 1990; Okada et al., 1996; Zhu et al., 2004a). Clinically, the acupuncture feeling remains several hours to even a few days after withdrawal of the acupuncture needles, supporting major involvement of C-type afferents in acupuncture analgesia.

Taken together, the peripheral afferent mechanisms underlying acupuncture analgesia produced by EA and MA are homologous, but some differences exist. Most studies indicate that A β / δ -type afferents preferentially mediate EA analgesia, whereas all types, particularly C-type afferents, preferentially mediate MA analgesia. Accordingly, when EA and MA are simultaneously used, there is more potent analgesia than when only one is applied (Kim et al., 2000).

3. Central mechanisms underlying acupuncture analgesia

3.1. Acupuncture analgesia as a result of sensory interaction

One of traits of acupuncture analgesia is that it lasts long after the cessation of the needling stimulation, suggesting the involvement of central summation. In general, pain can be alleviated by various procedures, such as acupuncture, forceful pressure, vibration and heating as well as white noise and flicker. Consequently, it is considered that one kind of sensation may be suppressed by another kind. It is well known that many areas in the CNS, particularly the reticular formation, receive a convergence of heterosensory impulses from various sources. On the basis of these facts, Chang (1973) raised a fascinating postulate that under certain conditions any innocuous sensory input may have some inhibitory effects on pain, but the characteristic sensory impulses produced by acupuncture probably are the most effective. A large body of evidence in the following chapters shows that acupuncture analgesia is essentially a manifestation of integrative processes at different levels of the CNS between the afferent impulses from the pain regions and impulses from acupoints (Chang, 1973, 1980; Du and Zhao, 1976; Kerr et al., 1978; Liu et al., 1986; Liu and Wang, 1988; Pomeranz and Cheng, 1979; Shen et al., 1975; Takeshige et al., 1993; Wu et al., 1974).

3.2. Spinal segmental mechanisms: the functional specificity of acupoints

Clinically, the different acupoints are selected according to the therapy. Traditional acupuncturists emphasize the functional specificity of acupoints according to the meridian theory. A general principle is when the sites of pain are located in the upper body, such as the head, neck and arm, acupoints on the arms are

usually used for treatment, whereas acupoints on the legs are used to relieve pain in the lower body, such as sciatica and abdominal pain. Consequently, it is consistent with the principle of spinal segmental innervation in modern neurophysiology. The segments of the body comprise dermatomes, myotomes, sclerotomes and viscerotomes, in which the same level of innervation and sensory input enter the spinal dorsal horn. The findings of an electrophysiological experiment provide strong support for this view. Inhibition of nociceptive responses, which were evoked by noxious thermal stimulation to the cat hindpaw, in spinal dorsal horn neurons was more powerful when the acupoints were located in the same segmental innervation regions, such as “Zusanli” (ST-36) than that those in remote spinal segments, such as “Hegu” (LI-4). The most efficacious inhibition occurred when acupoints were selected in the same nerve innervating the receptive field of the source of pain (Wu et al., 1974). Similar results were obtained when electroacupuncture at ST-36 decreased the expression of Fos in the superficial dorsal horn of spinal cord by noxious thermal stimulation to the hindpaw in a rat model of neuropathic pain (Dai et al., 2001). As a result, we believe that the spinal segmental relationship between pain site and acupoints partially underlies their functional specificity of acupoints (Bing et al., 1991a). Despite the importance of the spinal segmental principle, many acupoints distant from the pain sites are efficient for relieving pain (Bing et al., 1990; Wu et al., 1974; Zhu et al., 2004a).

The analysis of synaptic mechanism hints at the involvement of both pre- and post-synaptic inhibition in EA-induced inhibition of nociceptive responses in spinal neurons. The size of the antidromic compound potential of the sural nerve evoked by a testing stimulus was measured as an index of afferent terminal excitability mediating presynaptic inhibition (Rudomin, 1999). Using this method (Fung and Chan, 1976; Li et al., 1993), researchers found that EA stimulation at “Huantiao” (GB 30) and “Yanglingquan” (GB 34) or “Zusanli” (ST-36) induced significant enlargement of antidromic C-waves of the sural nerve by strong electrical stimulation, suggesting that EA-induced enhancement of depolarization in presynaptic primary C-afferent terminals resulted in inhibition of release of transmitters, such as substance P and glutamate, from terminals. Moreover, EA at ST-36 was capable of inhibiting noxious stimulation-induced impulses from single fibers of the dorsal root mediated by the sympathetic nerve (Hu et al., 1982). In addition, EA stimulation at ST-36 produced inhibitory post-synaptic potentials (IPSPs) and a long-lasting membrane hyperpolarization in nociceptive neurons of the spinal dorsal horn resulting in inhibition of nociceptive responses by noxious heating to the hindpaw in the cat. This suggests possible involvement of post-synaptic inhibition in EA analgesia (Wu et al., 1978).

3.3. Neural pathways

The main ascending and descending pathways of pain are well-documented (Millan, 1999, 2002). There are two leading ascending pain pathways: the spinoparabrachial tract and the spinothalamic tract. The former originates from the superficial dorsal horn in the spinal cord and projects to the parabrachial nucleus connecting to brain areas mainly involved in processing pain emotion, whereas the latter originates in the superficial and deep dorsal horn and projects to the thalamus connecting to the cortical areas involved in the sensory discrimination and emotion of pain. Clinical observations and experimental studies suggest that the pathways of acupuncture signals are interwoven with pain pathways. As shown in Fig. 2, convergence of impulses originating from pain sites and acupoints occur in the spinal dorsal horn and medial thalamus (such as the nucleus parafascicularis, Pf), where

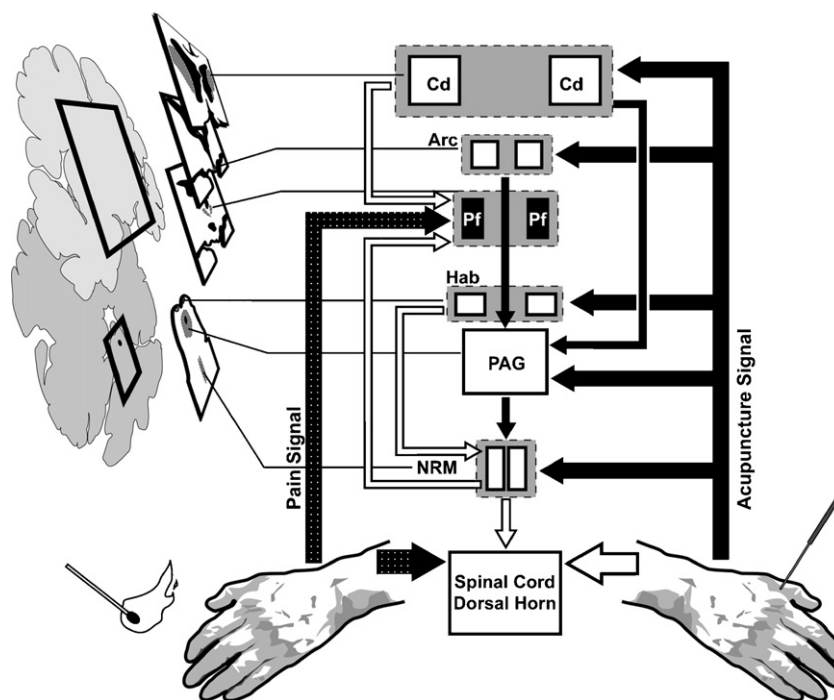


Fig. 2. Putative central circuit underlying acupuncture analgesia. Fundamental nuclei are involved in processing signals from acupoints. Acupuncture signals from acupoint conduct to the brain via the ventrolateral funiculus (VLF) to activate and deactivate the different nuclei and region. *Abbreviations:* CN, Caudate nucleus; Arc, arcuate nucleus; Pf, nucleus parafascicularis; Hab, habenular nucleus; PAG, periaqueductal grey; NRM, nucleus raphe magnus. Solid arrow: Excitatory input; open arrow: inhibitory input.

integration of two kinds of impulses takes place. For example, axons from Cd and NRM neurons project to the pain-sensitive neurons in the Pf. Activity of Cd and NRM neurons by EA significantly inhibits nociceptive responses in the Pf resulting in the analgesic effect.

Lines of clinical observation provide crucial evidence for understanding the pathways of acupuncture analgesia (Cao, 2002). Some patients suffering from tabes dorsalis involving the posterior column, infantile paralysis and amyotrophic lateral sclerosis, who represent a deficit of deep sensation or degeneration of spinal motor neurons, still retained the “De-Qi” feeling at all affected acupoints. Compared with the long-lasting sensation in normal subjects, their “De-Qi” feeling quickly disappeared after ceasing acupuncture stimulation. However, patients with syringomyelia involving the anterior commissure and posterior horn in the spinal cord not only presented pain and temperature deficits, but their acupuncture feeling was markedly weakened or completely abolished in the related acupoints. To test these clinical observations, experimental studies were done. Sections of the spinal dorsal columns failed to affect acupuncture stimulation-induced inhibition of nociceptive responses in thalamic neurons. The acupuncture-induced increase in pain threshold to noxious heating was similar in chronically dorsal chordotomized and intact animals. However, with unilateral section of the ventral of two-thirds of the spinal lateral funiculus at segments T₁₂-L₁, the analgesia induced by stimulation of acupoints located in the contralateral, but not the ipsilateral, leg was almost abolished (Chiang et al., 1975). Collectively, these data suggest that impulses from acupoints ascend mainly through the ventrolateral funiculus, which is the spinal pathway of pain and temperature sensation.

3.4. Relevant brain areas

In the 20th century, the endogenous descending inhibitory system in the CNS was one of the most valuable findings in

understanding pain (Fields et al., 2005). The descending inhibitory system consists of many brain regions, such as the rostral ventromedial medulla (RVM) (mainly nucleus raphe magnus, NRM), periaqueductal gray (PAG), locus coeruleus (LC) and arcuate nucleus (Arc). Intriguingly, in accordance with opiate analgesia and brain stimulation-induced analgesia, this system plays a vital role in processing acupuncture analgesia. An early study showed that when the dorsal lateral funiculus of the spinal cord was sectioned at T₂₋₃, analgesia by EA at “Zusanli” (ST-36) in the hindleg was lessened or abolished in the cat, indicating participation of the descending inhibitory system in acupuncture analgesia (Hu et al., 1980; Shen et al., 1974, 1975, 1978). Subsequently, studies of brain lesions further revealed that the source of descending inhibition is predominately attributed to the NRM and its adjacent structures (Du and Zhao, 1975, 1976). A recent report showed that EA at Huantiao (GB30) significantly inhibited Fos expression in laminae I-II of the spinal cord in the sham-operated rats, but not in those with dorsolateral funiculus lesions (Li et al., 2007).

In the last decades, by means of various techniques, such as classical physiological approaches of stimulation and lesion of brain nuclei, acupuncture-induced Fos expression and functional imaging, many studies have shown involvement of many brain structures in the modulation of acupuncture analgesia, including the RVM (mainly NRM), periaqueductal gray (PAG), locus coeruleus (LC), arcuate nucleus (Arc), preoptic area (Po), centromedian nucleus (CM), nucleus submedius (Sm), anterior pretectal nucleus (APTn), habenular nucleus (Hab), nucleus accumbens (Ac), caudate nucleus (Cd), septal area (Sp), amygdale, anterior cingulate cortex (ACC), and hypothalamic paraventricular nucleus (PVH). Except for the Hab, stimulation of these nuclei potentiates EA analgesia, whereas lesions attenuate it (Bing et al., 1991b; Guo et al., 1996; Hui et al., 2005; Lee and Beitz, 1993; Takeshige et al., 1991, 1993; Wu et al., 1995; Yan et al., 2005; Yang et al., 1992). Based on a large amount of relevant data, several complicated schematics of the possible physiological mechanism of acupuncture analgesia have been

drawn (Han, 1989; He, 1987; Takeshige, 1989; Stener-Victorin, 2002). Their elegant schematics provide a comprehensive understanding for central pathways of acupuncture analgesia from the different angles. But, given that reciprocal connections exist between these nuclei making up intricate neural circuits, the accumulative data of acupuncture analgesia are much insufficient to make a clear schematic of interaction of these nuclei in acupuncture analgesia. Focusing on several important nuclei modulated pain, Fig. 2 is a simplified schematic of central mechanisms underlying acupuncture analgesia.

It is well-documented that off- and on-neurons in the RVM are closely related to the noxious heat-induced tail-flick reflex in the rat, in which just prior to the occurrence of tail-flick elicited by noxious heat, the on-neurons and off-neurons exhibit a burst of discharges and a cessation of discharges, respectively (Fields et al., 2005). Synchronous recording of activity in the RVM neurons and tail-flick reflexes by noxious heat was performed in the rat. EA at bilateral “Ciliao” (BL 32) acupoints inhibited the tail-flick reflex by noxious heat with a significant increase in spontaneous discharges of most off-neurons, but not on-neurons. These results provide evidence that the off-neurons may be the main efferent neurons in the RVM involved in EA analgesia (Wang and Chen, 1993).

Stimulation of the NRM potentiated the acupuncture-induced inhibitory effect, whereas lesion of this nucleus, or the dorsolateral funiculi (DLF) containing descending fibers from the NRM, significantly reduced the acupuncture effect (Du and Zhao, 1975). However, when EA at high intensity exciting C afferent fibers was delivered, EA facilitated spontaneous firing in some NRM neurons responsive to noxious stimuli, but inhibited their nociceptive responses. After transection of the DLF, the NRM neurons were still activated by EA, but the post-inhibitory effects of EA on the nociceptive responses were clearly reduced. It is suggested that the EA can activate the NRM, a supraspinal area mediating a negative feedback circuit modulating pain, thus inducing analgesia via descending inhibition (Liu et al., 1986, 1990).

EA at “Zusanli” (ST-36), “Neiguan” (PC 6) or “Neiting” (ST-44) induced clear analgesic effects in behavioral tests and Fos expression, specifically in the ventrolateral PAG (vIPAG) (de Medeiros et al., 2003; Guo et al., 2004; Sheng et al., 2000). Also, similar results were found in the Arc, CM, Cd, Ac and amygdala.

The Cd is well-known as a constitutive part of the extrapyramidal system in modulation of motor function. Compelling evidence has shown that the head of the Cd has a role in the relief of pain in animals (Acupuncture Anesthesia Group, 1976; Department of Physiology, 1979; Schmidek et al., 1971; Zhang et al., 1980) and patients (Acupuncture Anesthesia Coordinating Group, 1977). Also, further studies found that lesions of the head of the Cd attenuated EA analgesia (Zhang et al., 1978), indicating that it plays crucial role in this process (He and Xu, 1981; He et al., 1985).

The Hab, an important relay station between the limbic forebrain and brainstem, receives afferent fibers originating from various areas of the limbic forebrain and sends out efferent fibers to extensive regions in the brainstem (Herkenham and Nauta, 1977, 1979; Wang and Aghajanian, 1977). The lateral habenula (LHab) is implicated in processing pain (Department of Physiology, 1977). Nociceptive responses in LHab neurons were evoked by noxious stimulation, and the excitation of the LHab by microinjection of L-glutamate decreased the pain threshold (Wang et al., 1987). The electrophysiological results showed that electrical stimulation and lesion of the LHab produced a decrease and increase of spontaneous discharges of neurons in the NRM, respectively, suggesting that excitation of the LHab has an inhibitory action on the NRM (Wang et al., 1980). Consequently, the LHab may play a negative regulatory role in acupuncture

analgesia. Activity of LHab neurons induced an antagonistic effect on acupuncture analgesia. Given that the Hab projects to the NRM contributing to descending control, the effects of interrupting the NRM on LHab-inhibited acupuncture analgesia were found. The LHab excitation-induced antagonistic effect on acupuncture analgesia was abolished by microinjection of lidocaine into the NRM (Liu and Wang, 1988), suggesting that acupuncture might promote the descending inhibitory action of the NRM by inhibiting LHab, thus strengthening the analgesic effect.

3.5. Functional imaging studies in acupuncture analgesia

The above brain regions associated with acupuncture analgesia were predominantly identified in animal experiments. Recently, the studies on correlations between neuroimages and acupuncture in the human brain have increased by means of functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) (Fang et al., 2004; Hsieh et al., 2001; Hui et al., 2000; Kong et al., 2002; Wu et al., 1999, 2002; Yan et al., 2005; Zhang et al., 2003a,b, 2004), which greatly deepen our insight into the central mechanisms underlying acupuncture analgesia and drive further exploration of acupuncture.

Several fMRI studies have revealed that needling stimulation of Hegu (LI-4), Zusanli (ST-36), or Yanlingquan (GB 34) is capable of modulating activity in the human central nervous system including cerebral limbic/paralimbic and subcortical structures (Li et al., 2000; Wu et al., 1999; Yan et al., 2005). When the “De-Qi” feeling was evoked by acupuncture, many brain regions including the PAG and NRM in the midbrain, insula, dorsomedial nucleus of the thalamus, hypothalamus, nucleus accumbens, and primary somatosensory-motor cortex were activated, while some regions exhibited deactivation, such as the rostral part of the anterior cingulate cortex, the amygdala and the hippocampal complex (Wu et al., 1999). As mentioned above, De-Qi sensation is closely associated with the efficiency of acupuncture analgesia in patients; therefore, De-Qi-induced changes in brain images will contribute to understanding the central mechanisms of acupuncture. A recent study further showed that the acupuncture-induced De-Qi feeling without sharp pain elicited widespread signal decreases in several brain areas, including the frontal pole, VMPF cortex, cingulate cortex, hypothalamus, reticular formation and the cerebellar vermis, whereas the acupuncture-induced De-Qi feeling with sharp pain elicited signal increases in several areas, including the frontal pole and the anterior, middle and posterior cingulate (Hui et al., 2005). A similar finding was acquired by scanning in the 3-D mode of PET, in which acupuncture activated many brain regions, including the ipsilateral anterior cingulate, the insulae bilaterally, the cerebellum bilaterally, the ipsilateral superior frontal gyrus, and the contralateral medial and inferior frontal gyri (Biella et al., 2001).

To address whether the stimulation of acupoints in the same spinal segments could induce different central responses, brain imaging with fMRI was used in 12 healthy, right-handed subjects. Stimulation of the Zusanli/Sanyinjiao (ST-36/SP6) acupoints specifically activated orbital frontal cortex and de-activated hippocampus, whereas stimulation of the Yanglingquan/Chengshan (GB34/BL57) acupoints activated dorsal thalamus and inhibited the primary motor area and premotor cortex. Thus, stimulation of acupoints at the same spinal segments induced distinct though overlapping cerebral response patterns. This supports the notion that acupoints are relatively specific (Zhang et al., 2004). Intriguingly, a further study showed that stimulation of classical acupoints elicited significantly higher activation over the hypothalamus and primary somatosensory-motor cortex and deactivation over the rostral segment of anterior cingulate cortex

than stimulation of non-acupoints. When vision-related acupoints located in the lateral aspect of the foot, which are used to treat eye diseases in traditional Chinese medicine, were stimulated bilaterally, activation of the bilateral visual cortex (occipital lobes) was found by fMRI (Siedentopf et al., 2002). Stimulation of the eye by direct light results in similar activation in the occipital lobes by fMRI. But there was no activation in the occipital lobes following stimulation of non-acupoints (Wu et al., 2002).

Taken together, acupuncture activates some structures, such as PAG and NRM contributing to descending inhibitory modulation (Liu et al., 2000) and deactivates multiple limbic areas contributing to modulating pain emotion, such as the insula, anterior cingulate cortex (ACC), etc. (Lei et al., 2004; Gao et al., 2004; Price, 2000). Increasing evidence demonstrates that the ACC plays an important role in the descending facilitatory modulation (Zhang et al., 2005b). It is, therefore, suggested that acupuncture is effectively capable of modulating central homeostasis to produce analgesia in the treatment of patients, supporting the notion that acupuncture regulates the balance of “Yin” and “Yang” in the ancient meridian theory.

4. Roles of transmitters and modulators in acupuncture analgesia

In the early '70s, an elegant study from Han's group revealed that when the cerebrospinal fluid of donor rabbits given acupuncture was infused into the cerebral ventricles of recipient rabbits, the pain threshold of recipients was increased, strongly suggesting the involvement of central chemical mediators in acupuncture analgesia (Research Group, 1974). From then on, many findings in human and animal studies have demonstrated that acupuncture analgesia is a complex physiological process mediated by various transmitters and modulators. Han and his colleagues have made important contributions to this field (Han and Terenius, 1982; Han, 2003).

4.1. Opioid peptides

It is well known that the three main groups of opioid peptides, β -endorphin, enkephalins and dynorphins, and their μ -, δ - and κ -receptors are widely distributed in peripheral primary afferent terminals and areas of the CNS related to nociception and pain, and play a pivotal role in peripheral and central antinociception (Basbaum and Jessell, 2001; Fields et al., 2005). The discovery of endogenous opioid peptides intensified exploration of the role of opioid peptides in acupuncture analgesia. In 1977, the first stimulating finding showed that naloxone, a specific opioid receptor antagonist, partially reversed the analgesic effect of acupuncture on electrical stimulation-induced tooth pulp pain in human subjects (Mayer et al., 1977). This result was swiftly evaluated by sensory detection theory in healthy subjects and confirmed in patients with chronic pain (Jiang et al., 1978). Further observations showed that EA increased the content of β -endorphin-like immunoreactive substances in the ventricular CSF of patients with brain tumours (Chen and Pan, 1984) and in the lumbar CSF of patients with chronic pain (Sjolund et al., 1977) as well as in the rabbit PAG (Zhang et al., 1981). Also, naloxone blocked EA-induced inhibition of nociceptive responses in dorsal horn neurons in the cat (Pomeranz and Cheng, 1979) and reversed EA analgesia in the monkey (Ha et al., 1991). But, conflicting results were also reported in human subjects (Chapman et al., 1983).

In addition, CXBK mice deficient in opiate receptors showed poor EA-induced analgesia (Peets and Pomeranz, 1978). Further, acupuncture analgesia was potentiated by protection of endogenous opioid peptides using peptidase inhibitors, such as D-amino

acids, D-phenylalanine and bacitracin (Ehernpreis et al., 1978; Han et al., 1981; Zhou et al., 1984). Subsequently, on the basis of these findings, the effects of opioid peptides on acupuncture analgesia have been studied widely.

4.1.1. Peripheral opioid peptides

The involvement of the peripheral opioid system in modulating inflammatory pain has been well-documented (Stein, 1991; Stein et al., 2003). In rats with CFA-induced inflammation, intraplantar, but not intraperitoneal, injection of naloxone methiodide, a peripherally acting opioid receptor antagonist, eliminated the analgesic effect at 30 min after EA treatment (Sekido et al., 2003). Intraplantar injection of an antibody against β -endorphin and a corticotropin-releasing factor antagonist also reduced EA analgesia, strongly suggesting that peripheral opioids released by EA at the inflammatory site are involved in modulating inflammatory pain (Zhang et al., 2005a).

4.1.2. Central opioid peptides

Compelling evidence demonstrated that frequency-dependent EA analgesia is mediated by the different opioid receptor subtypes (Han, 2003; Kim et al., 2004; Wang et al., 2005; Zhang et al., 2004a). Direct evidence comes from a study using radioimmunoassay of spinal perfusates from the rat. EA at low-frequency (2 Hz) facilitates the release of enkephalin, but not dynorphin, while EA at high frequency (100 Hz) stimulates the release of dynorphin, but not enkephalin in the rat (Fei et al., 1987; Han, 2003). These findings were fully confirmed in humans (Han et al., 1991). By intrathecal administration of various specific antagonists or antisera of opioid receptor subtypes, further studies showed that 2 and 100 Hz EA-induced analgesic effects were differentially reduced by blockade of μ -/ δ -, and κ -receptors, strongly suggesting that low- and high-frequency EA are mediated by μ -/ δ - receptors and κ -receptors in the rat spinal cord, respectively, under the physiological pain conditions (Fig. 3) (Han, 2003; Wang et al., 2005). It has shown that endomorphin is a newly characterized opioid peptide with high selectivity for μ -receptors (Zadina et al., 1997). Since 2 Hz EA is highly effective in accelerating the release of β -endorphin and enkephalins with high selectivity for μ - and δ -receptors in the CNS, 2 Hz EA should be effective in accelerating the release of brain endomorphin exerting an antinociceptive effect. Intracerebroventricular injection of endomorphin antiserum or μ -receptor antagonist CTOP dose-dependently antagonized 2 Hz, but not 100 Hz EA analgesia in mice (Huang et al., 2000).

However, in pathological conditions, κ -receptors seem not to be involved in EA analgesia. In rats with inflammatory pain, both 2 and 100 Hz EA-induced analgesia are mediated by μ -/ δ -receptors, but not κ -receptors (Zhang et al., 2004b). In rats with neuropathic pain, 2 Hz EA induced a robust and longer lasting effect than 100 Hz. Also, μ -/ δ -receptors, but not κ -receptors, mediate the relieving effects on pain behaviors induced by 2 Hz EA (Kim et al., 2004; Sun et al., 2004). Further studies showed that lesions of the arcuate nuclei abolished low-frequency EA-induced analgesia but not high-frequency EA, whereas selective lesions of the parabrachial nuclei attenuated high-frequency EA-induced analgesia but not low-frequency EA (Wang et al., 1990, 1991). It appears that low- and high-frequency EA-induced analgesia may be mediated by different brain nuclei expressing opioid receptors.

Apart from the relationship between EA frequency and brain nuclei, different EA intensities might be associated with brain nuclei expressing opioid receptors. Microinjection of naloxone into the thalamic nucleus submedius (Sm) blocked high-intensity EA analgesia, but not low-intensity, whereas the opposite result occurred when naloxone was injected into the anterior pretectal

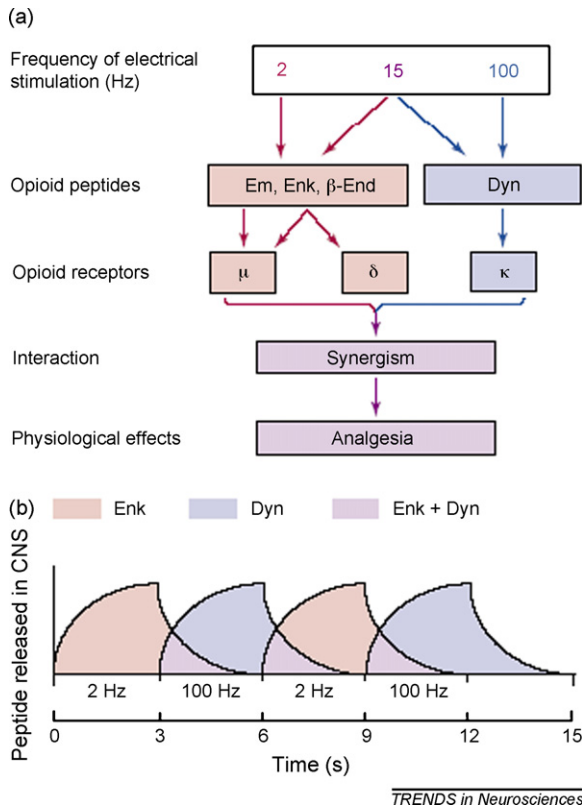


Fig. 3. Opioid peptides and opioid receptors involved in analgesia elicited by electroacupuncture of different frequencies: (a) 2 Hz (red), 100 Hz (blue), 15 Hz (purple). Dyn (dynorphin A); β-End (β-endorphin); Em (enkephalins). Synergism: simultaneous activation of all three types of opioid receptor elicits a synergistic analgesic effect. (b) Model for the synergistic analgesic effect produced by alternating low- and high-frequency stimulation. Stimulation at 2 Hz facilitates the release of enkephalin (red); that at 100 Hz stimulates the release of dynorphin (blue). The overlapping areas (purple) indicate the synergistic interaction between the two peptides. (with permission from Han, 2003).

nucleus (APtN) in the rat (Zhu et al., 2004b). These results suggest that opioid receptors in the Sm and APtN may be involved in Aδ/C and Aβ afferent fiber-mediated EA analgesia, respectively.

As mentioned above, many brain nuclei and regions are involved in processing acupuncture signals, and most of them, such as the Sm, Cd, Sp, Ac, Arcu, PAG and NRM, contain opioid peptides and μ-, δ- and κ-receptors. At the supraspinal level, decrease in acupuncture analgesia was demonstrated when activity of opioid receptors of given areas were blocked by opioid receptor antagonists (He, 1987).

A pioneering work revealed that the PAG and periventricular grey matter of the rabbit are targets for morphine analgesia (Tsou and Jang, 1964). Subsequently, Reynolds (1969) reported that electrical stimulation of the PAG produced analgesia potent enough to allow exploration of internal organs in rats. A number of studies have well-documented that the PAG is a critical region in the descending pain inhibitory system (Millan, 2002). Given that the PAG contains a high density of opioid receptors, blockade of opioid receptors in the PAG by naloxone or antibody against μ- or δ-receptors significantly attenuated EA analgesia (Han et al., 1984; Xie et al., 1983). Further, acupuncture analgesia was potentiated by preventing hydrolyzing enzyme-induced degradation of endogenous opioid peptides by microinjection of a mixture of three peptidase inhibitors (amastatin, captopril and phosphoramidon) into the PAG (Kishioka et al., 1994). It is documented that the axons of PAG neurons project to the NRM. When the PAG was stimulated, the firing rate of NRM neurons was enhanced following analgesia

in rats. Further, EA at “Zusanli” (ST-36) activated NRM neurons and produced analgesia, which was weakened by microinjection of naloxone into the PAG in rats (Liu, 1996).

Similarly, blockade of EA analgesia was found with microinjection of naloxone into the preoptic area, habenula, septal area, nucleus accumbens, amygdala and caudate nucleus (Cd) in rabbits. EA elicited an increase in pain threshold and a rise in opioid peptide levels in the perfusate of the anterodorsal part of the head of caudate nucleus (Cd) in rabbits. The EA analgesia was readily reversed by microinjection of a μ-receptor antagonist, but not δ- and κ-receptor antagonists, into the anterodorsal part of the head of Cd (He et al., 1985). It is most likely that the Cd is involved in EA analgesia through μ-receptors, but not δ- and κ-receptors.

The arcuate nucleus (Arc) is an important structure in the endogenous opioid peptide system, in which β-endorphin-containing neurons are densely located. Their axons project to the lateral septal area, nucleus accumbens, PAG and LC (Akil et al., 1984; Bloom et al., 1978), suggesting that the Arc is implicated in mediating acupuncture analgesia. In a behavioral and electrophysiological study, EA analgesia and EA-induced responses of neurons in the dorsal raphe nucleus were significantly increased by Arc stimulation, whereas EA-induced responses of neurons in the locus coeruleus were decreased by Arc stimulation. These effects were reversed by i.p. injection of naloxone (Yin et al., 1988). Arc stimulation-induced excitation of NRM neurons was blocked by a section of the β-endorphinergic tract or microinjection of naloxone into the PAG. In support of results of Arc stimulation, further evidence revealed that lesions of the Arc almost completely blocked EA analgesia (Wang et al., 1990). These results indicate the important role of the Arc-PAG-NRM-dorsal horn pathway mediated by the opioid system in acupuncture analgesia (Take-shige et al., 1992).

The thalamic anterior nuclei (AD), which densely express opioid receptors, are regarded as a part of the limbic system, which may be involved in pain emotion (Mark et al., 1963; Pert et al., 1976). An electrophysiological study found that iontophoresis of opiate strikingly inhibited nociceptive responses of AD neurons, which were blocked by naloxone. Similar to the action of opiates, EA also produced naloxone-reversible inhibition of nociceptive responses in AD neurons (Dong et al., 1987), suggesting that EA may modulate pain emotion via opioid receptors in AD neurons.

Despite some contention, orphanin FQ (OFQ) is considered to be an endogenous opioid peptide. It is the ligand for the orphan opioid receptor-like-1 (ORL1) receptor. Studies indicate that OFQ is involved in the modulation of not only nociception (Grisel et al., 1996; Tian et al., 1997a; Xu et al., 1996; Shu and Zhao, 1998), but also EA analgesia (Zhu et al., 1996; Tian et al., 1997b; Huang et al., 2003). Administration of OFQ (i.c.v.) induced a dose-dependent antagonism of the analgesia induced by EA (100 Hz) in the rat, whereas antisense oligonucleotides (i.c.v.) to OFQ mRNA potentiated EA-induced analgesia. Comparable to this result, microinjection of OFQ into the PAG remarkably antagonized EA analgesia in a dose-related manner (Wang et al., 1998). Taken together, it is conceivable that endogenous OFQ in the brain exerts a tonic antagonistic effect on EA-induced analgesia. However, intrathecal administration of OFQ enhanced rather than antagonized EA-induced analgesia in the spinal cord. These findings are consistent with the experimental results obtained in rats where morphine-induced analgesia is antagonized by i.c.v. OFQ and potentiated by i.t. OFQ (Tian et al., 1997a). Apart from mediating acute pain, it has been shown that OFQ is involved in neuropathic pain. In the sciatic nerve chronic constriction injury model, EA decreased expression of preproorphanin FQ mRNA and increased OFQ immunoreactivity in the NRM of rats, suggesting that EA modulated OFQ synthesis

and OFQ peptide levels in the NRM in neuropathic pain (Ma et al., 2004).

4.2. Cholecystokinin octapeptide (CCK-8)

CCK-8 is widely distributed in various brain areas and the spinal cord and exerts many physiological functions. It is the most potent neuropeptide involved in processing anti-opioid activity via the CCK₈ receptor (Ito et al., 1982; Faris et al., 1983; Han, 1995, 2003; Watkins et al., 1985). Compelling evidence has clearly shown the involvement of CCK-8 in processing EA analgesia (Chen et al., 1998; Huang et al., 2007; Ko et al., 2006; Lee et al., 2003). In a behavioral test, intrathecal administration of CCK-8 and CCK₈ receptor antagonists significantly depressed and potentiated electroacupuncture-induced antinociception, respectively (Huang et al., 2007). Further study showed that rats with weak EA-induced analgesia, so-called non-responders, had a remarkable increase in CCK release, whereas rats with strong EA-induced analgesia, so-called responders, had little increase in CCK release in the spinal cord (Zhou et al., 1993). A recent study showed that CCK receptor mRNA in the rat hypothalamus was increased by high-frequency EA in non-responders (Ko et al., 2006).

The responders and non-responders to acupuncture represent individual variations in acupuncture analgesia. It is very interesting that following i.c.v. microinjection of antisense oligonucleotides to CCK mRNA, the CCK-mRNA as well as the CCK-8 content in rat brain was decreased, and particularly non-responders were converted into responders for EA analgesia and morphine analgesia (Tang et al., 1997). Further work indicated when the tail-flick response by radiant heat was performed to quantify analgesic effects after MA at the Zusanli (ST-36) in rats, the expression of the mRNA level of the CCK-A receptor was significantly higher in non-responders than responders, but the mRNA level of CCK-B receptor expression was not significantly different (Lee et al., 2002). Collectively, this suggested that both CCK release and the density of CCK receptors are closely associated with individual sensitivity to acupuncture.

As mentioned in Section 1.3, placebo is a potentially beneficial treatment for EA. Compelling evidence has shown that placebo analgesia is mediated by endogenous opioids (Benedetti and Amanzio, 1997). Consistent with the effect on EA and opiate analgesia, the blockade of CCK receptors potentiates the placebo analgesic response (Benedetti, 1996). Given the anti-opioid action of CCK, it is suggested that EA analgesia may, at least partially, share a common mechanism with placebo analgesia. A potentiation of the endogenous opioid systems is implicated in EA and placebo analgesia.

4.3. 5-Hydroxytryptamine (5-HT)

5-HT and its receptor are densely expressed in the CNS and are implicated in modulating nociception (Kayser et al., 2007; Liu et al., 2007; Millan, 2002). The role of 5-HT in mediating acupuncture analgesia has been well-summarized (Han and Terenius, 1982). It is acknowledged that the nuclei raphe magnus (NRM) contains an abundance of 5-HT and is a crucial site in the descending pain modulation system (Millan, 2002). EA increased the central content of 5-HT and its metabolic products, particularly in the NRM and the spinal cord (Han et al., 1979a,b; Ye et al., 1979; Zhu et al., 1997a,b). 5-HT immunoreactivity was potentiated in the NRM with EA analgesia (Dong and Jiang, 1981). The electrolytic lesion of the NRM or selective depletion of brain 5-HT by 5,6-dihydroxytryptamine (5,6-DHT) remarkably attenuated EA analgesia in the different species (Baumgarten et al., 1972; Dong et al., 1984; Du and Zhao, 1976; Du et al., 1978; Kaada et al., 1979;

McLennan et al., 1977; Han et al., 1979a; Shen et al., 1978; Yih et al., 1977). The blockade of 5-HT biosynthesis by *p*-chlorophenylalanine, a 5-HT synthesis inhibitor, and degradation by pargyline, a monoamine oxidase inhibitor, produced inhibition and potentiation of acupuncture analgesia, respectively (see references [35–38,45] in Han and Terenius, 1982). Further, blockade of 5-HT receptors by cinaserine, cyproheptadine or methysergide, 5-HT receptor antagonists, almost abolished acupuncture analgesia (Han et al., 1979a,b; Chang et al., 2004). These compelling data clearly verify that both serotonergic descending and ascending pathways originating from the NRM are implicated in mediating acupuncture analgesia.

Recent studies have well-documented that there are multiple 5-HT receptor subtypes in the nervous system (Millan, 2002). 5-HT_{1A} and 5-HT_{1B} receptors are densely present in neurons of the dorsal horn in the spinal superficial laminae where primary nociceptive afferent fibers terminate (Hamon and Bourgoin, 1999). 5-HT_{2A} and 5-HT₃ receptors are expressed by primary nociceptive afferent fibers (Carlton and Coggeshall, 1997; Hamon et al., 1989). An electrophysiological study using 5-HT receptor subtype antagonists (i.v.) showed that 5-HT₁, 5-HT₂ and 5-HT₃ are likely involved in EA-induced inhibition of acute nociceptive responses evoked by tooth pulp stimulation in the trigeminal nucleus caudalis of rabbits (Takagi and Yonehara, 1998). In a study on 5-HT release, tooth pulp stimulation slightly increased 5-HT release and significantly increased SP release in the trigeminal nucleus caudalis. EA remarkably enhanced 5-HT release and suppressed SP release evoked by tooth pulp stimulation. NAN-190 (i.v.), a 5-HT_{1A} receptor antagonist, further enhanced the increase in tooth pulp stimulation-induced 5-HT release in the presence of EA and could significantly reduce the amount of SP release. The incremental effect of NAN-190 on 5-HT release may depend on presynaptic 5-HT_{1A} receptors (autoreceptors) that exert an inhibitory control on nerve activity to increase 5-HT release (Hjorth, 1993). These results suggest that EA-induced activation of 5-HT_{1A} receptors regulates tooth pulp stimulation-induced SP release in the trigeminal nucleus caudalis, which is likely involved in mediation of EA-induced inhibition of acute nociceptive responses (Yonehara, 2001). However, intrathecal injection of antagonists of 5-HT_{1A} and 5-HT₃ receptors, but not 5-HT_{2A} antagonists, significantly blocked EA-induced depression of cold allodynia in the neuropathic rat and reduced spontaneous pain behaviors (Kim et al., 2005). Similarly, intracerebroventricular administration of antagonists of 5-HT_{1A} and 5-HT₃ receptors reduced EA analgesia in the rat formalin-induced pain test (Chang et al., 2004). These data suggest that 5-HT_{1A} and 5-HT₃ receptor subtypes play important roles in mediating EA analgesia through modulation of SP release.

4.4. Noradrenalin (NA)

It is well-documented that the NA-containing neurons reside in A₁, A₂, A₄₋₇ nuclei of the brain stem, which axons bilaterally project to the forebrain via the dorsal or ventral bundle and to the spinal cord via the dorsolateral funiculus contributing to modulation of pain (Ungerstedt, 1971; Millan, 2002). A line of studies revealed that EA-induced analgesia and decreased NA content in the rat brain (Dong et al., 1978; Han et al., 1979a; Zhu et al., 1997b). Further studies suggested that NA seems to exert different actions at the spinal and supraspinal levels. When DOPS, a precursor of NA, was intracerebroventricularly administered, EA analgesia was inhibited, whereas following intrathecal administration, EA analgesia was potentiated (Xie et al., 1981). Given that the NA-ergic terminals in the locus coeruleus originate from the dorsal raphe nucleus (Rd), microinjection of 6-OHDA into the Rd produced an increase in

efficacy of acupuncture analgesia resulting from the destruction of NA-ergic terminals, suggesting that NA may induce inhibition of acupuncture analgesia in brain nuclei (Dong et al., 1984).

It has showed that spinal $\alpha 2$ -adrenoceptors play a crucial role in inhibitory descending pain control by noradrenergic projections from supraspinal nuclei, such as the LRN and LC to the dorsal horn (Liu and Zhao, 1992; Millan, 2002; Zhao and Duggan, 1988), particularly in modulating neuropathic pain (Yu et al., 1998). A recent report indicated involvement of spinal $\alpha 2$ receptors in EA analgesia. Intrathecal injection of $\alpha 2$ receptor antagonist yohimbine, but not $\alpha 1$ receptor antagonist prazosin, significantly blocked EA analgesia in neuropathic rats (Kim et al., 2005). Taken together, the data support the notion that NA may induce inhibition of acupuncture analgesia in brain nuclei, but potentiation in the spinal cord.

4.5. Glutamate and its receptors

Excitatory amino acids, such as glutamate and aspartate, are abundant in nociceptive primary afferent fiber terminals, and NMDA, AMPA/KA and metabotropic receptors are distributed densely in the superficial dorsal horn of the spinal cord where primary nociceptive afferents terminate (Coggeshall and Carlton, 1998; Li et al., 1997; Liu et al., 1994b). It is well-documented that glutamate and its receptors play a pivotal role in spinal transmission of nociceptive information and central sensitization in physiological and pathological conditions (Aanonsen et al., 1990; Dougherty and Willis, 1991; Du et al., 2003; Hu and Zhao, 2001; Millan, 1999; Ren et al., 1992; Song and Zhao, 1993a,b).

Increasing evidence suggests that blockade of NMDA and AMPA/KA receptors is capable of reinforcing acupuncture analgesia. In the rat spinal nerve ligation model, EA decreased nerve injury-induced mechanical allodynia (Huang et al., 2004). Immunohistochemical studies further revealed that nerve ligation increased the expression of NMDA receptor subtype NR1 immunoreactivity in the spinal superficial laminae, which could be reduced by low-frequency electroacupuncture (EA) in the rat spinal nerve ligation model (Sun et al., 2004). Also, in the CFA-induced inflammation model, EA lessened both inflammation agent-induced pain responses and expression of NR1 and NR2 GluR1 in the spinal cord (Choi et al., 2005a,b) as well as the number of DRG neurons with IB4 and NR1 double-labeling (Wang et al., 2006).

Pharmacological studies provide further evidence for blockade of NMDA receptors boosting up acupuncture analgesia. A combination of low-dose ketamine, a NMDA receptor antagonist, with EA produced more potent anti-allodynic effects than that induced by EA alone in a neuropathic pain model (Huang et al., 2004). A similar phenomenon was demonstrated in the carrageenan- or CFA-induced inflammation model. Both 10 and 100 Hz EA combined with a sub-effective dose of MK-801, a NMDA receptor antagonist, showed prolonged anti-hyperalgesia with no side effects (Zhang et al., 2005d). Although neither i.t. injection of the NMDA receptor antagonist AP5 (0.1 nmol) nor the AMPA/KA receptor antagonist DNQX (1 nmol) alone had an effect on inflammation-induced thermal hyperalgesia, both significantly potentiated EA-induced analgesia in carrageenan-injected rats, especially AP5. Simultaneously, when a combination of electroacupuncture with AP5, DNQX or kynurenic acid (KYNA, a wide-spectrum glutamate receptor antagonist) was used, the level of Fos expression in the spinal cord induced by carrageenan was significantly lower than electroacupuncture or i.t. injection of AP5, DNQX or KYNA alone (Zhang et al., 2002, 2003). These results demonstrate that electroacupuncture and NMDA or AMPA/KA receptor antagonists have a synergic anti-nociceptive action against inflammatory pain.

4.6. γ -Amino-butyric acid (GABA)

GABA is an important inhibitory transmitter in the CNS and is involved in multiple physiological and pathological functions. There are three GABA receptor subtypes: GABA_A, GABA_B, and GABA_C. It has been known that GABA_A and GABA_B receptors contribute to modulation of pain (Millan, 1999, 2002). But its role in acupuncture analgesia is still obscure and the relevant results are contradictory.

4.6.1. GABA_A receptor

Early pharmacological studies found that systemic administration of a GABA_A receptor antagonist reduced acupuncture analgesia (McLennan et al., 1977), whereas intrathecal diazepam binding to GABA_A receptors potentiated EA analgesia (Pomeranz and Nguyen, 1987). Microinjection of muscimol, a GABA_A receptor agonist, or 3-MP, a GABA synthesis inhibitor, into the PAG markedly suppressed and potentiated acupuncture analgesia, respectively (Han, 1989). EA at “Zusanli” (ST-36) induced analgesia with an increase in expression of GABA in the PAG (Fusumada et al., 2007). Acupuncture raised the rat pain threshold with an increase in GABA concentration in the habenula, suggesting that an increase of GABA concentration in brain is the cause of increasing the pain threshold through its inhibitory effect on habenular activity (Tang et al., 1988).

4.6.2. GABA_B receptor

Intracerebroventricular administration of GABA_B, but not GABA_A, receptor antagonists clearly decreased both acupuncture analgesia and GABA_B receptor agonist-induced analgesia. Moreover, intrathecal administration of both GABA_A and GABA_B receptor antagonists partially blocked acupuncture analgesia (Zhu et al., 1990a,b, 2002). In support of the behavioral results, in an electrophysiological study from the same laboratory, microiontophoretic administration of a GABA_A receptor antagonist markedly inhibited acupuncture-induced inhibition of nociceptive responses of the spinal dorsal horn neurons and EA-induced depolarization of C-afferent terminals, suggesting involvement of presynaptic inhibition (Li et al., 1993). These data suggested that only GABA_B receptors in supraspinal structures contribute to mediating acupuncture analgesia, whereas both GABA_A and GABA_B receptors in the spinal cord are associated with acupuncture analgesia (Zhu et al., 2002).

4.7. Other bioactive substances

In addition to the above transmitters, interruption of functions of the following bioactive substances could positively or negatively influence acupuncture analgesia. Except that substance P, an important pain signal molecule, may be a target of acupuncture, the following others might be involved in mediating acupuncture analgesia.

4.7.1. Substance P (SP)

It is well-documented that SP is one of the most important signal molecules mediating peripheral (Zhang et al., 2007b) and spinal nociception (Hunt and Mantyh, 2001). Various noxious stimuli induce SP release in the spinal cord (Yaksh et al., 1979; Duggan et al., 1987) and peripheral inflammation enhanced expression of SP in nociceptors in the cat (Xu et al., 2000). Low-intensity EA at bilateral “Huantiao” (GB30) for 30 min did not change the SP content in the perfusate of the spinal cord, but clearly reduced noxious stimulation-induced elevation of SP (Zhu et al., 1991). Immunohistochemical studies showed that EA at “Zusanli” (ST-36) depressed the pain response and increased

SP-immunoreactivity possibly due to the inhibition of its release (Du and He, 1992). Moreover, EA induced a decrease in tooth pulp stimulation-induced SP release in the trigeminal nucleus caudalis and A δ afferent-mediated evoked potentials in the rabbit, which was reversed by a SP receptor antagonist (Yonehara et al., 1992). Given that opiates inhibited SP release (Yaksh et al., 1980) and acupuncture induced release of opioid peptides in the spinal cord (Han, 2003), it is conceivable that pain stimulation itself may activate the endogenous opioid mechanism to inhibit SP release and acupuncture is able to enhance the process. This might be one of the mechanisms underlying acupuncture analgesia.

4.7.2. Angiotensin II (All)

This neuropeptide is widely distributed in the CNS and exerts multiple physiological and pathological functions including modulation of pain (Han et al., 2000; Tchekalarova et al., 2003; Toma et al., 1997). Preliminary work showed that the role of All in EA analgesia is comparable with that of CCK. EA at 100 Hz increased All-ir content in the spinal perfusate. Intrathecal administration of saralasin, an All receptor antagonist, induced a significant potentiation of the analgesia induced by 100 Hz EA. EA at 100 Hz probably accelerated the spinal release of All as a brake for EA analgesia (Shen et al., 1996).

4.7.3. Somatostatin (SOM)

SOM, an endogenous non-opioid neuropeptide, is located in the peripheral and central nervous system and contributes to modulation of nociception (Sandkuhler et al., 1990; Song et al., 2002). A few studies showed that SOM might be involved in EA analgesia (Zheng et al., 1995) and other functions of EA, such as modulating meal-stimulated acid secretion (Jin et al., 1996). To validate the effect of SOM on EA analgesia, a recent study showed the effects of EA on the expression of SOM peptide and preprosomatostatin (ppSOM) mRNA in a rat model of neuropathic pain induced by chronic constriction injury (CCI) to the sciatic nerve, using immunohistochemistry and RT-PCR. EA significantly enhanced SOM expression in DRG and spinal dorsal horn as well as ppSOM mRNA level in DRG of rats with neuropathic pain (Dong et al., 2005a). The results indicated that endogenous SOM might play a role in EA analgesia for neuropathic pain.

4.7.4. Arginine vasopressin (APV)

An early study revealed that the hypothalamic paraventricular nucleus (PVH) plays an important role in acupuncture analgesia (Yang et al., 1992; Yang and Lin, 1992). Microinjection of L-glutamate, which only excites PVH neurons, into the PVH, dose-dependently enhanced EA (Zusanli, ST-36) induced analgesia and increased the AVP, but not oxytocin (OXT), concentrations in the PVH perfuse liquid using radioimmunoassay. Intraventricular injection of anti-APV serum completely reversed the glutamate-induced enhancement of EA analgesia (Yang et al., 2006a). Furthermore, EA increased APV, but not OXT, concentrations in the PVH perfuse liquid and decreased the number of AVP, but not OXT, immunoreactive neurons in the PVH (Yang et al., 2006b). The data suggested that AVP, and not OXT, in the PVH may be important in the modulation of EA analgesia.

4.7.5. Neurotensin (NT)

NT-ergic fibers and NT receptors are distributed in the PAG, which is involved in modulation of nociception (Li et al., 2001; Tershner and Helmstetter, 2000). The effect of activity of NT receptors in the PAG on EA analgesia was assessed in the rat tail-flick test (Bai et al., 1999). Microinjection of NT into the PAG remarkably enhanced EA analgesia, which was attenuated by

microinjection of naloxone into the PAG. Opioid receptors in the PAG may participate in NT-induced potentiation of EA analgesia.

4.7.6. Dopamine (DA)

Several reports revealed that DA receptor antagonists potentiated EA analgesia (Han et al., 1979b; Wang et al., 1997; Wu et al., 1990; Xu et al., 1980). Receptor binding studies provided further support, in which DA receptor antagonists enhanced EA analgesia and up-regulated opioid receptors in many brain regions including the Cd, Po, PVH and PAG, suggesting that activity of opioid receptors may be one of the mechanisms in the potentiating action of haloperidol on acupuncture analgesia (Wang et al., 1994; Zhu et al., 1995). It appears that activity of DA receptors, particularly DA1 receptor, may reduce EA analgesia (Wang et al., 1999).

In addition, several reports showed that other bioactive substances (GDNF, GFR α -1, IL-1, adenosine, acetylcholine and Ca²⁺) are probably involved in processing acupuncture analgesia. In the neuropathic pain model, mRNA levels of GFR α -1 were increased in the rat DRG. EA significantly reduced thermal hyperalgesia and potentiated GFR α -1 elevation, which were blocked by antisense oligodeoxynucleotide (ODN) specifically against GFR α -1. Also, EA potentiated neuropathic pain-induced increase of GDNF in the spinal dorsal horn. It is suggested that EA activates the endogenous GFR α -1 and GDNF system in neuropathic pain (Dong et al., 2005b, 2006). EA reduced peripheral inflammation-induced increase of expression of IL-1 receptor I mRNA in rat periaqueductal gray (Ji et al., 2003). EA significantly attenuated cancer cell inoculation-induced thermal hyperalgesia, and inhibited the upregulation of IL-1beta and its mRNA compared to the sham control. Intrathecal injection of IL-1ra, IL-1 receptor antagonist, significantly inhibited cancer-induced thermal hyperalgesia, suggesting that EA alleviates bone cancer pain, at least in part by suppressing IL-1beta expression (Zhang et al., 2007c). Intrathecal administration of adenosine P1 receptor antagonists depressed EA-induced inhibition of nociceptive responses in spinal WDR neurons and blocked EA-induced increases in pain threshold in the rat (Liu et al., 1994a,b). EA significantly increased both pain threshold and the content of ACh in the cortex, caudate nucleus, hypothalamus and brainstem (Guan et al., 1984), and enhanced the activity of acetylcholinesterase (AChE) in the spinal cord (Ai et al., 1984). EA or morphine-induced analgesia with a clear increase in mitochondrial protein-bound Ca²⁺ in the PAG and hypothalamus, which was blocked by i.c.v. administration of ruthenium red. It is suggested that Ca²⁺ transport across the neuronal cell membrane may be implicated in EA analgesia (Xie et al., 1988).

5. Miscellaneous

5.1. Glial function in acupuncture analgesia

Increasing evidence has revealed that spinal cord glia (microglia and astrocytes) make important contributions to the development and maintenance of inflammatory and neuropathic pain (Deleo et al., 2004; Ledebuer et al., 2005; Ma and Zhao, 2002; Song and Zhao, 2001; Sun et al., 2007a; Watkins et al., 2005, 2007; Zhang et al., 2005c, 2007c). Also, recent studies found that spinal glia has an intimate relationship with EA analgesia (Kang et al., 2007; Sun et al., 2006, 2007b). Intrathecal application of fluorocitrate, a glial metabolic inhibitor, alone had no effect on basal thermal hyperalgesia and mechanical allodynia in monoarthritic rats. EA at unilateral “Huantiao” (GB30) and “Yanglingquan” (GB34) considerably reduced thermal hyperalgesia and mechanical allodynia. Moreover, when fluorocitrate was simultaneously administered with EA, EA analgesia was significantly potentiated, strongly suggesting that EA and disrupting glial

function synergistically suppress inflammatory pain (Sun et al., 2006). Also, intrathecal injection of minocycline, a microglial inhibitor, or EA stimulation of ipsilateral “Huantiao”(GB30) and “Yanglingquan” (GB34) significantly suppressed CFA-induced nociceptive behavioral hypersensitivity and spinal microglial activation. Furthermore, combination of EA with minocycline significantly enhanced the inhibitory effects of EA on allodynia and hyperalgesia (Sun et al., 2007b). A similar result was found in a MPTP-induced Parkinson disease (PD) model. Acupuncture attenuated the increase in MAC-1, a marker of microglial activation, and reduced the increases in cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression in the PD model. Acupuncture could be used as a neuroprotective intervention for the purpose of inhibiting microglial activation and inflammatory events in PD (Kang et al., 2007). In addition, the gap junction protein connexin 43 (Cx43) is extensively expressed in the CNS, and serves in signal transmission between glia and neurons (Sa’ez et al., 2005). In connexin 43 gene knock-out mice, EA analgesia was partially reduced without change in pain threshold in comparison with wild-type mice (Yu et al., 2007).

These data provide direct evidence for the involvement of spinal microglial functional state in the anti-nociception of EA, suggesting that analgesic effects of EA might be associated with its counter-regulation to spinal glial activation, and thereby provide a potential strategy for the treatment of arthritis.

5.2. Possible molecular mechanisms

Although molecular techniques have been used in the study of acupuncture analgesia, understanding of the molecular mechanisms underlying acupuncture analgesia is preliminary and fragmentary.

G protein-coupled receptors, such as opioid receptors, are a focus of attention as pain targets. G proteins are classified mainly as Gs and Gi/o, which stimulate and inhibit the membrane effector adenylate cyclase, respectively. It has been demonstrated that pertussis toxin (PTX), an inhibitor of Gi/o protein signal transduction, prevents the antinociception mediated by opioid receptors (Przewlocki et al., 1987). In a recent report, the effect of PTX on EA analgesia was assessed (Liu et al., 2005). Spinal Gi/o-protein function was destroyed by 7 days of intrathecal pretreatment with pertussis toxin (PTX). In this condition, EA failed to inhibit hyperalgesia in the CFA inflammatory pain model, but did not affect either baseline pain threshold or CFA-induced hyperalgesia in the rat. These data suggest that PTX-sensitive Gi/o proteins and the spinal endogenous opioid-mediated signaling pathways may be implicated in anti-hyperalgesia by EA.

Extracellular signal-regulated protein kinase (ERK) is a mitogen-activated protein kinase (MAPK), which mediates intracellular signal transduction involved in cell proliferation, differentiation, and neuronal plasticity. Increasing numbers of studies have demonstrated ERK phosphorylation in the nociceptive pathway (Ji et al., 2002; Obata and Noguchi, 2004). Recently, a preliminary study found that EA at contralateral Zusanli (ST-36) dramatically decreased intraplantar formalin-induced increase of pERK1/2-positive neurons in the ipsilateral superficial dorsal horn of the rat, compared with the formalin group without EA. In EA alone without formalin treatment, a few positive-neurons were found. The results suggest that EA might produce inhibition of ERK1/2 phosphorylation in the spinal dorsal horn (Song et al., 2006).

Genes are regulated primarily at the transcriptional level. Moreover, transcription factors control target genes. A line of evidence has demonstrated that EA markedly induces a rapid expression of the *c-fos* gene in the spinal cord and various brain regions, suggesting that transcription factors are also involved in

processing acupuncture signals. A family of possible targets is the three opioid genes: preproenkephalin (PPE), preprodynorphin (PPD) and pro-opiomelanocortin (POMC). EA is known to induce *c-fos* expression, which precedes PPE gene expression (Ji et al., 1993a,b). Using antisense oligodeoxynucleotide ODNs of *c-fos* and/or *c-jun*, researchers addressed (Guo et al., 1996) the role of Fos and Jun proteins in EA-induced transcription of the opioid genes preproenkephalin (PPE), preprodynorphin (PPD) and proopiome-lanocortin (POMC). EA-induced Fos and Jun expression was blocked efficiently and specifically by *c-fos* and *c-jun* antisense ODNs, respectively. The antisense ODNs markedly decreased EA-induced PPD, but not PPE, mRNA expression. These results suggest that Fos and Jun proteins are involved in PPD rather than PPE gene transcription activated by EA stimulation.

Nuclear factor-kappa B (NF- κ B) is another crucial regulator of inducible genes, and, as a transcriptional activator, is implicated in multiple functional processes including inflammation-induced hyperalgesia (Chan et al., 2000; Wood, 1995). The NF- κ B family consists of NF- κ B1 (p50/p105) and NF- κ B2. A study of NF- κ B1 knock-out mice showed that deletion of the NF- κ B1 gene induced a significant decrease of both low- and high-frequency EA-induced analgesia, compared with that in wild-type mice. The results imply that NF- κ B1 takes part in EA analgesia despite the fact that the mechanism is still unclear (Park et al., 2002).

In the rat neuropathic pain model, cDNA microarray analysis was used to compare the expression of 8400 genes and mRNAs that were pooled from the spinal cord in rats. Sixty-eight genes were differentially expressed more than two-fold in the neuropathic rat model compared to the normal, and restored to the normal expression level after EA treatment. By a dot-blot analysis, among the 68 genes, the mRNA expression level of 8, such as the opioid receptor sigma gene, MAP kinase, zinc finger protein, and tyrosine phosphatase, was down-regulated in the neuropathic pain model compared to the normal rat, and the mRNA expression level of these genes was restored to the normal state following EA treatment. Therefore, multiple signaling pathways, including opioid receptor- and MAP kinase-mediated pathways, as well as other gene expression, might be involved in pain development and EA analgesia (Ko et al., 2002).

As mentioned above, there are individual differences and EA frequency-dependency in acupuncture analgesia. Using inbred strains of rodents, Mogil (1999) found that robust strain differences in analgesic sensitivity suggest a role of inherited genetic factors. Therefore, a revealing study analyzed the effect of genotype on sensitivity to EA analgesia in 10 common inbred mouse strains (129P3 (129), A, AKR, BALB/c (B/c), C3H/He (C3H), C57BL/6 (B6), C57BL/10 (B10), C58, RIIS (R3) and SM) (Wan et al., 2001). Among them, the B10 strain was the most sensitive, and the SM strain was the least sensitive to EA frequency both at 2 and 100 Hz. However, the relative sensitivities of the other strains to these two EA frequencies suggested some genetic dissociation between them as well. An intriguing finding is the significant difference in 2 Hz EA-induced analgesia between C57BL/6 (B6) and B10 strains. It is known that there is allelic variation in microsatellites of the delta gene between sublines B10 and B6. This raises the possibility that B10 and B6 may have different allelic forms of the gene encoding delta and hence show variation in their analgesic responses to EA.

A recent study was conducted to identify and characterize the genes that differ between high-responders and low-responders to acupuncture stimulation in human volunteers, using cDNA microarrays. Fifteen participants were stimulated at “Hegu” (LI-4), and the finger withdrawal latency as the pain threshold was used to classify high- and low-responders. The pain threshold was significantly elevated by acupuncture in the high-responders,

whereas there was little increase in the low-responders. In the high-responder group, 353 and 22 genes were up- and down-regulated, respectively. However, a measure of psychological variation did not differ significantly between the two groups (Chae et al., 2006). These findings provide a role for inherited genetic factors as a possible explanation of individual differences in acupuncture analgesia.

6. Conclusions and consideration

Acupuncture, an age-old healing art, has been accepted to effectively treat various diseases, particularly chronic pain. Despite the involvement of psychological factors in acupuncture treatment of patients and stress in animal behavioral tests, a large volume of evidence clearly demonstrates that acupuncture analgesia has physiological, anatomical and neurochemical bases.

- (1) Acupuncture analgesia is manifest only when the intricate feeling of acupuncture (soreness, numbness, heaviness and distension), so-called “De-Qi”, occurs. Such a “De-Qi” feeling mainly originates from acupuncture-induced impulses from local muscle contraction beneath the acupoint.
- (2) Types of afferent nerve fibers activated by acupuncture depend upon the manipulation methods and individual differences in sensitization. Manual acupuncture (MA): all types (A β , A δ and C) of afferent fibers are activated to conduct the signal. Electroacupuncture (EA): electrical current via acupuncture needles at intensities strong enough to excite A β - (group II) and part of A δ -type afferents (group III) can induce an analgesic effect.
- (3) Acupuncture analgesia is essentially a manifestation of integrative processes at different levels of the CNS between afferent impulses from the pain regions and impulses from acupoints. Segmental mechanisms in the spinal cord contribute to the functionally relative specificity of acupoints.
- (4) Spinal pathways of acupuncture impulses from acupoints ascend mainly through the ventrolateral funiculus. A complex network of many brain structures is involved in processing acupuncture analgesia, including the VRM (mainly NRM), PAG, LC, Arc, Po, Sm, AptN, Hab, Ac, Cd, Sp, amygdala, etc. Most of nuclei are constitutive parts of the endogenous descending inhibitory system in the CNS. Activity in most nuclei mediates acupuncture analgesia, except for the Hab and LC, which antagonize it.
- (5) Various signal molecules are implicated in acupuncture analgesia, such as opioid peptides (μ -, δ - and κ -receptors), cholecystinin octapeptide (CCK-8), glutamate (NMDA and AMPA/KA receptors), 5-hydroxytryptamine and noradrenalin. Among them, opioid peptides and their receptors play a pivotal role in mediating acupuncture analgesia. The release of opioid peptides evoked by EA is frequency-dependent. EA at 2 and 100 Hz induce release of enkephalin and dynorphin in the spinal cord, respectively. In accord with the effect of morphine analgesia, CCK antagonizes acupuncture analgesia.

Further considerations associated with acupuncture analgesia include:

- (1) Serious basic research on “acupuncture analgesia” begun at the end of the 1960s when “acupuncture anesthesia” was used in surgical operations in China. Scientists are devoted to explaining this mysterious phenomenon. Notwithstanding the fact that acupuncture can induce an analgesic effect, no evidence shows that it has any anesthetic effect. Strictly speaking, the term “acupuncture anesthesia” is vague. In fact, when acupuncture alone, without adjunct drugs, is performed

for surgery, the effectiveness is not satisfactory in most cases. Given the progress in pain research, it is well-documented that some receptor antagonists and agonists depress pain transmission. Some studies have indicated that a combination of acupuncture and adjunct drugs (antagonists or agonists) can powerfully strengthen acupuncture analgesia (Cao, 1997; Zhang et al., 2004b; Zhu et al., 1997a,b). It is, therefore, conceivable that the development of “acupuncture-drug-assisted anesthesia,” acupuncture with adjunct drugs, may be a hopeful route for surgery (Han, 1997; Wu, 2005).

- (2) Acupuncture can effectively treat chronic pain in patients. In other words, the development of acupuncture analgesia depends on the treatment of chronic pain. However, most previous studies on acupuncture analgesia focused on physiological pain in normal animals and human volunteers. Increasing evidence has demonstrated that the mechanisms underlying pathological pain are much more complex than physiological pain (Scholz and Woolf, 2007). Moreover, a notable experiment revealed that the analgesic effects of acupuncture in normal rats and rats with inflammation were evidently different (Sekido et al., 2003). There were so-called responders and non-responders for acupuncture analgesia in normal rats, whereas all those with inflammation hyperalgesia were responders and manifested acupuncture analgesia, strongly suggesting that diverse mechanisms underlie acupuncture analgesia in physiological and pathological pain. To substantially explore the mechanisms of acupuncture analgesia, adequate models of chronic pain should be selected for research.
- (3) Conflicting results of acupuncture analgesia are probably attributed to various experimental conditions (pain models, MA or EA, acupoints, and insertion depth). For instance, EA current parameters (frequency, intensity, duration and polarization of pulses) differ in different studies. Particularly, EA intensity is rarely monitored. Generally, which types of afferent nerve fibers were excited was unknown in most of the previous studies, and this might be an important reason for the conflicting results. How to make experimental conditions as identical as possible will play a key role in acupuncture analgesia research.

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