

## Acupuncture Analgesia: A Review of Its Mechanisms of Actions

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**Abstract:** The mechanism of acupuncture analgesia (AA) has been widely explored since the 1970s. Early studies investigated the relationship between acupuncture and endogenous opiates ( $\beta$ -endorphin, enkephalin, endomorphin and dynorphin). Before the 1990s, most experts agreed on the concept that in normal animal models, lower frequency electroacupuncture (EA) stimulates the release of  $\beta$ -endorphin, enkephalin and endomorphin, which in turn activates the  $\mu$ - and  $\delta$ -opioid receptors, and that higher frequency EA stimulates dynorphin which activates the  $\kappa$ -opioid receptor. Besides endogenous opiates, our studies have focused on serotonin. The serotonergic descending inhibitory pathway is suggested to be an important mechanism of acupuncture analgesic, collaborating with endogenous opiates. Many efforts have been made to clarify these mechanisms, but to date no satisfactory consensus has been reached. In the late 1990s, researchers began to focus on the different analgesic effects of EA between normal and hyperalgesic animal models. Published data from these studies imply that normal and hyperalgesic animals respond differently to EA. Results from experiments on the anti-hyperalgesia effect of EA have raised a new issue about the influences of EA on receptors to excitatory amino acid in the spinal cord level. Results from various studies have shown that these receptors play a role in the mechanism of AA. Recently, research on the autonomic nervous system (ANS) seem to indicate its connection with acupuncture. The inflammatory reflex (via the ANS) might be a crucial part of anti-hyperalgesia elicited by acupuncture, and this reflex, which regulates the immune system in the organism, can elucidate not only the mechanism of AA but also the mechanism of acupuncture applied to other inflammatory conditions. Innovation of functional image study enables us to analyze the responses of cortex on living human body to acupuncture. However, results of these experiments are still controversial. After 30 years of acupuncture research, there are still many puzzles left to be solved regarding the mechanism of AA.

**Keywords:** Acupuncture; Acupuncture Analgesia; Mechanism.

## Introduction

Acupuncture is widely used to induce analgesia and is approved nowadays; however the mechanism of acupuncture analgesia (AA) is still poorly understood. There are two different strategies of performing acupuncture therapy, manual acupuncture (MA) and electroacupuncture (EA). EA is a modified form of traditional MA. The advantage of EA is its combined therapeutic effects of transcutaneous electric nerve stimulation (TENS) and MA. Most studies use EA because EA can be standardized by frequency, voltage, wave form, length, etc. Although standardization is essential for modern research, some experts do not agree that EA can be a substitution for MA.

De-Qi or De-Chi is a sensation of heaviness, soreness or numbness “recognized” by the cortex during acupuncture needling. Therefore, it is generally accepted that cortical involvement follows acupuncture stimulation (Kimura *et al.*, 2006; Ernst *et al.*, 2007; Kou *et al.*, 2007). A number of evidences demonstrates that AA induces its effect via neuronal mechanisms correlated with central nervous system (CNS) (Clement-Jones *et al.*, 1980; He, 1987; Tsai *et al.*, 1989a; Pomeranz *et al.*, 1977). The most well-known mechanism is endogenous opiates and their receptors. Early works have shown the role of endogenous opiates in the CNS on AA. Different kinds of endogenous opiates, such as  $\beta$ -endorphin, enkephalin, endomorphin and dynorphin, have been reported to be frequency-dependent factors (Han *et al.*, 1999; Huang *et al.*, 2000).

As studies continue around the world, serotonin and related descending pain inhibitory pathway theories have been also developed (Tsai *et al.*, 1989a). Later in our studies, we elucidated the relationship between serotonin and AA, and it is increasingly clear that EA evokes some regions of upper brain stem and hypothalamus to release endogenous opiates and serotonin. However, in the 1990s researchers were not satisfied with the results of studies on normal animal models. Some speculated that there were differences in the effect of AA between normal and inflammatory animal model (Sekido *et al.*, 2003). Inflammation will induce the state of hyperalgesia, in turn will decrease the pain threshold. Acupuncture can regulate this state of hyperalgesia, thus reducing the degree of pain. Although no consensus has been reached, these studies using inflammatory animal models have demonstrated that AA may be related to a sort of anti-inflammatory mechanism.

Moreover, acupuncture therapy is used not only to relieve pain but also to treat various medical conditions in traditional Chinese medicine (TCM) (Ma *et al.*, 2006; Tseng *et al.*, 2006; Shin *et al.*, 2007). Some experiments have found the relationship between acupuncture and the ANS (Hsieh *et al.*, 1999; Hsu *et al.*, 2006). The inflammatory reflex via the ANS could be a possible explanation for acupuncture’s diverse therapeutic strategies (Tracey, 2002). Many disorders are thought to be inflammation-related. It is hypothesized that acupuncture can modulate these inflammatory conditions through inflammatory reflex. The hypothalamus is the pivot for both hormonal and neuronal systems. Therefore, the hypothalamus might play a key role in AA.

Although EA has been investigated extensively, these studies are of limited clinical relevance to traditional application. Most clinicians, especially in oriental society, use MA instead of EA. In those studies with EA, it is not clear whether the analgesia effect is through

needling itself or through electric stimulation. An investigator found that electric stimulation of peripheral nerve elicits analgesic effect (Hsieh *et al.*, 2000). Therefore, the fundamental question of acupuncture and the existence of specific points, cannot be answered by results from studies which intend to explore the effect of EA.

### Endorphins Theory

Endogenous opiates play important roles in AA. In the 1970s and early 1980s, acupuncture was regarded as a novel pain-killer. Naloxone, an antagonist to opiate (morphine-like substrate), was shown to attenuated analgesic action of acupuncture in humans (Mayer *et al.*, 1977) and mice (Pomeranz and Chiu, 1976); the release of morphine-like substrate in central nerve system (CNS) was hypothesized to be a possible mechanism. Many investigations took place to clarify the definite mechanism of AA during this period. Pomeranz has suggested that EA induces release of endogenous opiates from the pituitary gland into plasma. Plasma endogenous opiates were shown to sufficiently cause analgesia in the CNS (Cheng *et al.*, 1979).

In the early 1980s,  $\beta$ -endorphin and enkephalin were purified and they were suggested to play roles in AA in humans and animals. It was revealed the elevated level of endorphin in the cerebrospinal fluid (CSF) in cat after auricular EA (Pert *et al.*, 1981). In human, elevated levels of CSF,  $\beta$ -endorphin (Clement-Jones *et al.*, 1980) and plasma enkephalin (Kiser *et al.*, 1983) were also observed after acupuncture. Soon after that, the relationship between AA and different kinds of endogenous opiates was explored in detail. For example, Pomeranz's group first described the possibility that there were different mechanisms of analgesia when EA was applied with different frequencies (Cheng and Pomeranz, 1979). This finding inspired later researches. The most well-known group is Han JS's. The role of different frequencies of EA was not elucidated until the experiments were performed by his group. They reported that in EA, frequency is a basic determinant for different endogenous opiates secretion (Chen and Han, 1992). By using a cross-tolerance technique (to evaluate whether two kinds of drugs bind to the same receptor), they found that lower frequency (2 Hz) EA analgesia is mediated by  $\mu$ - and  $\delta$ -opioid receptors and that higher frequency (100 Hz) EA analgesia is mediated by the  $\kappa$ -opioid receptor. They concluded that 2 Hz EA stimulates the release of  $\beta$ -endorphin, enkephalin and endomorphin (Han *et al.*, 1999; Huang *et al.*, 2000) within the network of the CNS and that 100 Hz EA releases dynorphin (Han, 2003).

Although the release of endorphin into plasma from the pituitary gland is a presumed mechanism of AA (Pomeranz *et al.*, 1977), auricular EA does not result in increased endorphin levels in animal plasma (Pert *et al.*, 1981). In contrast, endorphin was regarded as a neurotransmitter in the CNS instead of hormone in the plasma (He, 1987). These two mechanisms (hormone and neurotransmitter) were not mutually exclusive. Although no further evidence supports, we assume that differences between auricular and somatic EA may have caused these conflicting results. In addition, a recent report concluded that not only frequency but also pulse width of EA can influence the analgesic effect (Lao *et al.*, 2004). Our study also finds that intermittent EA stimulation is a valid way to prevent the occurrence

of tolerance to AA (Lin *et al.*, 1993). Taken together, the stimulation protocol may elicit different effects. However, most early papers did not mention the precise protocol, including pulse width and stimulation mode. Therefore, it is not easy to compare the results of early studies and deduce a mechanism of AA. Nonetheless, most early studies concluded that EA produces analgesic effect by both hormonal and neuronal pathways. A hormonal pathway may be involved in AA elicited by lower frequency EA (discussed later). It is traditionally believed that hormone produces its analgesic effect via anti-inflammation in a gradual mode. However, little detail was known about the relationship between EA and anti-inflammation in early experiments.

More recently researches focus on the anti-hyperalgesic effect of acupuncture in inflammatory animal models (Koo *et al.*, 2002; Sekido *et al.*, 2003; Huang *et al.*, 2004; Lao *et al.*, 2004). Injured neurons will express more peptides (substance P, somatostatin and calcitonin gene-related peptide) related to pain transmission, commonly referred to as neuronal plasticity (Hokfelt, 1991). With persistent inflammation, neuronal plasticity causes hyperalgesia (hyperexcitable to pain). Dr. Berman's and Dr. Zhang's group found the phenomenon that acupuncture analgesia differs between chronic pain animal models (induced by persistent inflammation) and normal animal models. Derm-sap is a toxin specific to  $\mu$ -opioid receptor (MOR). Fourteen days after Derm-sap had been injected into the intrathecal space, rats were administered with complete Freund's adjuvant (CFA) in their hind paw. This was a good hyperalgesic rat model without MOR. Compared with animals with MOR, anti-hyperalgesic effect of 10 Hz EA declines in those without MOR; that is, the MOR gets involved in anti-hyperalgesia produced by lower frequency EA (Zhang *et al.*, 2005b). This result is consistent with a previous study in which naloxone was used to antagonize MOR (Sekido *et al.*, 2003). These researchers further found that with CFA injection, the anti-hyperalgesic phenomenon at both lower (10 Hz) and higher (100 Hz) frequency EA was reversed by  $\mu$ - and  $\delta$ -opioid receptors antagonist; the  $\kappa$ -opioid receptor antagonist did not alter the anti-hyperalgesic phenomenon at any frequency EA (Huang *et al.*, 2004). Hence, they concluded that in hyperalgesic rats, both lower (10 Hz) and higher (100 Hz) frequency EA decreased paw withdrawal latency through  $\mu$ - and  $\delta$ -opioid receptors (Zhang *et al.*, 2004a); in normal animals, only lower frequency EA but not higher frequency EA is through  $\mu$ - and  $\delta$ -opioid receptors. Higher frequency EA is found to elicit its analgesic effect via  $\kappa$ -opioid receptor (Han, 2003). These researches show that there are different mechanisms in studies conducted with different animal models.

However, this issue has not been settled yet. Another group of researchers found that intraperitoneal injection of high dose naloxone (which acts as a  $\kappa$ -opioid receptor antagonist) can attenuate the anti-hyperalgesia effect of higher frequency stimulation (100 Hz) (Huang *et al.*, 2004). In other words, anti-hyperalgesia on chronic pain models at 100 Hz EA is the same as the analgesic effect in normal animal models. This conflict implies that different treatment protocols (timing of needling and selection of acupoints) and tools of assessment can influence the results even when the stimulating frequency is similar.

In peripheral inflamed tissue, immune cells express endogenous opiates and bind with opioid receptors on peripheral afferent nerves (Stein, 1995). This binding of opiates and their receptors can inhibit the transmission of noxious signal from the peripheral nerve system

(PNS) to CNS. Local peripheral opioid receptors on nerve endings are up-regulated during inflammation and these peripheral receptors are likely more determinant than those central receptors to AA (Sekido *et al.*, 2003). From Sekido's research, local blockade instead of systemic blockade of opioid receptors decreased the analgesic effect of EA. Not long ago, there was debate about whether endogenous opiates act as neurotransmitters or hormones. The neuroimmune link is a good answer for this dispute. Within CNS, endorphins are neurotransmitters and within peripheral tissue, they are hormones. Given these complexities, AA cannot be explained by any single mechanism.

### **Serotonin and the Descending Pain Inhibitory Pathway Theory**

In addition to opioids, our group focuses on the role of central monoaminergic systems on AA. Particular emphasis is given to serotonin. Serotonin (5-HT, 5-hydroxytryptamine) was speculated to be an analgesic transmitter in an early study (Cheng and Pomeranz, 1979). With 2 Hz EA, threshold of tail pressure pain was shown to increase in SD rats. This analgesic effect diminished after p-chlorophenylalanine (serotonin synthesis inhibitor) injection (Tsai *et al.*, 1989a). Therefore, serotonin is thought to have a role in AA. Evidence suggests that serotonin levels increase in spinal cord (Tsai *et al.*, 1989b) and that its precursor (5-hydroxytryptophan) responds to enhanced analgesia at 2 Hz EA (Chang *et al.*, 2004).

To elucidate which receptor subtypes serotonin acts upon at different frequencies of EA, we used antagonists for 5-HT<sub>1a</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptor subtypes to analyze the change in analgesic effect of EA. It has been found that EA analgesia is blocked by 5-HT<sub>1a</sub> and 5-HT<sub>3</sub> antagonists at both low and high frequencies; whereas, EA analgesia is enhanced by 5-HT<sub>2</sub> antagonist at high frequency (100 Hz) (Chang *et al.*, 2004). These results suggest the importance of the serotonergic pathway in AA and are supported by those reported in later studies (Baek *et al.*, 2005; Kim *et al.*, 2005). There are many serotonin releasing nuclei (serotonergic neurons) in the CNS. The nucleus raphe magnus (NRM) is one of them in the lower brainstem (from lower pons to medulla) and it is associated with the descending pain inhibitory pathway. The axons of these serotonergic neurons terminate at the level of spinal cord, so they are referred to as the serotonergic raphe-spinal neurons. EA can activate these serotonergic raphe-spinal neurons in the NRM (Liu *et al.*, 1986). Serotonin is released from the terminals of the serotonergic raphe-spinal neurons, and it then binds to the receptors on the surface of the inhibitory interneurons. There are several 5-HT receptor subtypes. The 5-HT<sub>1</sub> and 5-HT<sub>3</sub> subtypes are located in the dorsal horn of the spinal cord and their agonists have been found to reduce pain (Danzebrink and Gebhart, 1991; Eide and Hole, 1993). The 5-HT<sub>1a</sub> subtype is also an autoreceptor within the NRM. The binding of serotonin to its receptor can activate the inhibitory interneurons in the spinal cord, which contain enkephalin (enkephalinergic interneurons). Enkephalin and  $\mu/\delta$ -opioid receptors are thought to play important roles in inhibiting pain sensation signals. These enkephalinergic interneurons presynaptically block the signal transmission of peripheral nociception by releasing enkephalin. EA, in both lower and higher frequencies, can activate this mechanism mentioned above.

In contrast, the 5-HT<sub>2</sub> receptor subtype may increase the transmission of pain signal (Eide and Hole, 1993). It is also an excitatory receptor in the cortex and the hippocampus. Higher frequency EA might decrease the serotonin concentration within the cortex, therefore acting as a sedative. As a result, higher frequency EA may elicit analgesic effect via both the descending pain inhibitory pathway and the cortex. A study had suggested distinct neuronal pathways elicited by EA at different frequencies (Guo *et al.*, 1996).

Now we have the concept of how EA works through the descending pain inhibitory pathway associated with serotonin from the lower brainstem to the spinal cord level. However, from the diencephalon to the upper brainstem, this pathway is mediated by endogenous opiates. The periaqueductal gray (PAG) in the upper brainstem (midbrain) and the NRM in the lower brainstem are the central parts of this pathway. They act as a group during needling (He, 1987; Lee and Beitz, 1993). During the 1980s, many reports concerning the details of this pathway were published in Chinese. Some of them found that the arcuate nucleus of hypothalamus activates the PAG mediated by  $\beta$ -endorphin. Han concluded that the PAG is fired by  $\beta$ -endorphin and enkephalin but not dynorphin in lower frequency EA (Han, 2003). As a group with the PAG, the NRM then descends its serotonergic axons and project to the spinal cord. Enkephalinergic interneurons, especially in laminae I and II, which are responsive to nociception, are activated by serotonin from the NRM. These interneurons then release enkephalin and presynaptically inhibit primary sensory neurons in the spinal cord (Choi *et al.*, 2005). Therefore, the arcuate nucleus-PAG-NRM-spinal cord axis is activated by lower frequency EA. Chemical interference with the axis will partially block AA (He, 1987). In higher frequency EA, the PAG is activated by different structures within the CNS. The parabrachial nucleus (PBN) in the pons is activated by 100 Hz EA and there is an increasing expression of gene related to dynorphin (Guo *et al.*, 1996; Han, 2003). It is suggested that the neurons in the PBN project axons and release dynorphin to the PAG and the descending pain inhibitory pathway is activated.

### **Modulation of Nociception**

Inhibitory interneurons in the spinal cord might modulate the expressions of excitatory amino acids and their receptor in the spinal cord. Glutamate receptors are involved in nociception and EA has been found to down-regulate their expression (Choi *et al.*, 2005). Excitatory amino acid antagonists can enhance the anti-hyperalgesic effect of EA (Zhang *et al.*, 2002, 2003). Combined with the serotonin theory discussed above, it is hypothesized that the descending inhibitory pathway terminates at the enkephalinergic interneurons; these interneurons release enkephalin which is bound to opioid receptors on spinal cord C-fibers; upon presynaptic binding with enkephalin, C-fibers reduce the amount of excitatory amino acid release, resulting in an anti-hyperalgesic effect. EA can facilitate this pathway.

### **Inflammatory Reflex**

Recently, more and more experts pay attention to inflammation. It has been shown that long term EA reduces activities of T and B cell in the lymph nodes of collagen-induced arthritic

(CIA) mice (Yim *et al.*, 2007). In another study, the activity of splenic natural killer (NK) cells in mice was enhanced after long term EA. EA seems to be an immune modulator. This effect is eliminated by  $\beta$ -endorphin antagonist (naloxone) injection (Yu *et al.*, 1998). In a hyperalgesic animal model, inflammatory responses (edema and hyperalgesia) are reduced by EA (Zhang *et al.*, 2004b; Choi *et al.*, 2005; Zhang *et al.*, 2005a). This effect may be explained by endogenous opiates. Injection of naloxone at the site of injury can attenuate anti-inflammatory action caused by EA. Therefore, the role of peripheral opioid receptors on anti-inflammation is suggested (Sekido *et al.*, 2003). However, the immediate anti-inflammatory effect of EA still exists after intraperitoneal injection of naloxone. Using different study protocols, anti-inflammatory effect of EA was shown to act via non-opioid mechanism in CNS (Sekido *et al.*, 2003; Zhang *et al.*, 2004b). These discordances demonstrate that there are different mechanisms between long term and immediate effects of EA on anti-inflammation.

Electroacupuncture (EA) can modulate the imbalance between innate and acquired immune systems. EA has been shown to be able to adjust the pattern of leukocytes (granulocyte and lymphocyte) in human subjects (Moria *et al.*, 2002). This effect is thought to be associated with the hypothalamus-pituitary-adrenal (HPA) axis in several lines of evidences (Son *et al.*, 2002; Sung *et al.*, 2004; Chiu *et al.*, 2003; Lee *et al.*, 2004; Lao *et al.*, 2004). In the hypothalamus, the expression of mRNA of proinflammatory cytokines by lipopolysaccharide stimulation is reduced after EA (Son *et al.*, 2002). Proteomic analysis has revealed that inflammatory protein levels normalize in the hypothalamus after EA stimulation (Sung *et al.*, 2004). Functional MR images have revealed that the number of activated pain-modulation areas of the hypothalamus increases after EA on analgesic acupoints (Chiu *et al.*, 2003). Therefore, the hypothalamus plays an important role in immune modulation and this modulation is essential for AA. It has been reported that lower frequency EA (4 Hz) produces less of an analgesic effect in animals that had their pituitary gland removed (Pomeranz *et al.*, 1977). Moreover, the AA effect partially decreased in adrenalectomized rats (Lee *et al.*, 2004). Glucocorticoid level increases when 10 Hz EA is applied and 10 Hz EA is found to produce a longer analgesic effect than EA at 100 Hz (Lao *et al.*, 2004). These results suggest that lower frequency EA elicits a stronger anti-inflammatory effect (through HPA axis) and a longer-lasting analgesic effect than higher frequency dose.

Compared to slow-acting hormonal system, nervous system acts in a faster manner. Tracey K.J. was the first to postulate that the relationship between acupuncture and the anti-inflammatory reflex is mediated through the ANS (Tracey, 2002). This reflex leads to a new insight into the studies of mechanism of acupuncture. Although no direct evidence about the relationship of the ANS and AA has been provided, many researchers have found that acupuncture can regulate the function of ANS (Ernst and Lee, 1985; Hsieh *et al.*, 1999; Hsu *et al.*, 2006). Chemical blockade of the parasympathetic system antagonizes anti-inflammatory effect of 2 Hz EA (Baek *et al.*, 2005).

## Conclusion

Many researchers have tried to investigate whether there is a specific pathway involved in AA. However, to date, no consensus has been reached. The endorphin theory is probably the

most admitting one among others. Lower frequency EA acts by stimulating the release of  $\beta$ -endorphin, enkephalin and endomorphin, which then bind to the  $\mu$ - and  $\delta$ -opioid receptors; higher frequency EA stimulates dynorphin which binds to the  $\kappa$ -opioid receptor. However, in clinical settings, acupuncture is performed in patients and not in healthy volunteers. There are many disorders of inflammatory origin, including pain syndromes. Inflammation induces hyperalgesia and in hyperalgesic animal models. Some researches have found that there are different responses to acupuncture in normal and hyperalgesic animal models. The frequency-dependent phenomenon might not appear in hyperalgesic animals. Serotonin and the related descending inhibitory pathway may regulate hyperalgesic status through enkephalin-interneurons on spinal cord level. With endogenous opiates, serotonin completes the whole pathway. The hypothalamus is the common center for ANS and the hormonal system. Acupuncture might act through it to influence these two systems. The HPA axis adjusts the immune system by secreting hormones, such as endogenous opiates and glucocorticoids. The ANS offers a new concept of inflammatory reflex. In this reflex, acupuncture can harmonize ANS and suppress inflammation.

The analgesic effect of acupuncture is hypothesized to be through immune, hormonal and nervous systems. Within these systems, several pathways have been postulated, including the HPA axis, the ANS and the descending inhibitory pathway (hypothalamus-PAG-raphe nucleus- spinal cord).

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