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Activation of the Enkephalinergic System by Acupuncture

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Ever since the discovery of enkephalins by Hughes, Kosterlitz, et al. [1], several laboratories in China have been working on the involvement of endogenous opioid peptides in acupuncture analgesia, notably Han's group in Beijing [2] and Zhang's group [3] in Shanghai. My laboratory first studied 5-hydroxytryptamine (5-HT) involvement in acupuncture analgesia, and then attention was focussed on the role of enkephalins.

The role of 5-Hydroxytryptamine in Acupuncture Analgesia

The acupuncture analgesia project was started in 1973 with the role of 5-HT. Morphine was always used as an experimental control. Using rabbit as the experimental model, the release of 5-HT during acupuncture was studied [4]. Pain sensitivity was measured by potassium ionophoresis on the ear tips through two cotton wick electrodes, one on each ear. The current passing through the ears was increased steadily, and the current that caused a sudden struggle of the rabbit was recorded as the pain threshold. Plastic cannulas were implanted in the rabbits, one in the lateral ventricle and another in the aqueduct. The rabbits were suspended in cloth slings. The ventricular system was perfused with [³H]5-HT-containing artificial CSF, during which time [³H]5-HT was presumably taken up by the serotonergic nerve endings. The perfusion was then continued with [³H]5-HT-free CSF. Under control conditions, the radioactivity in the perfusates (collected at 10-min intervals) decreased in a time-dependent fashion. If the rabbits were manually acupunctured by twirling needles in the St.36 Zusanli points of both hind legs for 20 min, the radioactivity in the perfusates increased concomitantly with the elevation of the pain threshold. Iv injection of morphine (5 mg/kg), by contrast, produced a prominent and long-lasting elevation of the pain threshold without any increase of radioactivity in the perfusates. Therefore, there is a distinct difference between acupuncture and morphine analgesia with regard to 5-HT.

The effect of chemical lesioning of serotonergic neurons on acupuncture analgesia was also studied [5]. The 5-HT neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) was first injected into the lateral ventricle of the rat 1 week prior to assessment of the analgesic effect of electroacupuncture. The pain threshold was measured using the tail-flick test. Electroacupuncture was induced by application of high- and low-frequency impulses through needle electrodes inserted in the Gb.30 Huantiao points on both hind legs. This procedure caused a conspicuous

increase in the tail-flick latency in control rats. The analgesia of electroacupuncture was significantly attenuated in 5,6-DHT-treated rats. Histochemical examination of the raphe nucleus in these rats revealed degeneration of the cell bodies, swelling of axons, and loss of fluorescence in the nerve terminals.

The Role of Opioid Peptides in Acupuncture Analgesia

After the important discovery of enkephalins, I started working on the role of enkephalins in acupuncture analgesia. I was further encouraged by the work of Mayer et al. [4] that naloxone could partially antagonize acupuncture analgesia and the paper by Sjolund et al. [5] that electroacupuncture increased the level of endogenous opioids in the lumbar CSF in patients. Since either naloxone or radio-receptor binding assay was used in these two papers, it was not possible to identify which opioid peptide(s) was involved. Therefore, a radioimmunoassay was developed for Leu- and Met-enkephalin [8]. The immunogens were prepared by condensation of polylysyl succinic acid with the *N*-terminals of the two enkephalins. My Leu-enkephalin antiserum could detect 20 pg Leu-enkephalin, while the Met-enkephalin antiserum could detect 150 pg Met-enkephalin.

Alteration of Brain Regional Enkephalin Contents by Acupuncture

Using these antisera, the regional contents of Leu- and Met-enkephalins were measured before and after acupuncture [9]. In the rabbit experiments, after alternately twirling acupuncture needles in the bilateral St.36 Zusanli points for 20 min, the pain threshold usually increased more than 80% as measured by potassium ionophoresis on the rabbit ear. The rabbit was then decapitated and the brain dissected into the following regions: hypothalamus, striatum, hippocampus, thalamus, brain stem, and cortex. The enkephalin contents were measured by radioimmunoassay with these antisera. In the control group, enkephalin contents were high in the hypothalamus and the striatum. After acupuncture, there were prominent increases in these two regions of about 1.6–1.8-fold. No significant changes were found in other regions. In the rat experiments, the pain threshold as measured 5 s after 30 min of electroacupuncture increased 92%. Most prominent changes in enkephalin levels were again found in the hypothalamus and the striatum. Compared with the control group, Met-enkephalin increased 3.5 times, while Leu-enkephalin increased 1.4–2 times.

Immunohistochemical data obtained independently by Watson et al. [10] and Bloom et al. [11] showed that the β -endorphin neuronal system is located separately from the enkephalin neurons. The β -endorphin cells are located mainly in the basal hypothalamus. The Halasz knife was therefore used to isolate the basal hypothalamus from the rest of the brain. The effect of such an isolation on acupuncture analgesia and the Met-enkephalin content in the basal hypothalamus was studied. Thirty rats were divided into the following groups: (a) control, (b) hypothalamus isolation, (c) hypothalamus isolation plus electroacupuncture, (d) sham operation, and (e) sham operation plus electroacupuncture. Rats in group e

showed a prominent increase of the pain threshold. Rats in group c showed less effective analgesia. The Met-enkephalin content in the isolated hypothalamus was about the same in the hypothalamus of sham-operated rats, but electroacupuncture failed to increase the Met-enkephalin content in the isolated hypothalamus, indicating that the incoming impulses were essential for the acupuncture-induced enkephalin elevation [12].

Effect of the Peptidase Inhibitor Bacitracin on Acupuncture Analgesia

It is well-known that acupuncture only produces a transient analgesia in animals and that enkephalins degrade rapidly after release. If enkephalins are indeed involved in acupuncture analgesia, delaying their degradation should prolong the analgesia. Therefore I injected the peptidase inhibitor bacitracin 50 μ g/50 μ l into the lateral ventricle of the rabbit and found that the acupuncture analgesia was conspicuously prolonged. The elevated pain threshold of rabbits injected with saline returned to the preacupuncture level 30–40 min after the cessation of acupuncture, while the pain threshold in the bacitracin plus acupuncture group was still markedly elevated [13]. When the rabbits were killed 30–40 min after the cessation of acupuncture, the enkephalin contents in the hypothalamus and the striatum were found to be highest in the bacitracin plus acupuncture group and lowest in the bacitracin control. The saline plus acupuncture group showed a residual elevation. These results indicate that the enkephalinergic neurons have a low level of activity during the resting state. Iv administered naloxone was capable of reversing the acupuncture analgesia during the bacitracin-prolonged analgesia period, implying that the prolongation is due to endogenous opioid substances.

Release of Brain Enkephalins by Acupuncture

To discover whether acupuncture releases brain enkephalins, the Met-enkephalin content in the rabbit CSF was measured [14]. Experiments were performed in bacitracin alone and bacitracin plus acupuncture groups. The pain threshold was still highly elevated in the latter group 30 min after the cessation of acupuncture. Cisternal CSF was sampled at this time, and the Met-enkephalin content was found to be significantly higher in the bacitracin plus acupuncture group, indicating that acupuncture activated the enkephalinergic neurons to release enkephalin into the CSF. Likewise, a similar increase of Met-enkephalin content in the CSF of the monkeys was found after electroacupuncture [15].

Dynamic Changes in the Release and Biosynthesis of Enkephalins After Acupuncture

It was reported that the protein synthesis inhibitor cycloheximide interfered with the incorporation of tritium-labelled tyrosine into enkephalins in vitro [16]. I studied its in vivo effect on acupuncture analgesia after intraventricular injection. The

dose of cycloheximide was 500 pg per rat. This drug alone had no discernible effect on the pain threshold but greatly attenuated the electroacupuncture analgesia. It also decreased the Met-enkephalin content by 47% in the hypothalamus and by 20% in the striatum. In the electroacupuncture group, the Met-enkephalin level increased 113% in the hypothalamus and 200% in the striatum, but in the cycloheximide plus acupuncture group, it increased only 28% and 120%, respectively. The amplitude of the Met-enkephalin increase by acupuncture was greatly reduced by cycloheximide, suggesting that acupuncture accelerates enkephalin biosynthesis [17].

To study further the dynamic changes in the biosynthesis and release of enkephalins in acupuncture, high-potassium-induced enkephalin release from the *in vitro* striatal slices of control and electroacupuncture-treated rats was studied [18]. Rats were decapitated immediately, 15, 30, or 60 min after the termination of 30-min electric acupuncture treatment. Striatal slices 150–200 μm thick and weighing 30 mg were perfused with Krebs solution in a perfusing chamber. Perfusates collected at 2-min intervals were used for Leu-enkephalin radioimmunoassay. When the striatal slices from control rats were perfused with a Krebs solution containing 30 mM potassium, Leu-enkephalin release was observed after a 2-min delay, attaining its peak rate in 3 min, decreasing gradually thereafter, and approaching the basal rate in 7 min. In the group of rats killed immediately after the termination of the 30-min electroacupuncture treatment, the striatal slices became refractory to high potassium stimulation. There was a partial recovery of potassium-induced Leu-enkephalin release in the group of rats killed 15 min after the cessation of treatment. A full recovery was seen in the 60-min group. In view of the fact that the enkephalin content was actually increased in the rats killed immediately, the demonstration of decreased potassium-induced Leu-enkephalin release was rather surprising. However, it might be explained if the possibility of enkephalin having two pools, is considered, one releasable and the other nonreleasable. A long period of electroacupuncture depletes the releasable pool, while accelerating the biosynthesis and the processing of the enkephalin precursors. The newly formed enkephalin would take 30–60 min to become releasable.

To substantiate this working hypothesis that acupuncture accelerates enkephalin biosynthesis, the level of high molecular weight enkephalin-containing precursors were directly measured after electroacupuncture treatment [19]. Leu-enkephalin and its precursors in the pooled striatal extracts of 10 rats were first fractionated on a Sephadex G-75 column according to Lewis et al. [20]. The eluates were collected at 2-min intervals. Two peaks representing large and small molecular weight peptides were monitored by absorbance at 254 nm. The first peak was in the void volume while the second peak was in the salt volume. Leu-enkephalin could be detected directly in the eluates corresponding to the second peak. The immunoreactivity in the first peak and the intermediate region was detected only after trypsin and carboxypeptidase B digestion. Leu-enkephalin immunoreactivities in the first peak, the intermediate region, and the second peak were counted as the enkephalin precursor, the processed intermediates, and the free Leu-enkephalin, respectively. Their levels were measured in the following groups, each containing 10 rats: control, killed immediately, 0.25, 0.5, 1, 2, 4, 8, 16, 24, 48, 96 h after the termination of 30-min electroacupuncture. The Leu-enkephalin level was very mark-

edly elevated in the 0-h group, decreased gradually in the 0.25-, 0.5- and 1-h groups. In the 2- and 4-h groups, the Leu-enkephalin level was below that of the control group. From 8 h onward, the Leu-enkephalin level again increased steadily but was never as high as in the 0-h group. The proenkephalin and intermediates levels showed no change in the 0-, 0.25-, and 0.5-h groups but started to increase after 1 h. Their levels became steadily higher until 96 h. This increase in the proenkephalin and intermediates levels preceded the second phase of Leu-enkephalin elevation.

Hybridization Approaches to Study the Role of Enkephalins in Acupuncture Analgesia

In order to learn whether or not acupuncture acceleration of enkephalin biosynthesis also involves the transcriptional level, the proenkephalin mRNA quantity in the rat striatum was measured by the recombinant DNA technique [21]. The proenkephalin mRNA was hybridized by a dot-blot procedure with a 918-base pair cDNA sequence complementary to human pheochromocytoma proenkephalin mRNA kindly donated by Prof. E. Herbert. Rats receiving electroacupuncture treatment showed a three- to fivefold increase of proenkephalin mRNA in the striatum, beginning 1 h after the cessation of the 30-min treatment period and lasting for at least 48 h. A threefold increase of proenkephalin mRNA in the pituitary was found immediately after treatment and a five- to sixfold increase at 1 or 24 h after treatment termination. A similar change in adrenal proenkephalin mRNA was also seen after electroacupuncture treatment.

For comparison, the proopiomelanocortin mRNA level in the pituitary and the hypothalamus was also measured by dot-blot hybridization with a ME 150 plasmid containing a 144-base pair cDNA sequence complementary to the lipotropin-coding portion of mouse proopiomelanocortin mRNA (a gift from Prof. E. Herbert). A 50% increase in the proopiomelanocortin mRNA level in the pituitary was observed 1 h after the cessation of the 30-min electroacupuncture treatment. This elevation continued for 96 h with a maximum of about 70% increase at 24 h [22].

Summary and Conclusion

Enkephalins are likely to play an important role in mediating acupuncture analgesia. Acupuncture has proven to be a powerful method to activate the enkephalin-ergic system. The biosynthesis and posttranslational processing of the enkephalin precursors are accelerated by acupuncture. This activation also involves the transcriptional level as shown by the increase in the proenkephalin mRNA levels in the brain, the pituitary, and the adrenal gland.

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In recent years, electroacupuncture (EA) analgesia has been the focus of intense multidisciplinary research. This research has been very productive, leading to the publication of numerous studies that have added to our understanding of the neurophysiology of pain perception and pain modulation. This article will review the results of some of these studies, from which the central roles in pain modulation played by the endorphins and various monoamines (serotonin, in particular) will become apparent. In conclusion, an integrated neurophysiological model of EA analgesia will be proposed.

Experimental Data Supporting the Acupuncture-Endorphin Hypothesis

Much experimental data support the hypothesis that the analgesia produced by EA is mediated by the release of endorphins into the central nervous system (CNS) and that this release may occur at several different levels within the CNS.

1. An early experiment (Cheng and Pomeranz 1976) involved recording the electrical activity of a single interneuron in lamina V (Rexed) of the spinal cord of an anesthetized cat subjected to peripheral noxious stimulation (i.e., Pinprick). Before discussing the experiment proper, however, it may be useful first to review briefly the functional neuroanatomy. Lamina V of the spinal cord is rich in large interneurons which respond to activity of all three of the main fiber components of the cutaneous nerves: A β , A γ , and C fibers. Thus, they respond to both nonnoxious and noxious stimuli and are hence called "wide dynamic range nociceptors." When stimulated by an appropriate noxious stimulus (such as pinpricking the skin, as in the experiment about to be considered), these cells activate ascending dorsal horn nociceptive neurons; the electrical recording of the activity of these interneurons in response to a painful stimulus can therefore be taken as an approximation of the activity of painful signals being sent up to higher brain centers.

Returning now to the EA experiment at hand, electrical recordings were made from such an interneuron in lamina V of the cat's spinal cord under various conditions: subjected to light mechanical stimulation or to noxious pinpricking over the receptive field of the interneuron, both with and without simultaneous application of EA to the same receptive field.

The results are presented in Fig. 1, which shows that: (a) the activity of the interneuron in response to a nonnoxious stimulus was unchanged by EA; and

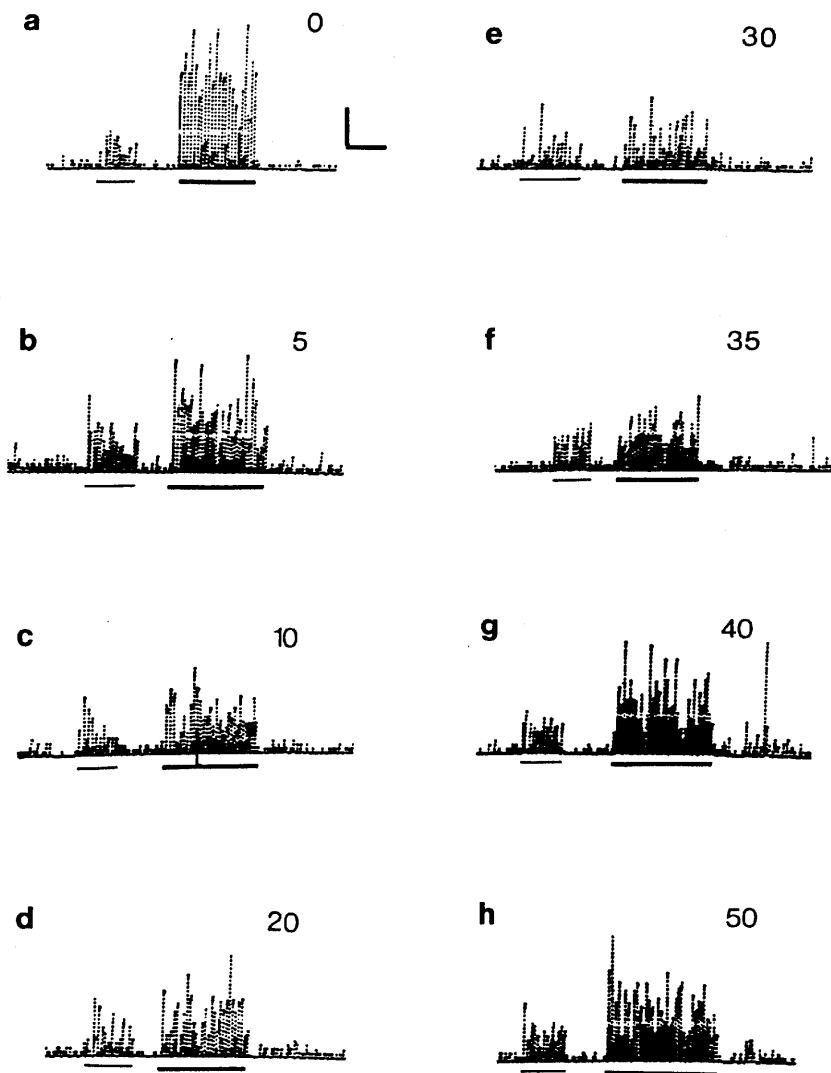


Fig. 1. Records (A to H) from a typical single interneuron in layer 5 of cat spinal cord. The output of an epochal rate meter, bin width 100 msec. is displayed on a storage oscilloscope, each dot representing a single action potential of this cell. As the oscilloscope sweeps from left to right the rate meter produces vertical dots in successively higher positions within bins of 100 msec. A-control before acupuncture; B to E are 5, 10, 20, 30 minutes after onset of acupuncture. F, G and H are recovery taken 35, 40 and 50 minutes; needles are removed at 30 minutes. Horizontal bars indicate the duration of the repetitive stimuli: Thin line is light mechanical stimulus, thick line is noxious pinprick. Scale is 5 sec. horizontal, 50 spikes/sec. vertical

(b) the interneuron's activity in response to noxious stimulation was considerably reduced by continuous EA.

EA-produced inhibition which builds up slowly over time (i.e., EA has a long induction time) and reaches a peak at 30 min; if EA is then discontinued, inhibition of the interneuron is maintained for a considerable length of time, indicating that EA has a prolonged after effect.

Finally, and most importantly, it was also shown (Fig.2) that the inhibitory

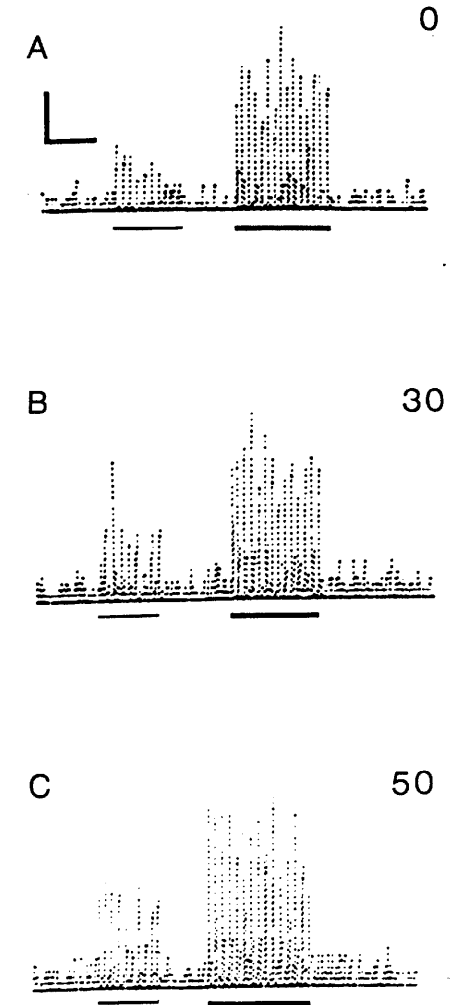


Fig. 2. Records (A to C) from a typical single interneuron in layer 5 of cat spinal cord. The output of an epochal rate meter, bin width 100 msec. displayed on a storage oscilloscope, each dot representing a single action potential of this cell. As the oscilloscope sweeps from left to right the rate meter produces vertical dots in successively higher positions within bins of 100 msec. A - control before acupuncture and injection of naloxone. C - recovery taken 20 min. after needles are removed (Note: electroacupuncture alone was first tested to have a reduction-effect on the noxious response of this typical cell.) B - 30 minutes after initiation of acupuncture and injection of naloxone

effect of EA could be blocked by parenteral administration of naloxone (0.3 mg/kg), which is a potent, almost purely opioid antagonist [24].

Taken together, these results suggest that EA has an inhibitory effect on spinal cord interneurons, that this effect is specific to activity related to the transmission of noxious signals, and that the inhibition may be mediated by opiatelike substances (i.e., endorphins).

2. Microinjections of naloxone (10 μ l) into areas of the CNS known to contain endorphins, such as the periaqueductal gray matter (PAG), the caudate nucleus, the nucleus accumbens, or the hypothalamus, all decreased acupuncture analgesia

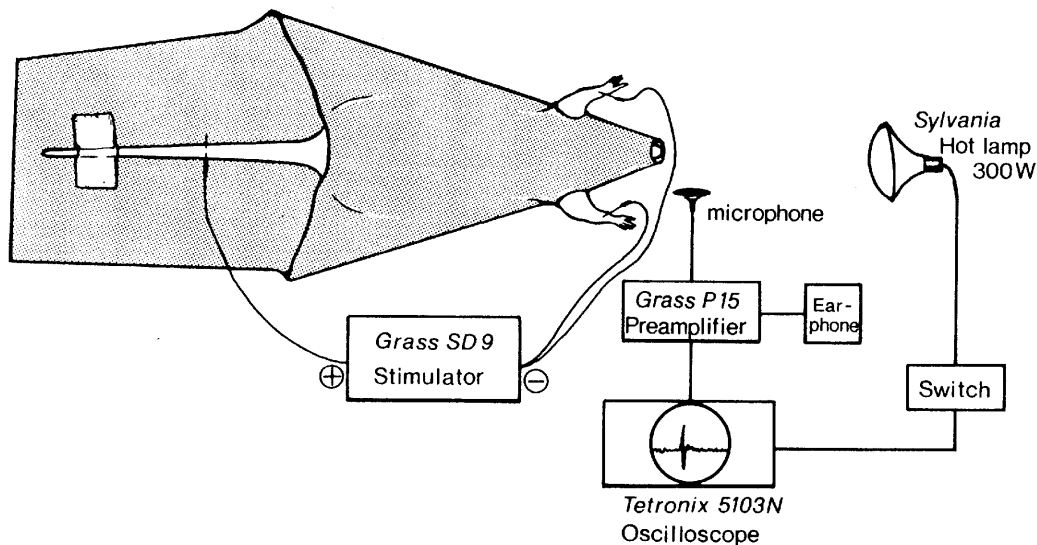


Fig. 3. A schematic diagram depicting the set-up of EA experiments of the mouse restrained in a paper receptacle. The forelimbs and the tail were immobilized by masking tape. The nose was also exposed to a radiant heat lamp at a distance of 9 cm. Squeak audiogram was recorded by an oscilloscope through a small microphone. Electrical currents were supplied by a Grass SD 9 stimulator. The negative pole was connected to the acupuncture needles inserted into the first dorsal interosseous muscles and positive pole was connected to the needle that was inserted through the middle of the tail

in rats and rabbits, while similar injections into other areas not containing endorphins had no effect [25]. This again suggests that acupuncture produces analgesia via activation of anatomically discrete, opiate-mediated, pain-relieving system that involve many levels of the CNS.

Another series of experiments [4] involving opiate antagonists also were revealing, and these experiments will now be reviewed in some detail.

In this study, the effects of various opiate antagonists on EA analgesia were investigated. EA-treated mice were injected with these various opiate antagonists, and their squeak responses to noxious heat stimulation were recorded; a shortening of the squeak latency period was taken as evidence that the given test substance inhibited EA analgesia. (Refer to Fig. 3 for the experimental set-up.) The animals used for these experiments were B6AF1/J female mice, selected for their reliable squeak response to noxious heat stimulation. The acupuncture needles were placed into the first dorsal interosseous muscles - a placement site required for good EA analgesia.

The experimental results (Figs. 4, 5, 6, 7) suggest that EA is mediated by stereospecific opiate receptors: The type 1 opiate antagonists (i.e., the almost purely opioid antagonists) L-naloxone, naltrexone, and cyclazocine all blocked EA analgesia in these mice, while their stereoisomer D-naloxone had no effect. These results are further evidence for a specific EA-endorphin relationship.

Using the same experimental set-up (Fig. 3), other studies were performed (Cheng and Pomeranz 1980) to examine the relationship between the frequency of EA and blockage of its analgesia by simultaneous administration of various

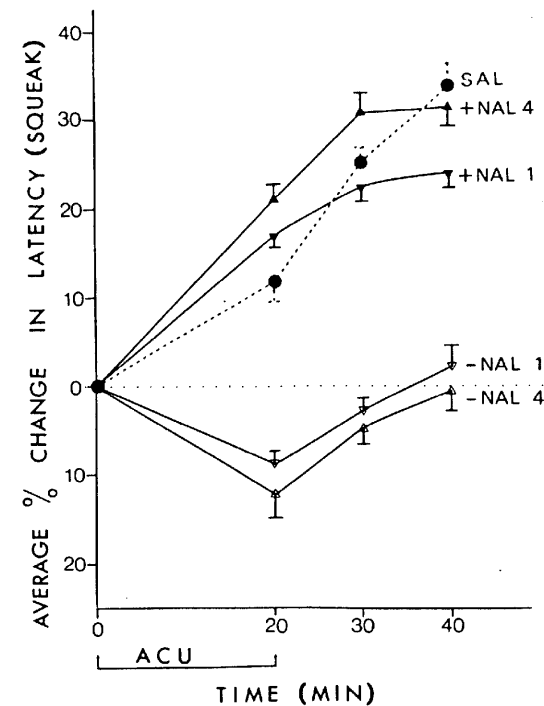


Fig. 4. Effect of (+) naloxone, (-) naloxone or saline on electroacupuncture analgesia in mice. Ordinate shows percentage change in latency to squeak as compared to zero time pretreatment control value. Positive values denote analgesia. +Nal 1 shows effect of electroacupuncture plus dextronaloxone (1 mg/kg). +Nal 4 shows EA plus dextronaloxone (4 mg/kg). Sal shows EA plus saline (0.9%). -Nal 1 shows levonaloxone (1 mg/kg) plus EA. -Nal 4 shows levonaloxone (4 mg/kg) plus EA. Each point is the mean for 15 mice. Bars show standard error. Arrows and 'ACU' indicate time or treatment: EA started after zero time and stopped at 20 minutes; injections were given at zero time and again at 20 minutes (booster). (-) naloxone blocks electroacupuncture analgesia, while saline (0.9%) and (+) naloxone do not

chemicals. It was found (Figs. 8-11) that low-frequency EA (4 Hz) induced an analgesia which could be blocked by naloxone, but that high-frequency EA (200 Hz) induced an analgesia which was not affected by this opiate antagonist. However, it was possible to block partially the high-frequency-induced EA analgesia which injections of parachlorophenylalanine (PCPA), which is an inhibitor of serotonin synthesis. The finding hinted at the existence of another system mediating EA analgesia, to be discussed shortly.

3. Peets and Pomeranz [22] demonstrated that mice (CXBK) genetically deficient in opiate receptors showed poor acupuncture-induced analgesia. They speculated that there are genetic variations in both humans and animals and that because of these a certain proportion (about 30%-40%) of any species will respond poorly to acupuncture due to a poor endorphin system. Support for this idea came when Takeshige [30] observed that those rats (40% of their cohort) demonstrating poor acupuncture analgesia had a deficiency in total brain endorphins, as measured by receptor binding assay.

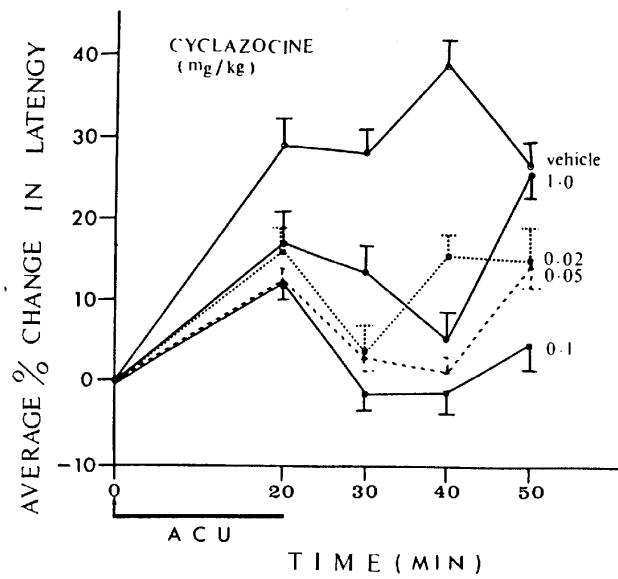


Fig. 5. Effect of cyclazocine on EAA in mice. This diagram is similar to figure 4 except that cyclazocine is administered once (immediately before EA). Doses of cyclazocine for each group of mice are indicated by the numerals 1, 0.1, 0.05 and 0.02 in mg/kg. Each curve represents the mean of 15 mice. Vehicle (0.5 ml/mouse, I.P.) is made by mixing 0.2 ml of 1 N hydrochloric acid and 0.2 ml of 1 N NaOH and the solution is brought to 10 ml with distilled water

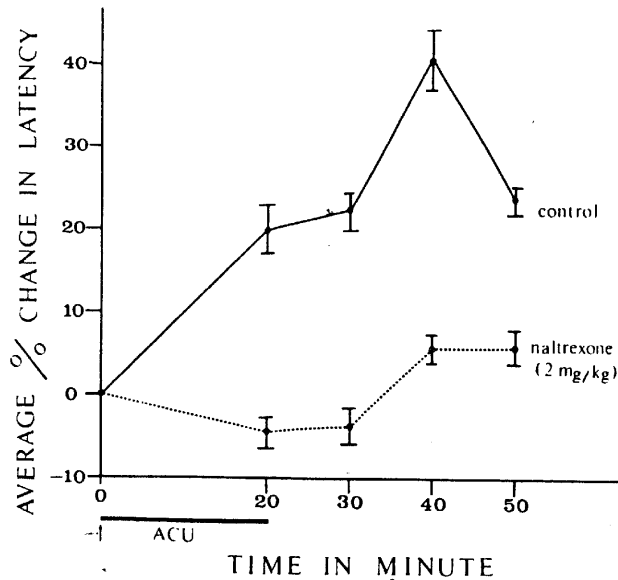


Fig. 6. Effect of naltrexone (2 mg/kg) on EAA in mice. This diagram is similar to Fig. 4 and 5 except naltrexone or 0.9% saline (control) is administered once (immediately before EA indicated by the arrow). Naltrexone reverses EAA in mice

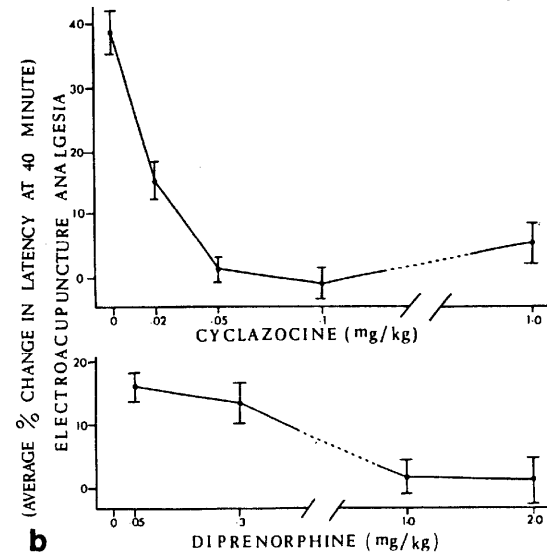
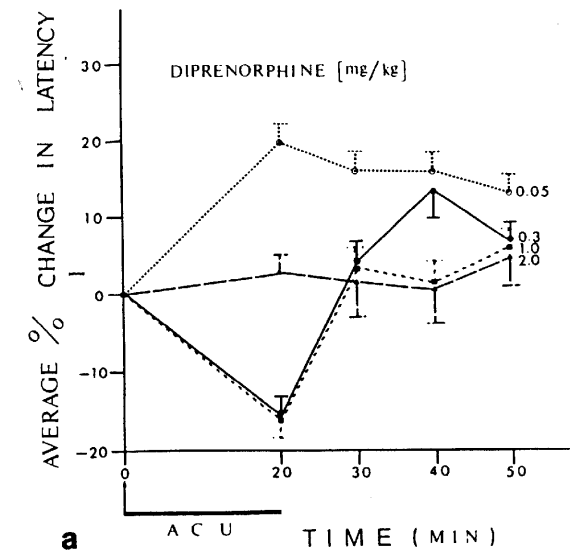


Fig. 7. a Effect of diprenorphine on EAA in mice. This diagram is similar to figure 4, 5 and 6. Diprenorphine was administered once (immediately before EA indicated by the arrow). Doses is indicated by the numerals 2, 1, 0.3 and 0.05 in mg/kg. **b** Dose-response curves showing that cyclazocine and diprenorphine reverses EAA in a dose-related manner in mice. Ordinate represents the EA effect (average % change of squeak latency at the 40 minute derived from Fig. 5 and 6). Abscissa indicates the dose concentration in mg/kg. Each point is mean of 15 mice. Upper curve shows the cyclazocine effect and lower curve shows the diprenorphine effect on EAA at various doses

4. More direct support of the acupuncture-endorphin hypothesis was obtained in 1977 when Sjolund, Terenius, and Erickson [29] reported that transcutaneous electroacupuncture (TE) on the lower lumbar spine (segmental TE) in humans elevated the lumbar cerebrospinal fluid (CSF) endorphins (fraction 1) concentrations, while TE to nonsegmental TE did not. This "fraction" endorphin extract was subsequently found to cross react with dynorphin antigens (Terenius, personal com-

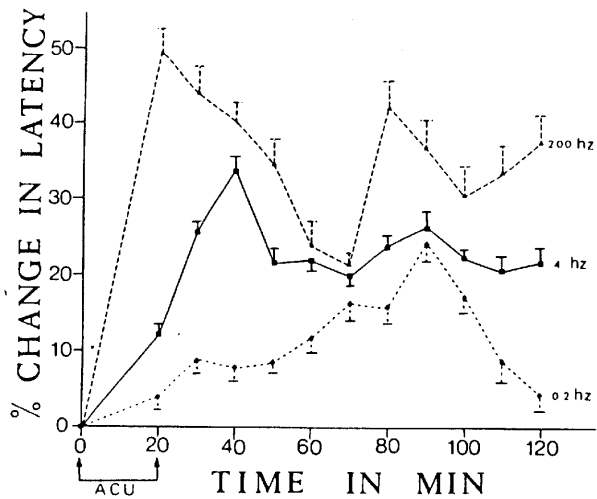


Fig. 8. Electroacupuncture analgesia induced by three different frequencies (200, 4 and 0.2 Hz) of electrical stimulation. Ordinate shows average percentage change in latency to squeak as compared to zero time pretreatment control values. Positive values denote analgesia. Abscissa shows the time of measurements. Top line shows EA effect induced by 200 Hz. Middle line shows EA effect induced by 4 Hz. Bottom line shows EA effect induced by 0.2 Hz. Arrows indicate the time of E. A. Bars indicate standard error. Each point is the mean of 15 mice

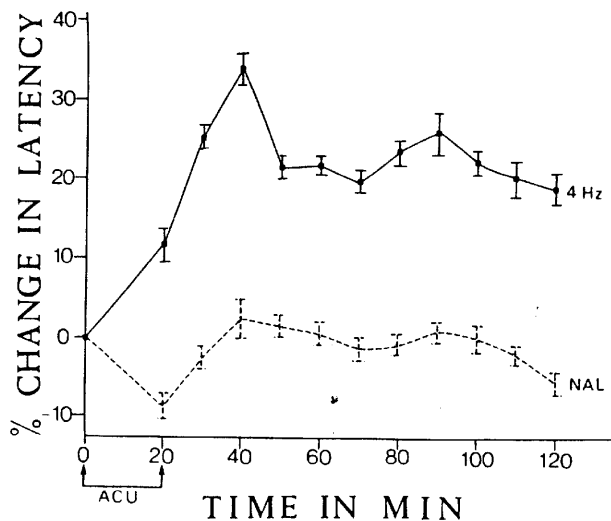


Fig. 9. Naloxone inhibits electroacupuncture (EA) analgesia which is induced by 4 Hz electrical stimulation. Coordinates same as Fig. 4. Upper line shows EA (at 4 Hz) effect on saline (0.9%) injected mice. Lower line shows EA effect on naloxone (1 mg/kg) injected mice. Injections were done twice: immediately before and after EA (at 0 and 20 minutes). Bars show standard errors. Arrows indicate the time of EA. Each point represents the mean of 15 mice

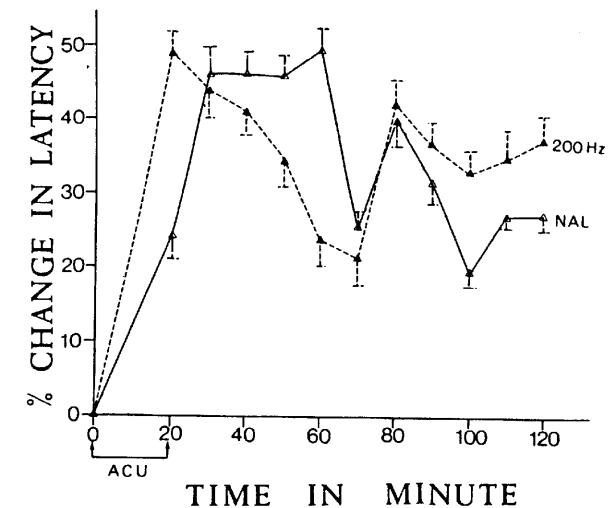


Fig. 10. Naloxone does not reverse the electroacupuncture (EA) effect at high frequency (200 Hz) stimulation. Coordinates same as Fig. 4. Dashed line (200 Hz) indicates EA in saline injected mice. Solid line (Nal) indicate EA in naloxone injected mice. Injections were given twice: immediately before and after EA. Arrows indicate the time of EA. Bars indicate standard errors. Each point is the mean of 15 mice

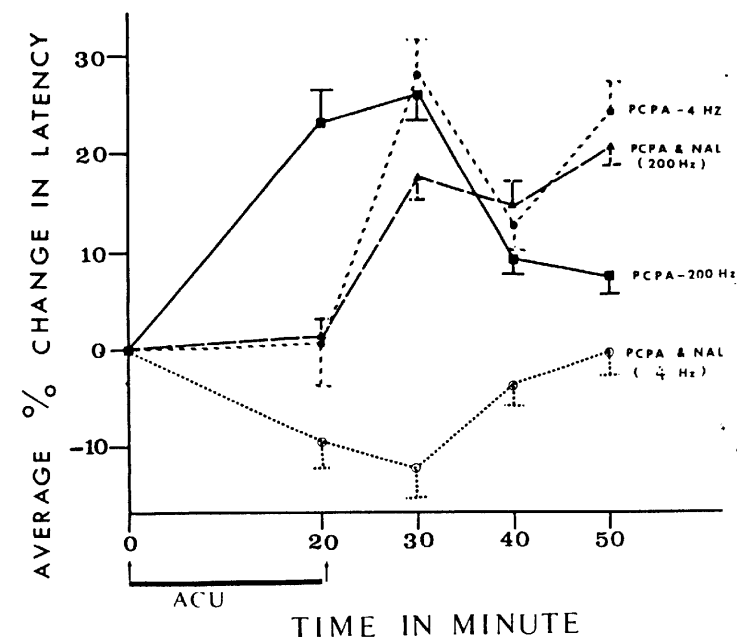


Fig. 11. Parachlorophenylalanine partially blocks high frequency (200 Hz) EA analgesia but not 4 Hz EA effect. Coordinates same as Fig. 4. 'PCPA-200 Hz' indicates high frequency EA treatment in PCPA treated mice. 'PCPA+Nal-(200 Hz)' indicates high frequency EA treatment in PCPA and naloxone treated mice. Treatment in PCPA treated mice. 'PCPA+Nal-(4 Hz)' indicates low frequency EA treatment in PCPA and naloxone injected mice. PCPA was injected 3 days before the experiment. Each point is the mean of 15 mice. Bars show standard error. Arrows indicate the beginning and end of EA and also the injections of naloxone or saline

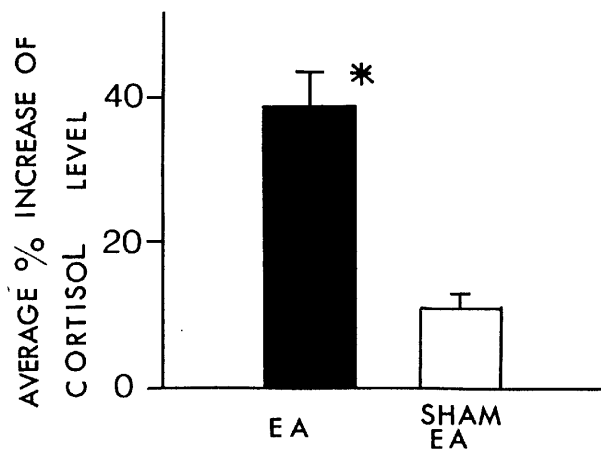


Fig. 12. The effect of electroacupuncture (EA) on blood cortisol level of horses. Ordinates indicate the average % increase of cortisol levels. Solid column indicates the average % increase of cortisol level of 15 horses after EA. Open column indicates the average % increase of blood cortisol level after sham EA in 15 horses. Bars indicate S.E. Star indicates statistical significance. EA significantly increases blood cortisol levels in horses

munication). Since dynorphin is found in the dorsal root ganglia [12], it was hypothesized that acupuncture can induce a local segmental release of endorphin (presumably dynorphin), which may then presynaptically inhibit the release of substance P. Substance P has been identified as one of the several potential neurotransmitters within primary nociceptive afferent neurons, whose cell bodies are located in the dorsal root ganglia. This substance is released at the central synapses of some primary afferent neurons following electrical stimulation of their high-threshold (C-fiber) axon. This release is blocked when morphine is applied in concentrations known to elicit analgesia: Han [17] further demonstrated that injection of dynorphin antibodies into rat spinal cord CSF inhibited EA in the 15–128 Hz frequency range. He speculated, therefore, that dynorphin mediated EA analgesia at the level of the spinal cord in this frequency range.

Tsou and colleagues [31] also found that EA caused an increase of endorphin (Fraction 1) levels in the cisternal CSF in rabbits; Zhang [35] demonstrated that fraction 1 endorphin release was elevated in the PAG of rabbits after acupuncture treatment. However, H. Akil has shown that injecting dynorphin into the PAG or ventricular CSF produces no analgesic effect in rats (personal communication), and thus the full role of dynorphin as an analgesic substance remains unclear.

Still, all the above results suggest that acupuncture releases endorphins into the CSF at several levels of the adding weight to the acupuncture-endorphin hypothesis.

5. It was demonstrated that D-phenylalanine and D-leucine produced naloxone-reversible analgesia in humans and mice [4, 5, 11]. It was postulated that these D-amino acids protected the endorphins from enzymatic degradation. Recent biochemical evidence supports this hypothesis; D-phenylalanine and D-leucine can cross the blood-brain barrier [21] and inhibit enkephalinases (demonstrated in guinea pig ileum assays [13]; It was then found [6] that combining these D-amino

acids and EA treatments produced analgesia in a greater number of mice in a test cohort than that produced by either treatment alone. Presumably, in keeping with the acupuncture endorphin hypothesis, EA released endorphins which were then protected from enzymatic degradation by the action of the D-amino acids.

6. It has been shown that hypophysectomy abolishes EA analgesia [1] and also that dexamethasone, which is known to inhibit the release of pituitary ACTH and β -endorphin, along with 2% saline treatment which depletes pituitary endorphin, all reduce EA analgesia [7]. These findings suggest that pituitary endorphin is at least partially involved in mediating EA analgesia. It has also been reported that hypophysectomy inhibits morphine analgesia (Katz 1980) and naloxone hyperalgesia [14]. This indicates that the pituitary is normally required for opiate analgesia. Finally, an indirect experiment indicated that EA may release endorphin and ACTH together into the blood, since plasma cortisol levels were elevated after EA analgesia in horses (7, 15) Fig. 12.

Evidently then, the acupuncture-endorphin hypothesis appears to implicate the existence of a highly complex neuroendocrine system for the mediation of EA analgesia (and by extension, for endogenous pain control). By integrating all the experimental data presented so far an internally consistent neurophysiological model for such a system can be proposed, but first another set of experimental data that implicates yet other complexities in the system must be considered. This set of data relates to the role of monoamines in EA analgesia.

Experimental Data Suggesting a Serotonin-Dependent System Mediating Electroacupuncture Analgesia

1. The raphe-serotonin descending inhibition system was first demonstrated by Shen and co-workers in 1975. They found that lesioning the dorsolateral fasciculus (DLF) completely abolished acupuncture analgesia in rabbits [27, 28]. In 1976, Du and Chao [10] showed that lesioning the raphe magnus also inhibited acupuncture analgesia. This result was repeated by McLennan [20], who showed that both electrical and chemical lesions of the raphe nucleus reduced EA in rabbits and in rats.

Injection of 5,6-dihydroxytryptamine (a chemical that destroys serotonin nerve endings) into the raphe nucleus inhibited EA analgesia [9]. Similarly, as described previously microinjection of naloxone into the PAG partially reversed EA analgesia [35]. Thus it is suggested that the raphe-DLF-serotonin system is linked in series to the enkephalinergic neurons in the raphe nucleus and PAG. (However, part of the PAG-EA analgesia effect may bypass the brain stem enkephalin system, as was previously suggested i.e., the PAG may be activated directly by the hypothalamus or the pituitary.)

In addition to 5,6-dihydroxytryptamine, many other chemicals that either deplete or antagonize the effects of brain serotonin have also been shown to abolish or reduce EA analgesia, while drugs that enhance the serotonin level in the CNS have been shown to increase EA analgesia [6, 16, 19]. These data all point to an important role of serotonin in EA analgesia. The results are presented in Table 1.

Table 1. Results of Acupuncture Research. Summary of monoaminergic drugs on EAA at 200 Hz. (Cheng R. 1981, Ph. D. Thesis)

Drug	Net Functional Change			EAA
	S↓	D↓	N↓	
1. TBZ	S↓	D↓	N↓	↓*
2. PCPA	S↓			↓*
3. AMPT		D↓	N↓	↓ ^a
4. Disulfiram	S↑		N↓	↓ ^a
5. TBZ + 5HTP	S↑	D↓	N↓	↓ ^a
6. TBZ + L-DOPA	S↓	D↑	N↓	R (Partial)
7. PCPA + 5HTP	Normal			R ^a
8. AMPT + L-DOPA (1 hr)		D↑	N↓	R
9. AMPT + L-DOPA (2 hrs.)		D↓	N↑	R (Partial)
10. 5HTP	S↑			↓ ^a
11. L-DOPA		D↑		↓ ^a
12. Probenecid	S↑	D↑		↓ ^a
13. Apomorphine		D↑		↓ ^a
14. Cinanserin	S↓			↓ ^a
15. Haloperidol		D↓		→
16. Pimozide		D↓		→
17. Yohimbine			N↓	→

S=Serotonin, D=Dopamine, N=Norepinephrine; R=Recovery of EAA after replacement drugs; TBZ=tetrabenazine; PCPA=parachloro-phenylalanine; AMPT=alpha-methyl-paratyroxine; 5HTP=DL-5-hydroxytryptophan; L-Dopa=L-3,4-dihydroxyphenylalaninmethylester.

^a only the data related to serotonin gave consistent EAA results

2. Another important piece of evidence in support of the EA-serotonin hypothesis is that serotonin and its catabolite SHIAA were found to be elevated in the CSF [34], raphe nucleus and locus ceruleus [16, 32], spinal cord [16], and in the whole brain [16, 19]. During EA analgesia. The above-mentioned elevation of serotonin and its metabolite was positively correlated to the extent of EA analgesia, higher levels occurring with greater EA analgesia. Han [16] further demonstrated that the serotonin content and its turnover rate increased in the telencephalon, diencephalon, brain stem, and spinal cord after 1 h of EA treatment in rats. They also observed that certain areas of the CNS behaved differently in response to the same EA stimulation. There was a marked increase in serotonin synthesis in the lower brain stem and spinal cord but a marked increase of serotonin turnover in the diencephalon and telencephalon; they concluded from this that the forebrain might also play an important role in serotonin-mediated EA analgesia.

3. Ionophoretically applied serotonin or norepinephrine was found to inhibit the response of dorsal horn cells evoked by nociceptive stimulation; intrathecal application of these same chemicals in the spinal cord also produced a profound analgesia in rats, rabbits, and cats [33]. These results suggest the existence of descending systems which may modify spinal sensory processing by means of serotonin or norepinephrine.

4. Restating a finding described earlier Cheng and Pomeranz [2] demonstrated that while low-frequency (4 Hz) EA analgesia might be mediated by endorphins, high-frequency (200 Hz) EA analgesia might be mediated by a separate, serotonin-dependent system; the evidence for the existence of these dual systems was that the 4-Hz EA analgesia was partially reduced only by PCPA, the serotonin synthe-

Table 2. Results of Acupuncture Research (Cheng R. 1981, Ph. D. Thesis)

Treatment	Action	EAA (4 Hz)	EAA (200 Hz)
1 Hypophysectomy*	Deplete pituitary beta-endorphin	↓	ND
2 Dexamethasone	Inhibit beta-endorphin release	(partial) ↓	ND
3 2% saline feeding	Deplete pituitary endorphin	↓	ND
4 Type I opiate antagonists (i) Levo-naloxone (ii) Naltrexone (iii) Cyclazocine (iv) Diphrenorphine	Block type I opiate receptors	↓	ND
5 Dextro-naloxone	Inactive isomer	→	ND
6 D-Leucine and D-Phenylalanine	May enhance endorphins	↑	ND
7 Two EA treatments at 3 hrs apart	Cumulative effect	↑	ND
8 Morphine addicted mice during withdrawal	Addiction	↑	ND
9 PCPA	Deplete 5-HT	→	↓
10 Cinanserin	5-HT receptor blocker	ND	↓
11 5-HTP	5-HT precursor	ND	↑

Key: ↓ reduce EAA; ↑ increase EAA; → no effect on EAA; ND=not done

sis inhibitor. Similarly, Han [16] found that naloxone only partly blocked EA analgesia in rats. When the rats were injected with PCPA, again EA analgesia was only partly reduced. However, when the investigators combined naloxone and PCPA treatments together, EA analgesia, was completely abolished. This supports the Cheng and Pomeranz evidence for the existence of dual systems mediating EA analgesia, one dependent on endorphin and the other on serotonin [2].

Zhang [35] found that "moderate EA analgesia" (7.5-8.0 mA) was readily reversed by naloxone alone in rabbits, a result that would seem to contradict the studies [16] in which naloxone alone was insufficient to abolish EA analgesia. However, when Zhang et al. used "super strength EA analgesia (12.5-15.0 mA), they too could no longer reverse EA analgesia with naloxone alone. One explanation for these results is that the higher current stimulations (Han [16, 17] had used 5.0 mA in their studies, which is very high in comparison with the 0.1 mA threshold of Aβ fibers) may activate, in addition to the "normal" endorphin-dependent system, a second stress-induced analgesia which would be mediated by the non-endorphin system; low-intensity stimulation (nonnoxious) may activate only the endorphin-dependent system. In contrast, the Cheng and Pomeranz study [2] indicates that the non-endorphin system can also be activated by a low-intensity, nonnoxious current (they used 0.1-0.2 mA impulse of 1-mS duration), provided one

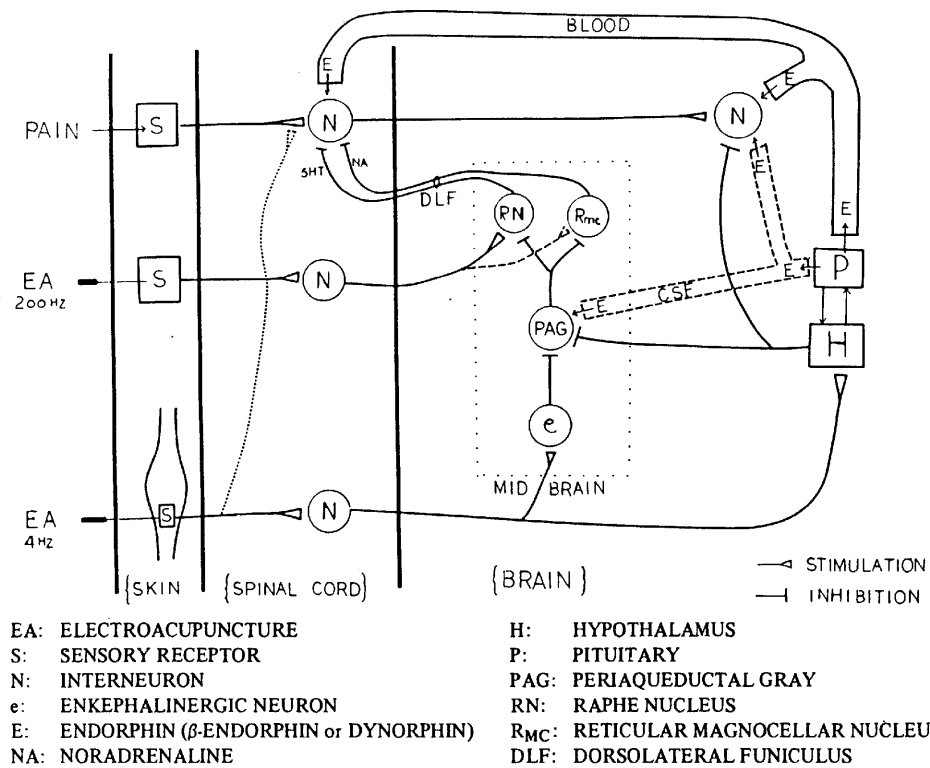


Fig. 13. At low frequency (4 Hz), EA may stimulate the midbrain (PAG) to release enkephalins which will indirectly stimulate the raphe nucleus (RN) and/or reticular magnocellular nucleus (Rmc) to send a descending inhibition on the spinal cord pain cells. Serotonin and noradrenaline are probably the neurotransmitters involved in the RN and Rmc systems respectively. In parallel, EA may also stimulate the hypothalamus and pituitary to release beta-endorphin or dynorphin. The pituitary endorphins may either go through the blood-brain barrier or backflow to the hypothalamus or CSF and bind to the opiate receptors in the spinal cord and the brain. In addition, low frequency (4 Hz) EA may cause the segmental release of endorphins from the spinal cord interneurons and bind to the opiate receptors in the pain transmission cells. High frequency (200 Hz) EA appears to stimulate directly the RN and Rmc descending inhibitory systems, bypassing the endorphin system

stimulated with high frequencies (they used 200 Hz); in their study, they could also trigger the endorphin system independently with low-intensity current of low frequency.

An Integrated Neurophysiological Model of Electroacupuncture Analgesia Is Proposed

Table 2 summarizes most of the data discussed in this article. From these data, a model of EA analgesia was constructed, which is presented in Fig. 13. Details of this model are further emphasized in Fig. 14 and 15, which isolate the postulated two pain-controlling systems from each other; these are the endorphin-dependent system and the serotonin-dependent system.

Briefly, it is proposed that low-frequency (4-Hz) EA stimulates the sensory receptors in deep muscle causing the midbrain PAG to release enkephalins. These

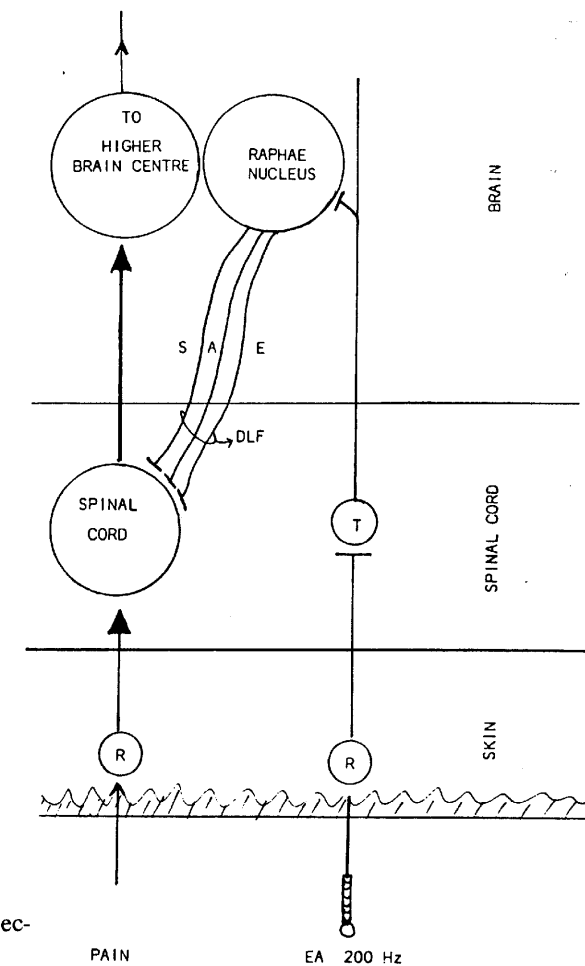


Fig. 14. Mechanism of high frequency electroacupuncture analgesia

enkephalins in turn activate the raphe nucleus (RN) and/or the reticular magnocellular nucleus (RMC), which then send descending inhibitory signals along the DLF to the spinal cord; the RN-DLF pathway may use serotonin as its neurotransmitter, while the RMC-DLF pathway may use norepinephrine. In the spinal cord the descending serotonergic and/or norepinephrinergic fibers would synapse with local enkephalinergic interneurons and activate (or disinhibit) these cells, leading to the release of enkephalins; these enkephalins may exert presynaptic inhibitory control over incoming substance P-containing primary afferent fibers concerned with pain transmission (Jessell and Iverson cellular model 1977). In parallel with this, low-frequency (4-Hz) EA may also stimulate the release of β -endorphin from the hypothalamus. These hypothalamic endorphin neurons project to different areas of the brain (e.g., PAG, periventricular nucleus of the thalamus, nucleus accumbens, amygdala) where they may release their β -endorphins and produce central pain relief. The EA-stimulated hypothalamus may also produce a releasing factor to stimulate the release of pituitary endorphins, and ACTH. These pituitary endorphins could then be released into the systemic circulation and be redistributed back to the brain and spinal cord by passing through

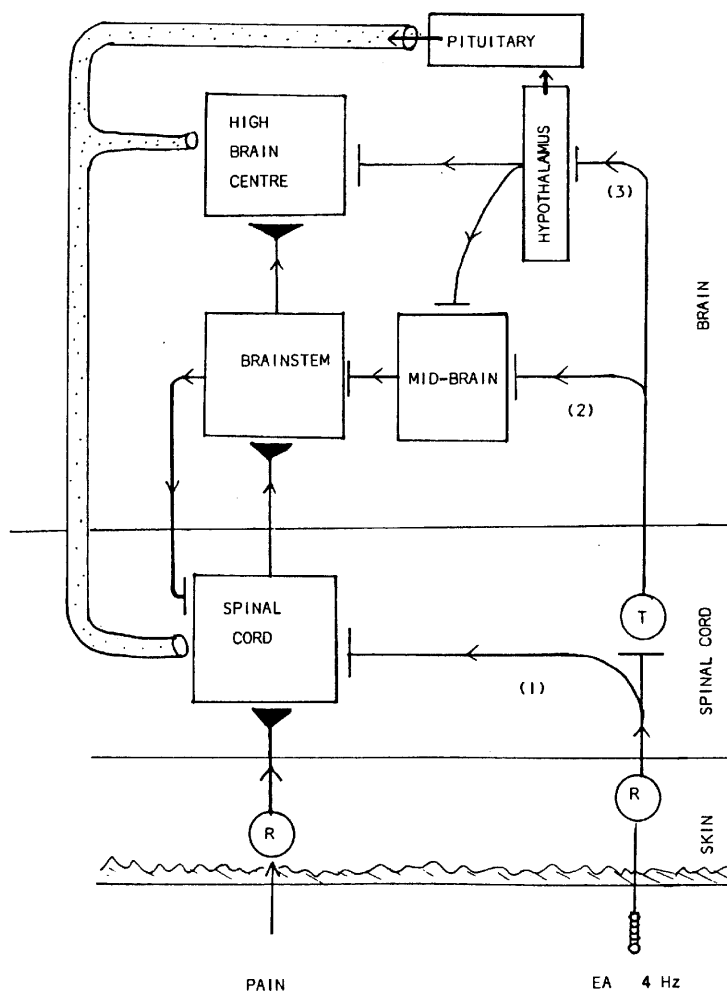


Fig. 15. Mechanism of low frequency electroacupuncture analgesia

the blood-brain barrier; at these locations they would bind to opiate receptors, causing analgesia. Low-frequency (4-Hz) EA may additionally cause segmental release of endorphins from the spinal cord interneurons and bind to the opiate receptors in the pain transmission cells, producing analgesia by presynaptic inhibition (suppressing the release of substance P). High-frequency (200-Hz) EA may activate sensory nerves and directly stimulate the DLF-serotonin/norepinephrine descending inhibitory systems, bypassing the PAG-endorphin system.

In summary, this model describes three anatomical levels of EA analgesia (Fig. 15):

1. Endorphin (dynorphin) release in the spinal cord would cause segmental or localized pain relief.
2. Activation of the midbrain and brain stem would induce a regional analgesia through the enkephalin-DLF-serotonin system. That is, EA to one part of the body would produce analgesia over a wide surface area which can be far removed from the site at which EA is applied. This can be understood if one

accepts the hypothesis advanced by Mayer et al. (1971) and Liesbeskind et al. (1973) that there exists a regional (Humunculus) mapping in the midbrain, whereby stimulation of a certain midbrain area will cause analgesia in a certain part of the body (e.g., in humans, stimulation of the first dorsal interosseous muscle in the hand produces analgesia in the facial area).

3. Activation of the hypothalamus and the pituitary would produce a generalized increase in the pain threshold. This effect would be mediated by the release of endorphins and ACTH into the systemic circulation; the endorphins so released would act via the pain-modulating systems already described while the ACTH would stimulate the release of cortisol, which would tend to reduce inflammation.

It is proposed that EA may stimulate the above levels individually, in part or all three together, resulting in different degrees of analgesia (as is observed in clinical practice). Higher analgesia and better clinical results can be obtained if acupuncture is done properly.

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