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Involvement of endogenous opioid peptides in acupuncture analgesia

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Introduction

Acupuncture, an important branch of Chinese traditional medicine, has been practiced for more than 2000 years and has been proved to be an effective weapon for combating pain. In ancient China, it was used not only in humans but also in domestic animals like horses and oxen. Acupuncture therapy traditionally refers to the procedure of inserting fine needles into particular points on the body and stimulating them by hand maneuver to produce a phenomenon called 'Teh-ch'i.' 'Teh-ch'i' involves two aspects: one is that the subject being needled gains a feeling of soreness, heaviness, swelling or numbness either localized in the acupuncture point or moving diffusely in certain directions and the other is that the acupuncturist has a feeling that something is pulling the needle like 'a fish on the hook.' In recent years, electroacupuncture (EA), utilizing electric current to stimulate acupuncture points via inserted needles, was developed. The stimulation parameter adopted is of low frequency and low intensity (in volts or milliamperes) to elicit slight, localized muscle twitches. EA is now widely used in China, especially in scientific studies, since it is easier to control.

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Successful application of acupuncture to certain cases of surgical analgesia in the late 1950s appealed to the people in medical circles. Though acupuncture cannot be expected to produce total abolition of pain perception and the best it can do is to lessen the sharpness of pain to a degree allowing certain operations, it does produce impressive results in various aches and pains. As a model of physiological methods to relieve pain, it has caused much research into its mechanism. A growing body of literature in the past 20 years has revealed that the essence of acupuncture analgesia (AA) is mainly the activation of the endogenous antinociceptive system to modulate pain transmission and pain response, resulting in the diminution of pain perception and aversive reactions. The neural process involves the integration of different transmitter and modulator systems at various levels of the central nervous system. The data accumulated cause the author to consider that the establishment of the participation of the endogenous opioid peptidergic system (EOPS) is the most important advance in the study of AA within the last decade. The present article gives a brief review in this respect and discusses the possible central circuit through which the endogenous opioid peptides (EOP) mediate AA. There are controversial viewpoints on the opioid mechanism. However, those different viewpoints are beyond the scope of this article to review and the reader may refer to Chapman et al. [11–13].

Evidence indicating the participation of EOP in AA

The discovery of EOP [56] in the brain has attracted intense interest in acupuncture research. Investigations on the mechanism of AA have concentrated on the involvement of an EOP mediated antinociceptive system that underlies the pain relieving effect of acupuncture. Substantial evidence has come from 3 main series of experiments: the use of narcotic antagonists as probes, the evaluation of EOP levels and the protection of EOP from degradation.

Antagonism by naloxone

Reversal by narcotic antagonists is a necessary condition to characterize an analgesic manipulation as narcotic. Mayer et al. [70,71] first reported that AA in humans was reversible by naloxone, a specific opiate antagonist. In their study, pain threshold estimated by electrical stimulation of the tooth was increased significantly by acupuncture of the Ho-Ku points in both hands with intermittent manual rotation. This increase was reversed by naloxone 5 min after injection. Jiang et al. [57] tested the effect of naloxone on AA evaluated by sensory decision theory and found that naloxone also blocked the acupuncture produced increase in pain threshold estimated by radiant heat and acupuncture produced inhibition on the finger vascular response to pain. In their study, acupuncture was performed first by a traditional Chinese acupuncturist to induce 'Teh-ch'i,' which was then maintained by a mechanical manipulator. A noxious stimulus was applied to the forearm.

Parallel results have been seen in animal experiments. Huang et al. [55] established a monkey model in which EA significantly prolonged the latent period of the

operant lever-pressing response to a noxious stimulus. They found that naloxone was more effective in blocking inhibition produced by stimulating distant segmental points with a frequency of 2 Hz than that produced by stimulating the same segmental points with 80 Hz. Ha et al. [38] reported an antagonism of the antinociceptive effect of acupuncture of Ho-Ku by naloxone in monkeys, the pain threshold being evaluated by the jaw-opening response to tooth pulp stimulation. Naloxone antagonism was demonstrated also in other animal models such as the rabbit [116] and the mouse [34,86]. In these studies different noxious stimuli were used and behaviorally defined nociceptive thresholds were estimated.

Experiments using neuronal responses for evaluation showed similar results. Pomeranz and Cheng [84] demonstrated that EA selectively depressed the nociceptive responses of single cells in lamina V of the cat spinal cord, and the depression was also naloxone reversible. He [45] demonstrated that the noxious neuronal response from nucleus lateralis anterior of the rabbit thalamus could be inhibited by iontophoretic etorphine and EA, and the inhibition was readily reversed by iontophoresis of naloxone.

The above data obtained from both humans and animals suggest that (electro-) acupuncture may activate certain processes to release EOP onto binding sites and a consequent interaction between them results in analgesia.

In contrast to the above suggestion, controversial results were also obtained. For example, Chapman et al. [13] were unable to demonstrate the reversal of inhibition from EA following the injection of naloxone using tooth pulp evoked potential methodology. The acupuncture points stimulated were located either in the face of the same neurologic segment as the test tooth or Ho-Ku in the hands. Their further investigation of pain threshold evaluated by dental pulp stimulation revealed similar results [12]. In rabbit experiments, McLennan et al. [72] also failed to show naloxone reversal of the inhibition of an aversive response by EA. To comment on the discrepancies between different laboratories is somewhat a subjective assertion. However, some experiments might be worth noting. Cheng and Pomeranz [18] found that EA at both 200 and 4 Hz was effective in depressing mouse squeak responses to noxious heat, but naloxone reversed only the effect induced by low frequency stimulation. Han et al. [41] reported that naloxone blockade of EA depends on the stimulation frequency being used. The ID_{50} of naloxone has been shown to be 0.5, 1 and 24 mg/kg for 2, 15 and 100 Hz EA analgesia, respectively, suggesting that high frequency stimulation may release some endogenous opioids relatively resistant to naloxone. They also demonstrated that low frequency (2–4 Hz) EA analgesia could be blocked by intrathecal injection of antiserum against met-enkephalin, but not by antiserum against dynorphin, whereas high frequency (100 Hz) EA analgesia could be blocked by dynorphin antiserum, but not by met-enkephalin antiserum. Zhao et al. [121] demonstrated in rabbits that under the same stimulation frequency of 2–3 Hz, low intensity EA, which kept the animal in a quiet state, produced naloxone reversible inhibition of the nociceptive withdrawal response while high intensity EA agitated the animal and produced inhibition insensitive to naloxone. In the latter condition, there was a significant elevation of plasma cortisol and cAMP levels bearing some similarities to the state of stress.

Therefore, to evaluate the significance of the naloxone test, experimental conditions like the location of points, stimulation parameters and naloxone dosage are important factors to be taken into consideration.

Release of EOP

Measurement of the EOP levels in the cerebrospinal fluid (CSF) is a potential approach to evaluate their release from the central nervous system (CNS). In clinical observations, Clement-Jones et al. [21] found that low frequency EA effectively alleviated recurrent pain and significantly increased the lumbar CSF β-endorphin level while leaving the met-enkephalin level unchanged. Chen and Pan [14] found that low frequency EA increased the content of β-endorphin-like immunoreactive substances in ventricular CSF in patients with brain tumors. There was a linear correlation between the percentage increase of β-endorphin-like immunoreactive substances and of pain threshold and the percentage increase of β-endorphin-like substances and of pain tolerance.

In a rabbit model, where potassium iontophoresis was used to give a noxious stimulus to the ear skin and the iontophoretic current necessary to elicit a withdrawal response was recorded as the pain threshold, Tsou et al. [102] estimated the cisternal CSF enkephalin contents before and after acupuncture. Their experiments were divided into 3 groups, i.e., control, intraventricular bacitracin (50 μg) and intraventricular bacitracin (50 μg) plus acupuncture. Bacitracin is an amino peptidase inhibitor which can be used to prevent the enzymatic degradation of enkephalins [74], thus enhancing the analgesic effect of met-enkephalin when intraventricularly administered [77,127]. The results were that bacitracin injection alone had no prominent effect, but bacitracin plus acupuncture markedly increased the pain threshold and CSF leu- and met-enkephalin. In addition, Huang et al. [54] found that EA increased the leu-enkephalin content of monkey CSF, accompanying the inhibition of the operant lever-pressing response to noxious stimulus.

Some studies on plasma EOP demonstrated that β-endorphin was released into the peripheral blood in humans [67,81] and horses [7] during EA. Xu et al. [112] found that acupuncture significantly decreased the sensory discrimination and increased the verbal report criteria with a correlation between the efficacy of AA and plasma content of opioid peptides. However, the peripheral EOP are unlikely to be analgesically active [cf., 98] and their plasma change does not seem related to the attenuation of pain during acupuncture either.

Protection of EOP from degradation

Tsou et al. [103] reported that acupunctural inhibition of rabbits' withdrawal responses to noxious stimuli was greatly prolonged when bacitracin was injected intraventricularly. Naloxone effectively antagonized acupunctural inhibition during a prolonged period, indicating that the prolongation was possibly due to the protection of released EOP by bacitracin. Estimation of enkephalins in various brain areas revealed that enkephalin contents in the striatum and hypothalamus were much higher in the bacitracin plus acupuncture group than in the saline plus acupuncture and bacitracin control groups. Since they found that after bacitracin

treatment EA increased the release of enkephalins into the CSF, as mentioned above, the data may suggest an increase of enkephalin biosynthesis. Further evidence was given by Wu et al., who studied the effect of cycloheximide on AA in rats [110]. Cycloheximide, a protein synthesis inhibitor, has been reported to be able to interfere with the incorporation of [^{3}H]tyrosine into enkephalin in vitro. Intraventricular injection of cycloheximide produced no conspicuous effect on pain threshold but greatly attenuated EA analgesia in rats as evaluated by the tail flick test. Cycloheximide decreased met-enkephalin content by 47% in the hypothalamus and by 20% in the striatum. EA increased met-enkephalin by 113% and by 222%, respectively, in these 2 brain areas. Following cycloheximide, EA increased met-enkephalin by only 28% and 123%, respectively. These results suggest that EA activates the biosynthesis of enkephalins and the latter may play an active part in the process of analgesia. Cheng and Pomeranz [19], measuring the latency of the nociceptive squeak response, observed that EA and D-amino acids together produced an additive analgesic effect. D-Amino acids are known as peptidase inhibitors. They found that 62% of the mice tested showed EA analgesia and 53% showed D-amino acid analgesia. However, 80% of the animals treated with EA plus D-amino acids showed marked analgesia. The combined effect was blocked by naloxone. Takeshige et al. [97] investigated the similarities among the effects of EA, 0.5 mg/kg morphine and periaqueductal gray (PAG) stimulation on the tail flick response in rats. Individual variations in the effectiveness of these 3 kinds of treatment were parallel and could be reduced by the administration of D-phenylalanine, suggesting a common basic mechanism underlying them. Inconsistently with these observations, Liang et al. [59] measured brain opiate-like substances by radioreceptor assay and found that EA increased their contents and that the increase was positively correlated with EA effectiveness estimated by the inhibition of the tail flick reflex.

Massayoshi et al. [69] showed that D-phenylalanine markedly prolonged the pain threshold raising effect of acupuncture in healthy subjects, improved the therapeutic effect on low back pain and increased the rate of AA in dental surgery.

EOPS mediating the central mechanism of AA

Studies done in the last few years indicate that there are a number of CNS sites rich in opiate receptors and opioid peptides participating in AA. The data from physiological, biochemical and pharmacological experiments will be briefly reviewed here to give a comprehensive description.

Spinal cord

Immunohistological studies on the distribution of EOP in the spinal cord have shown that the dorsal horn is rich in enkephalins, especially laminae I and II where numerous enkephalin-positive terminals and perikarya are localized [22,36]. Laminae I and II are also densely invested with opiate receptors [1]. The distribution of enkephalins and opiate receptors corresponds well with the region where neurons

respond maximally to noxious stimulation [23] and contribute to ascending tracts associated with pain transmission [99,107]. The enkephalin system of the spinal cord is considered to be a part of the endogenous antinociceptive system relevant to opiate analgesia and brain stimulation analgesia [2], thus raising the possibility of its participation in AA. Han et al. [42] injected intrathecally the IgG fraction from antisera against enkephalins prior to EA in rabbits and found that the EA effect was apparently seen on the head while that on the tail region was markedly attenuated. Basbaum et al. [5] reported that dynorphin was also present in the spinal cord, although much more limited in lamina I. Han and Xie [40] observed that intrathecal injection of dynorphin in rabbits elicited a very potent naloxone reversible inhibition of the tail flick response and intrathecal administration of anti-dynorphin antibody markedly attenuated EA analgesia at the tail, leaving the analgesic effect in the head region intact. Rabbits tolerant to EA by long term EA stimulation no longer exhibited inhibition of nociceptive response following dynorphin administration. The possible role of the spinal enkephalin and dynorphin in AA is thus suggested.

Lower brain stem

Nucleus raphe magnus (NRM). The NRM of the ventromedial medulla and its descending inhibitory pathway have been proved to constitute a fundamental component in the central circuit responsible for the expression of AA. The inhibitory effect of EA on nociceptive response was abolished almost completely after the transection of the spinal cord [26,85]. To evaluate the involvement of descending inhibition in AA, Shen et al. [92] observed the inhibitory effect of EA on the splanchnic evoked potential recorded from the orbital cortex in cats. The experiments showed that section of the dorsolateral funiculi (DLF) at T_{3-4} markedly attenuated the depression of the evoked potentials by stimulating points on either the forelimb or the hind limb. Chang et al. [10] reported similar results in rabbits. They found that section of the dorsal half of the spinal cord diminished the inhibitory effect of EA on the nociceptive response of the parafascicular nucleus. The DLF contains descending fibers from the NRM and adjacent reticular formation [3]. It was found that a median lesion in the medulla including the NRM resulted in a significant diminution of EA produced inhibition of viscero-somatic reflexes [26], whereas stimulation of the NRM potentiated the inhibition [27]. Liu et al. [65] recorded from the NRM the activity of raphe-spinal neurons, the axons of which contribute to the DLF. They found that some raphe-spinal neurons were excited by noxious stimulation and EA could activate their spontaneous activity but inhibit their nociceptive responses. After the transection of the DLF, the raphe-spinal neurons could still be activated by EA, but the post-inhibitory effect of EA on their nociceptive responses was markedly reduced. The results suggest that EA can activate the NRM, a supraspinal area mediating a negative feedback circuit modulating pain, thus inducing analgesia via descending inhibition.

The NRM region, although not rich in opiate receptors [1], contains numerous enkephalin perikarya and relatively few enkephalin containing fibers [22]. The NRM enkephalin system is suggested as a part of the central antinociceptive system

[2]. The relevance of the same NRM substrate for AA is also suggested by some findings, e.g., intravenous administration of naloxone blocked the activating effect of EA on NRM neurons [120] and microinjection of naloxone into the NRM blocked the inhibitory effect of EA on visceral pain [66].

There is evidence that the serotoninergic descending pathway in the DLF transmits the inhibitory influence from the NRM to the spinal cord and mediates AA. Du et al. [28] injected 5,6-dihydroxytryptamine into the cat ventricle or medullary raphe nucleus and found that, accompanying the depletion of 5-HT in the spinal cord, the inhibitory effect of EA on nociceptive viscero-somatic reflexes was reduced significantly. The 5-HT axons descending via the DLF project onto the dorsal horn where the distributions of enkephalins and opiate receptors overlap and the nociceptive projecting neurons are localized [30]. Thus the modulation of pain transmission may result from the functional interaction between the EOPS and the 5-HT system. The modulation may also result from the direct impingement of the 5-HT terminals on nociceptive neurons, as suggested by the observations of Hoffert et al. [51].

The above mentioned results indicate that ascending pain impulses can be blocked at the spinal level by acupuncture induced descending impulses from the NRM and that the EOPS is probably a component link in the spinal cord–medulla–spinal cord circuit.

Periaqueductal gray (PAG). The landmark microinjection studies by Tsou and Jang [101] stimulated a series of work leading to the finding of the PAG as a region sensitive to local application of the opiates [114]. In line with this finding, the PAG has been proved to contain a relatively high concentration of opiate receptors [1,82]. There are enkephalin cells and terminals concentrated ventrolaterally in the caudal PAG and shifting dorsally in the rostral part. Beta-endorphin terminals originating from the hypothalamus and dynorphin cells and terminals are also present [cf., 4]. It has been established that the PAG is a critical site for morphine analgesia and brain stimulation produced analgesia, and at least part of its pain modulating function is mediated by the ventromedial medulla [30]. It has also been established that acupuncture may utilize the same substrates.

It was found that stimulation of the PAG greatly potentiated the inhibitory effect of EA on tail flick and squeak responses [52] and a lesion of it greatly attenuated the effect [96]. Sun et al. [95] demonstrated that microinjection of naloxone into the rabbit's PAG partially reversed both EA analgesia and morphine analgesia. The dose of naloxone was only 1/10 of the intraventricular effective dose and 1/400 of the intravenous effective dose. Han et al. [42], taking advantage of the high specificity of antibody–antigen interactions, injected anti-β-endorphin IgG or anti-enkephalin IgG into the rabbit's PAG and found that either pretreatment could partially block the inhibitory effect of EA on nociceptive responses. However, anti-dynorphin antibody injection produced no significant effect [40]. The data imply that acupuncture activates the brain to release enkephalins and β-endorphin onto their binding sites. In fact, the release of EOP by acupuncture was demonstrated by Zhang et al. [116]. They used push–pull perfusion and radioreceptor assay to observe changes of EOP levels in perfusates of the rabbit's PAG during EA

and found that EA increased EOP content and the increase was related to the efficacy of EA produced inhibition on nociceptive responses. These findings suggest the participation of the EOPS of the PAG in AA from the presynaptic level to the receptor sites.

There is general agreement that the PAG exerts its analgesic action through the ventromedial medulla, of which the major afferent connections originate in the PAG [68]. On the other hand, some experiments have shown that EA activates the NRM partly through the PAG. Liu et al. [64] compared the effects of EA and PAG stimulation on neuronal activity in the rat's NRM. Most neurons tested were responsive to both of the stimulations, their spontaneous activity being augmented and nociceptive responses inhibited. Bilateral destruction of the PAG weakened the effect of EA. Further work of Liu and Zhang [63] showed that local application of naloxone into the PAG could block the modulating effect of EA on NRM neurons. Therefore, the PAG–NRM may act as a unit to exert a descending inhibition on pain transmission in the process of AA.

In addition to the descending inhibitory pathway, an ascending inhibitory pathway has also been suggested. Chang et al. [10] demonstrated that the inhibitory effect produced by stimulation of the midbrain raphe nuclei, including the nucleus raphe dorsalis (NDR), on the nociceptive response of some parafascicular neurons persisted after sectioning the dorsal half of the spinal cord. Qiao et al. [88] compared the extent of inhibition of nociceptive responses from the parafascicular nucleus elicited by PAG stimulation before and after a lesion of the dorsal half of the spinal cord and found that the lesion could only reduce but not abolish the inhibition. Both studies indicate the existence of ascending inhibition. Since the NDR receives afferents from the PAG [90] and sends efferent fibers to the parafascicular nucleus [80], it may serve as a route for an ascending inhibition from the PAG.

In general, the actions of exogenous opiates and enkephalins applied on their target neurons are inhibitory [8]. It is interesting to note that EA inhibits most of the opiate sensitive neurons in the PAG and that iontophoretic naloxone can readily block the inhibition [17,46]. The comparison between the responses induced by iontophoretic etorphine, an opiate agonist, and EA revealed a significant correlation. The participation of the EOPS in EA produced inhibition on PAG neurons is thus suggested [cf., 46]. Nicoll et al. [79] have proposed that an important action of enkephalins is the inhibition of inhibitory interneurons. It is relevant to postulate that acupuncture activates the PAG to release EOP onto their binding sites, resulting in an inhibition of inhibitory interneurons and a subsequent disinhibition of the output neurons to the NRM and NDR. The descending and ascending controls over pain transmission are thus accomplished.

Diencephalon

Arcuate nucleus. In the arcuate nucleus are clustered the cell bodies of the β-endorphin-containing neurons [6], the axons of which distribute in a number of structures related to AA such as the lateral septal nucleus, nucleus accumbens, PAG and locus ceruleus. Quo et al. [89] found that an electrolytic lesion or surgical

isolation of the arcuate nucleus weakened lip acupuncture analgesia. Zhu et al. [126] injected monosodium glutamate into the arcuate nucleus to destroy this region in neonatal rats and obtained similar results. This antinociceptive action of the arcuate nucleus is probably mediated via its functional connection with the PAG–NRM system. Gao and Ku [35] demonstrated that arcuate nucleus stimulation activated neuronal activity in the NRM and the excitatory effect of arcuate nucleus stimulation was greatly reduced by sectioning the β-endorphinergic tract from the arcuate nucleus to the PAG or by microinjection of naloxone/anti-β-endorphin serum into the PAG. Their further work demonstrated that surgical isolation of the arcuate nucleus, sectioning of the β-endorphinergic tract or microinjection of naloxone/anti-β-endorphin serum into the PAG could almost abolish the effect of EA. The results indicate the mediation of the connection between the arcuate β-endorphinergic system and the PAG–NRM in the acupuncture effect.

The arcuate nucleus might participate in AA partly via its connection with the locus ceruleus. Strahlendorf et al. [94] reported that stimulation of the cat's arcuate nucleus could inhibit neuronal activity in the locus ceruleus. The locus ceruleus has been suggested to be antagonistic to AA. Liu et al. [61] reported that stimulation of the locus ceruleus attenuated the inhibitory effect of EA on nociceptive neuronal responses in the spinal trigeminal nucleus in the cat, and Zhang et al. [119] demonstrated that a lesion of the ascending dorsal noradrenergic bundle potentiated EA produced inhibition on nociceptive squeaking in the rat. The locus ceruleus projects widely in the vast areas of the neuraxis [80], and its pathway related to AA has to be worked out.

Preoptic area. It has been reported that stimulation of the preoptic area is able to raise the pain threshold [91]. Opioid peptides and opiate receptors are distributed in the preoptic area with a comparatively high concentration [93]. Microinjection of morphine [83] or β-endorphin [100] into this area produced a significant analgesic effect. The participation of the preoptic area in AA is suggested by some studies. Wu et al. [108] found that local application of naloxone into the preoptic area could partially reverse EA produced inhibition of the rabbit's withdrawal response Their further work [109] showed that most neurons recorded from the preoptic area were responsive to EA and most neurons inhibited by iontophoretic etorphine were inhibited by EA as well. The inhibition of the neuronal discharge induced by EA could be reversed by iontophoresis of naloxone, suggesting that the effect of acupuncture is, at least partly, via the EOP in the preoptic area.

A functional connection between the preoptic area and the PAG via the EOPS is suggested by the finding that stimulation of the preoptic area inhibited the majority of the PAG neurons sensitive to etorphine and the inhibition was reversed by iontophoresis of naloxone [9].

Forebrain

Caudate nucleus (CN). Substantial evidence has been provided indicating the involvement of the CN in pain modulation, e.g., stimulation of the CN is able to raise the pain threshold [91] and to suppress pain responses [60] in monkeys. Stimulation of the CN also produces satisfactory pain relief in patients suffering

from intractable pain [16,29]. Recent research has revealed that the CN participates in AA. It was found in rabbits that a caudate nucleus lesion attenuated EA produced inhibition of nociceptive withdrawal responses while caudate nucleus stimulation enhanced the inhibition [48]. The caudate nucleus contains abundant opioid peptides and opiate receptors [93]. The possible role of an intracaudate opioid peptidergic system in AA was suggested by the finding that microinjection of naloxone into the CN temporarily blocked the inhibition of the nociceptive response by EA [48]. Zou et al. [128] estimated in rats and rabbits the contents of enkephalins in different brain areas and found that acupuncture increased enkephalins in the striatum and hypothalamus. Xie et al. [111] showed a positive correlation between the caudate content of met-enkephalin and the efficacy of EA evaluated by the tail flick reflex in rats. Lu and Pan [cf., 47] observed the release of EOP from the CN and found that EA increased EOP release from the anterior part of the head of the CN along with the increase in pain threshold, but no increased release was obtained from the posterior part. The result is consistent with Zhang et al.'s observation [118] that different portions of the head of the CN are functionally differentiated, and only the anterior part is related to antinociception. He et al. [47] noticed that there were caudate neurons responsive to both microiontophoresis of etorphine and EA, and the response to etorphine and EA could be reversed by iontophoretic naloxone, a phenomenon similar to that seen in the PAG and the preoptic area. These results suggest that the intracaudate opioid peptidergic system is activated by acupuncture.

In addition, several findings have revealed that cholinergic and dopaminergic systems also participate in AA [cf., 48]. The interrelationships among opioid peptides, acetylcholine and dopamine are to be clarified.

The antinociceptive function of the CN is found to be related to the PAG–NRM system. During caudate nucleus stimulation, accompanying the elevation of the pain threshold, more opioid peptides are released from the PAG, as shown by the increase of EOP in the perfusate from this brain area; microinjection of naloxone into the PAG reversed the analgesic effect of caudate stimulation temporarily [46]. Further investigation showed that some PAG neurons could be inhibited by both iontophoretic etorphine and caudate nucleus stimulation. Stimulation of the anterior part of the CN head was more effective than stimulation of the posterior part and the effect of caudate nucleus stimulation could be reversed by iontophoretic naloxone [25]. These results indicate that the opioid peptidergic system of the PAG is an important link in the production of caudate stimulation produced analgesia. On the other hand, caudate stimulation was found to activate spontaneous activity in the NRM and to depress the nociceptive responses in it, and these effects could be blocked in some of the NRM neurons by microinjection of naloxone into the PAG. That the PAG is an important link between the CN and the NRM is thus suggested [123].

It has been reported that the CN exerts an inhibitory effect on the nociceptive neuronal responses of the lateral habenular nucleus [33]. The habenular nucleus, a station for the connection of the limbic forebrain with the brain stem [49,50], is probably a structure antagonistic to AA. Some studies revealed that stimulation of the lateral habenular nucleus increased the spontaneous firing rate in the locus

ceruleus [106] but decreased that of the NRM [105] and NDR [104]. The inhibition of the lateral habenular nucleus might be one route through which the CN participates in AA.

Limbic nuclei. (1) Septal area. Clinical observation has shown that stimulation of the septal area produces analgesia for cancer pain [37]. Animal experiments have implicated this area in EA produced inhibition on the galvanic skin reflex elicited by a noxious stimulus [76]. This area is rich in opioid peptides and opiate receptors [31,93]. Naloxone microinjected into this area can partially block the inhibition of the nociceptive withdrawal response by morphine as well as EA [75]. The septal area is an important link in the limbic midbrain circuit. There are connections between the septal area and the PAG [78] and between the septal area and the habenular nucleus [49]. The antinociceptive effect of the septal area is possibly brought forth by the activation of the opioid peptidergic system in the PAG, since naloxone microinjected into the latter blocks the inhibition from septal stimulation [53]. In addition, the septal area may exert an antinociceptive function by its inhibitory effect on the lateral habenular nucleus as suggested by the findings that stimulation of it inhibited both spontaneous discharges and nociceptive responses of neurons in the lateral habenular nucleus [32].

(2) Nucleus accumbens. Recent studies have revealed that the nucleus accumbens has an antinociceptive function and participates in AA. It is an area rich in both opioid peptides and opiate receptors [93]. Zhang et al. [117] found that bilateral lesions of the nucleus accumbens reduced EA produced inhibition on tail flick and squeak responses in rats, and Zhou et al. [122] found that naloxone microinjected into this nucleus attenuated EA produced inhibition of the nociceptive withdrawal response in rabbits. Liu and Zhang [62] demonstrated that naloxone microinjected into the nucleus accumbens partially blocked the excitatory effect of EA on NRM neurons. Furthermore, they demonstrated that stimulation of the nucleus accumbens produced an excitatory effect on most of the NRM neurons tested, and this effect was partially blocked by microinjection of naloxone into the PAG. The results imply that the nucleus accumbens may exert a descending inhibition via the PAG–NRM circuit in the process of EA, the EOPS in situ and of the PAG playing an important role. The nucleus accumbens projects to the lateral habenular nucleus [87] and exerts an inhibitory effect on the latter as revealed by experiments in which stimulation of the nucleus accumbens inhibited most of the neurons recorded from the lateral habenular nucleus [58]. This is probably another mechanism through which the nucleus accumbens mediates AA. Recently, Han et al. [43,44] put forth the concept of a mesolimbic loop for the expression of analgesia. They postulated that the nucleus accumbens and the PAG are reciprocally innervated and the descending pathway from the nucleus accumbens may take a relay in the habenula. Exogenously administered or endogenously released opioids (e.g., during EA) may act on the EOPS of the nucleus accumbens and the PAG to push the loop into action and from the loop efferents are sent to other neuronal structures implicated in pain modulation [44].

(3) Nucleus amygdala. The nucleus amygdala is one of the forebrain structures containing the highest density of opiate receptors [1] and the highest concentration

of opioid peptides [93]. Its possible involvement in AA is suggested by the findings that a lesion of this nucleus [115] or microinjection of naloxone into it [122] results in the attenuation of EA produced inhibition on the rabbit's nociceptive withdrawal response.

Conclusion and prospects

An outline has been given for the mediation by EOPS of transmission in the central antinociceptive system involved in AA. In this system, the NRM and the spinal cord constitute a fundamental circuit, while the PAG occupies a strategic position to funnel all the influences from high structures and to collect information from the spinal cord. After processing various inputs, the PAG initiates descending and ascending inhibition, resulting in the reduction of pain. In the whole process,

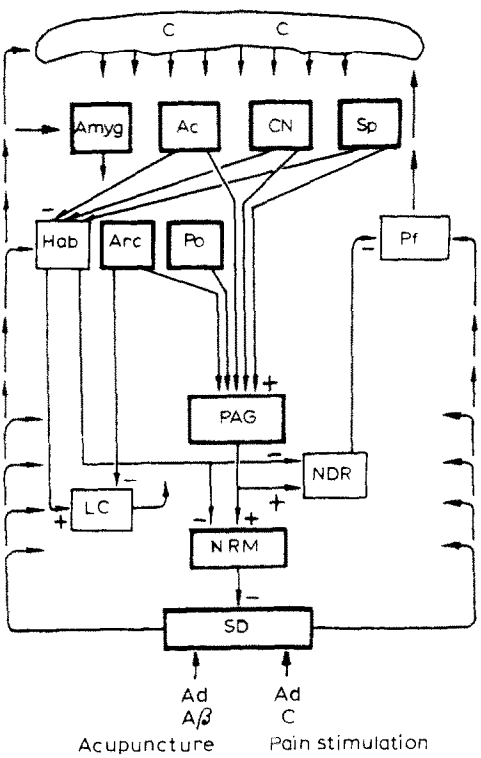

Fig. 1. Possible central circuits through which the endogenous opioid peptidergic system mediates acupuncture analgesia. Abbreviations: Ac = n. accumbens; Amyg = n. amyygdala; Arc = arcuate n.; CC = cerebral cortex; CN = caudate n.; Hab = habenular n.; LC = locus ceruleus; NDR = n. raphe dorsalis; NRM = n. raphe magnus; PAG = periaqueductal gray; Pf = parafascicular n.; Po = preoptic area; SD = dorsal horn of spinal cord; Sp = septal area.

the EOPS plays an important role at different levels of the CNS. The main activity of EOP might be the inhibition of inhibitory interneurons, thus bringing the output neurons into action. The possible central circuits through which the EOPS mediates AA are summarized in the schematic diagram (Fig. 1).

Evidence has been given to indicate that acupuncture signals are conveyed via the spinal ventrolateral funiculus where pathways for pain sensation are located. Clinical observations have revealed that in patients suffering from syringomyelia (in which the lesion involves the anterior commissure of the spinal cord and causes segmental deficits of pain and temperature), acupuncture sensation is either not obtainable or very weak when the affected area is needled. However, patients with a deficit of deep sensation or diseases of spinal motor neurons, such as sequelae of infantile paralysis and amyotrophic lateral sclerosis, could still experience acupuncture sensation while the affected area was being needled [15,24]. In rabbits, Chiang et al. [20] demonstrated that unilateral section of the ventral two-thirds of the spinal lateral funiculus at the level of T12–L1 almost completely abolished acupuncture inhibition of nociceptive withdrawal responses produced by stimulation of the Tzu-San-Li point in the contralateral hind limb, leaving the inhibition from the ipsilateral point unaffected. Animals with dorsal column section either unilaterally or bilaterally manifested pronounced acupuncture inhibition as usual. The results in animals support the observations in patients. It seems understandable that acupuncture signals take the ventrolateral funiculus as a route to the brain since acupuncture sensation is a kind of protopathic sensation also having characteristics of pain sensation such as the unpleasant feeling of soreness. Axons of the extralemniscal system of the ventrolateral funiculus on their way forward project to some brain areas related to algesia and analgesia [73]. These areas are particularly rich in opioid peptides and opiate receptors, the PAG being an example. There is evidence to show that pain is a physiological factor which activates the EOPS [113]. It is probable that acupuncture elicits a kind of sensory input similar to pain. Its significance is to enhance the antinociceptive processes of the brain to combat pain. This postulation might be a new aspect leaving much room for exploration.

AA is a complicated process resulting from the integrated activity of EOP, biogenic amines and other transmitters as demonstrated by some studies. For example, Han et al. [39] found in rats that after parachlorophenylalanine and 5,6-dihydroxytryptamine treatment, a reduction of brain 5-HT was correlated with an increased activity of brain morphine like substances (MLS) as measured by spectrofluorometry and radioreceptor assay, respectively, indicating possibly a compensation mechanism. In animals with low brain 5-HT, EA produced analgesia only when it increased MLS activity. The efficacy of EA was related to both 5-HT content and MLS activity as shown statistically by multiple linear regression. Zhu et al. [124] found that EA depressed the rabbit's nociceptive withdrawal response and decreased the contents of noradrenaline (NA) from perfusates of the preoptic area as estimated by radioenzymatic assay while microinjection of the β-antagonist phentolamine into the same brain area enhanced the depressive effect of EA, suggesting the antagonistic action of NA against EA. They further demonstrated that naloxone perfusion of the preoptic area did not influence nociceptive withdrawal

responses and the local release of NA but reversed the EA effect on both of them. It seems that acupuncture activates the activity of the EOP which inhibit the release of NA and that this is a mechanism underlying AA. Compared to the knowledge of individual functions of the central transmitters mediating AA, the integrated activity of them is rarely understood. More attention is being paid to this aspect at present, and it will certainly lead to a deeper insight into the mechanism of AA.

In conclusion, one important point should be mentioned: experimental studies either in man or in animals are liable to deviate from clinical conditions. Therefore, experiments must be carefully designed, especially in the method of acupuncture, e.g., point location, acupuncture manipulation and EA parameters. The method of EA varies in different laboratories and no criteria for evaluation have been established yet. How to unify the somatic stimulation in a way compatible with clinical acupuncture therapy remains a difficult problem. Nevertheless, the increasing exchange of scientific information among different laboratories will undoubtedly help us to obtain a solution, which is bound to promote more research and to better our understanding of AA. Taking acupuncture as a physiological measure to induce analgesia by eliciting sensory inputs, the research will definitely provide valuable clues about pain modulation.

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Appendix

Reference	Acupuncture manipulation		Evaluation of analgesia	Naloxone
	Point	Stimulation	Noxious stimulation and response	
Man				
13	(SI-18), (LI-20)	EA: 2 Hz, 11–18 mA, Chinese model 626 acupuncture stimulator	Electrical stimulation (tooth), evoked potential	0.4 mg i.v.
12	Ho-Ku (LI-4)	EA: 2 Hz, 3–4 V, Sanyo Denshi SD207 stimulator	Electrical stimulation (tooth), pain threshold	1.2 mg i.v.
21	San-Yan-Lo (TB-8), Szu-Tu (TB-9) Ho-Ku	EA: 3 Hz, 1–2 V, biopulse generator	Recurrent pain, pain relief	
57	Ho-Ku	Manual	Radiant heat or electrical stimulation (forearm), pain threshold, by sensory decision theory	0.8 mg i.v.
70, 71	Ho-Ku	Manual	Electrical stimulation (tooth), pain threshold	0.8 mg i.v.
112	San-Yan-Lo	Manual	Potassium iontophoresis (thoracic wall), sensory decision theory	
Monkey				
38	Ho-Ku	Manual	Electrical stimulation (tooth), jaw opening reflex	0.08 mg/kg i.v.
55	Ho-Ku Pi-Nao (LI-14)	EA: 2 or 80 Hz, 0.36 msec, 0.5–4 mA	Potassium iontophoresis (forearm), operant lever pressing response	0.05–0.1 mg/kg i.v.
Dog				
66	Hsin-Shu (Bl-15), Pi-Shu (Bl-20),	EA: 20 Hz, 3–4 mA, 57-6 North Naviaga-	Mechanical traction (stomach), visceral traction response	5 μg i.c.

Appendix (continued)

Reference	Acupuncture manipulation		Evaluation of analgesia	Naloxone
	Point	Stimulation	Noxious stimulation and response	
	Tsu-San-Li (St-36), Nei-Kuan (EH-6)	tion therapeutic stimulator (57-6 stimulator)		
Cat				
84, 85	Fu-Tu (St-32), Yang-Ling? (Gl-34)	EA: 4 Hz, 0.1 msec, 2–4 V, 4–6 V	Mechanical stimulation, nociceptive neuronal response from dorsal horn of spinal cord	0.3 mg/kg i.v.
Rabbit				
42	Zusanli (Tsu-San-Li), Sanyinjiao (San-Yin-Chiao, Sp-6)	EA: 2–15 Hz, 0.3 msec, 1 V	Radiant heat (skin around nostrils and mouth, or tail), head jerk or tail flick response	
45	Ho-Ku Wei-Kuan (TB-5)	EA: 4–5 Hz, 0.5 msec, voltage eliciting slight twitching of toes	Electrical stimulation (sural nerve), nociceptive neuronal response of thalamus	33 nA, iontophoresis
48	Same as 45	Same as 45	Potassium iontophoresis (ear) withdrawal response (head and limbs)	3 μg i.c.
72	Zusanli, Shang ju xu (Sang-Chu-Hsu, St-37)	EA: 3 Hz, 1–2 mA	Radiant heat (nose), aversive response	0.15–0.2 mg/kg i.v.
75	Ho-Ku, Wei-Kuan	EA: 3 Hz, voltage eliciting slight twitching of toes, G6805 acupuncture therapeutic stimulator (G6805 stimulator)	Same as 47	2 μg i.c.

95	Same as 75	EA: 2–4 Hz, 7.5–8 mA, G6805 stimulator	Same as 48	2 μg i.c.
102, 103, 128	Tsu-San-Li	Manual	Same as 48, withdrawal response (limbs)	
122	Kuenlun (Kun-Lun, Bl-60)?	Manual (finger pressing)	Same as 42	2 μg i.c., 4 μg i.c.v.
124, 125	Ho-Ku, Wei-Kuan	2–4 Hz, voltage eliciting slight twitching of toes, G6805 stimulator	Same as 48	15 μM in artificial CSF, brain perfusion
Rat				
41	Zusanli, sanyinjiao	EA: 2, 15 or 100 Hz, 1–3 V	Radiant heat (tail), tail flick response	ID_{50} 0.5, 1 or 24 mg/kg i.p.
59	Same as 41	EA: 2–15 Hz alternated, 57-6 stimulator	Same as 41	
64	Zusanli	EA: 20–40 Hz, 40–50 V unloaded, 57-6 stimulator	Electrical stimulation (splanchnic nerve or tail), nociceptive neuronal response from NRM	
Mouse				
18	Ho-Ku	EA: 4 Hz, 0.1 msec, 5–8 μA, 200 Hz, 0.1 msec, 0.8–1 μA	Noxious heat (nose), squeak response	1 mg/kg i.p.
19	Ho-Ku	EA: 4 Hz, 0.1 msec, 8–12 V	Same as 18	10 mg/kg i.p.
34	Tsu-San-Li	Manual, EA: 2 Hz, 1 msec, 1.2–1.5 mA	Phenylquinone i.p., writhing response	2 mg/kg s.c.

Abbreviations: i.c. = intracerebral injection; i.c.v. = intracerebroventricular injection; i.p. = intraperitoneal injection; i.v. = intravenous injection; s.c. = subcutaneous injection.

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