MECHANISMS OF ELECTROACUPUNCTURE ANALGESIA AS RELATED TO ENDORPHINS AND MONOAMINES; AN INTRICATE SYSTEM IS PROPOSED

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TABLE OF CONTENTS

TABLE OF CONTENTS ---------- (1)

ACKNOWLEDGEMENT ---------- (5)

ABSTRACT ---------- 1

INTRODUCTION

(A) GENERAL INTRODUCTION ---------- 6

(B) LITERATURE SURVEY

(i) PAIN ---------- 9

I. PRIMARY AFFERENT NEURONS ---------- 12

II. SPINAL CORD ---------- 16

III. ASCENDING PAIN-SIGNALLING PATHWAYS ------ 18

IV. ENDOGENOUS PAIN CONTROL MECHANISMS ------ 22

(ii) ENDORPHINS ---------- 28

I. OPIATE RECEPTORS ---------- 28

II. TYPES OF ENDORPHINS ---------- 30

III. LOCATIONS OF ENDORPHINS ---------- 33

1. ENKEPHALINS ---------- 33

2. BETA-ENDORPHIN ---------- 36

3. MORPHINE-LIKE COMPOUND ---------- 38

4. LEUCINE-ENDORPHIN ---------- 39

5. DYNORPHIN ---------- 39

6. OTHER RELATED SUBSTANCES ---------- 40

IV. FUNCTIONS OF ENDORPHINS ---------- 40

1. ANALGESIA ---------- 42

2. HOMEOSTASIS ---------- 46

3. MENTAL ILLNESS ---------- 47

4. BEHAVIOUR ---------- 50

5. ADDICTION ---------- 51

V. ENKEPHALINASE ---------- 52

(C) SPECIAL OBJECTIVES ---------- 55

MATERIAL AND METHODS OF ELECTROACUPUNCTURE IN MICE ---- 61

CHAPTER 1 ---------- 64

COMPARISON OF TWO TECHNIQUES OF BEHAVIORAL MEASUREMENTS IN MICE USING NOXIOUS RADIANT HEAT STIMULATION

CHAPTER 2 ---------- 70

ELECTROACUPUNCTURE ANALGESIA IS MEDIATED BY STEREOSPECIFIC OPIATE RECEPTORS AND IS REVERSED BY ANTAGONISTS OF TYPE I RECEPTORS

CHAPTER 3 ---------- 88

DEXAMETHASONE PARTIALLY REDUCES AND 2% SALINE ABOLISHES ELECTROACUPUNCTURE ANALGESIA: THESE FINDINGS IMPLICATE PITUITARY ENDORPHINS

CHAPTER 4 ---------- 99

ELECTROACUPUNCTURE ELEVATES CORTISOL LEVEL IN NAIVE HORSES, SHAM TREATMENT HAS NO EFFECT

CHAPTER 5 ---------- 106

ELECTROACUPUNCTURE ANALGESIA COULD BE MEDIATED BY AT LEAST TWO PAIN-RELIEVING MECHANISMS; ENDORPHIN AND NON-ENDORPHIN SYSTEMS

CHAPTER 6 ---------- 120

MONOAMINERGIC MECHANISMS OF ELECTROACUPUNCTURE ANALGESIA

CHAPTER 7 ---------- 160

CORRELATION OF GENETIC DIFFERENCES IN ENDORPHIN SYSTEMS WITH ANALGESIC EFFECTS OF D-AMINO ACIDS IN MICE

CHAPTER 8 ---------- 170

A COMBINED TREATMENT WITH D-AMINO ACIDS AND ELECTROACUPUNCTURE PRODUCES A GREATER ANALGESIA THAN EITHER TREATMENT ALONE; NALOXONE REVERSES THESE EFFECTS

CHAPTER 9 ---------- 182

TWO ELECTROACUPUNCTURE TREATMENTS THREE HOURS APART CAN INDUCE A HIGHER ANALGESIA THAN ONE TREATMENT ALONE

CHAPTER 10 ---------- 191

ELECTROACUPUNCTURE TREATMENT OF MORPHINE DEPENDENT MICE REDUCES SIGNS OF WITHDRAWAL, WITHOUT SHOWING CROSS-TOLERANCE

GENERAL DISCUSSION ---------- 204

I. LINES OF EVIDENCE SUPPORTING THE EAA-ENDORPHIN HYPOTHESIS ---------- 204

II. LINES OF EVIDENCE SUGGESTING EAA-SEROTONIN MECHANISMS ---------- 208

III. OTHER CONSIDERATIONS ---------- 210

IV. AN INTRICATE SYSTEM IS PROPOSED FOR EAA ----- 214

V. POSSIBLE ANATOMICAL SITES OF EAA MECHANISMS - 216

(A) LOCAL ---------- 216

(B) REGIONAL ---------- 218

(C) GENERAL ---------- 220

SUMMARY ---------- 222

REFERENCES FOR THE WHOLE THESIS ---------- 228

APPENDIX

(A) STATICAL ANALYSIS OF THE NORMAL (CONTROL) SQUEAK THRESHOLDS OF B6AF1/J MICE ---------- 264

(B) LIST OF ABBREVIATIONS ---------- 266

(C) GENERAL HISTORY OF ACUPUNCTURE IN CHINESE MEDICINE 268

(D) TRADITIONAL THEORY OF ACUPUNCTURE ---------- 272

Abstrait ---------- 275

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ABSTRACT

It has been hypothesized (Cheng, M.Sc. thesis, 1977) that electroacupuncture analgesia (EAA) is mediated by endorphins, the endogenous morphine-like peptides. According to this previous hypothesis electroacupuncture (EA) stimulates the periaqueductal gray (PAG in the midbrain) to release enkephalins which will activate the raphe-DLF (dorsolateral fasciculus) descending inhibitory system to block the pain messages (nociception) at the spinal cord level. In parallel, EA may stimulate the pituitary to release endorphins to produce analgesia.

To further explore this hypothesis, several investigations were carried out on the mechanisms of EAA as related to endorphins and the monoamines (serotonin, dopamine and norepinephrine). EA experiments were carried out on B6AF1/J mice which were put in paper receptacles. Noxious responses to radiant heat were measured by the latencies to squeak. EA was applied by inserting stainless steel needles into the acupuncture point, HoKu (the first dorsal interosseus muscles). Drug injections (intraperitoneally) were done in a blind manner. Results suggest that EAA is mediated by stereospecific opiate receptors; the chief component may be the Type I opiate receptors (the latter are located mainly in the analgesic areas in the central nervous system and may be responsible for opiate analgesia). Evidence also demon-

stated that EAA may involve pituitary endorphins and ACTH which are usually released together. Dexamethasone, a cortisone derivative, suppressed EAA probably by a negative feedback inhibition of pituitary release. Two percent saline, which depresses pituitary endorphins also reduced the effectiveness of EAA. The ACTH released by EA causes elevated cortisol levels and a study shows that EA increases blood cortisol levels in horses. Several experiments (including one in this thesis) suggest that the D-amino acids (DAA), D-phenylalanine and D-leucine, produce analgesia by protecting endorphins from enzymatic degradation. The combined treatments with EA and DAA produced a higher analgesia in mice than either treatment alone. This suggests that EA may release endorphins which are protected by DAA, and hence, EA plus DAA together produce a higher analgesia. The present study also shows that EAA does not show cross-tolerance with morphine and that EA reduces signs of withdrawal in the morphine addicted mouse. In another experiment it was found that low frequency (4 Hz) EAA may be mediated by endorphins while high frequency (200 Hz) EAA may be mediated by serotonin. Further EA experiments show that dopamine and norepinephrine modifying drugs do not give coherent results while those with serotonin manipulations are very consistent.

By combining the results in this thesis with those in the literature I propose an intricate system for the mechanisms of EAA (Figure A). EA at 4 Hz may stimulate the sensory afferent nerves to several brain regions. In the midbrain PAG, these stimuli release enkephalins which activate the raphe nuclei to send descending inhibition along the DLF to the spinal cord and block incoming pain messages. The DLF-descending inhibitory system may be partly mediated by the neurotransmitter, serotonin. In parallel, EA (4 Hz) may stimulate the beta-endorphin neurons in the hypothalamus and pituitary. The pituitary endorphins may be released into the blood circulation, or may backflow directly into the CSF (the endorphins in the circulation have to pass through the blood-brain barrier to bind to the opiate receptors in the brain for analgesia). If the acupuncture points and the painful areas are in the same segmental levels, EA (4 Hz) may also directly stimulate the release of endorphins in the spinal cord. High frequency (200 Hz) EAA may activate sensory nerves which directly stimulate the DLF-descending serotonin inhibitory systems, thus by-passing the PAG-endorphin systems.

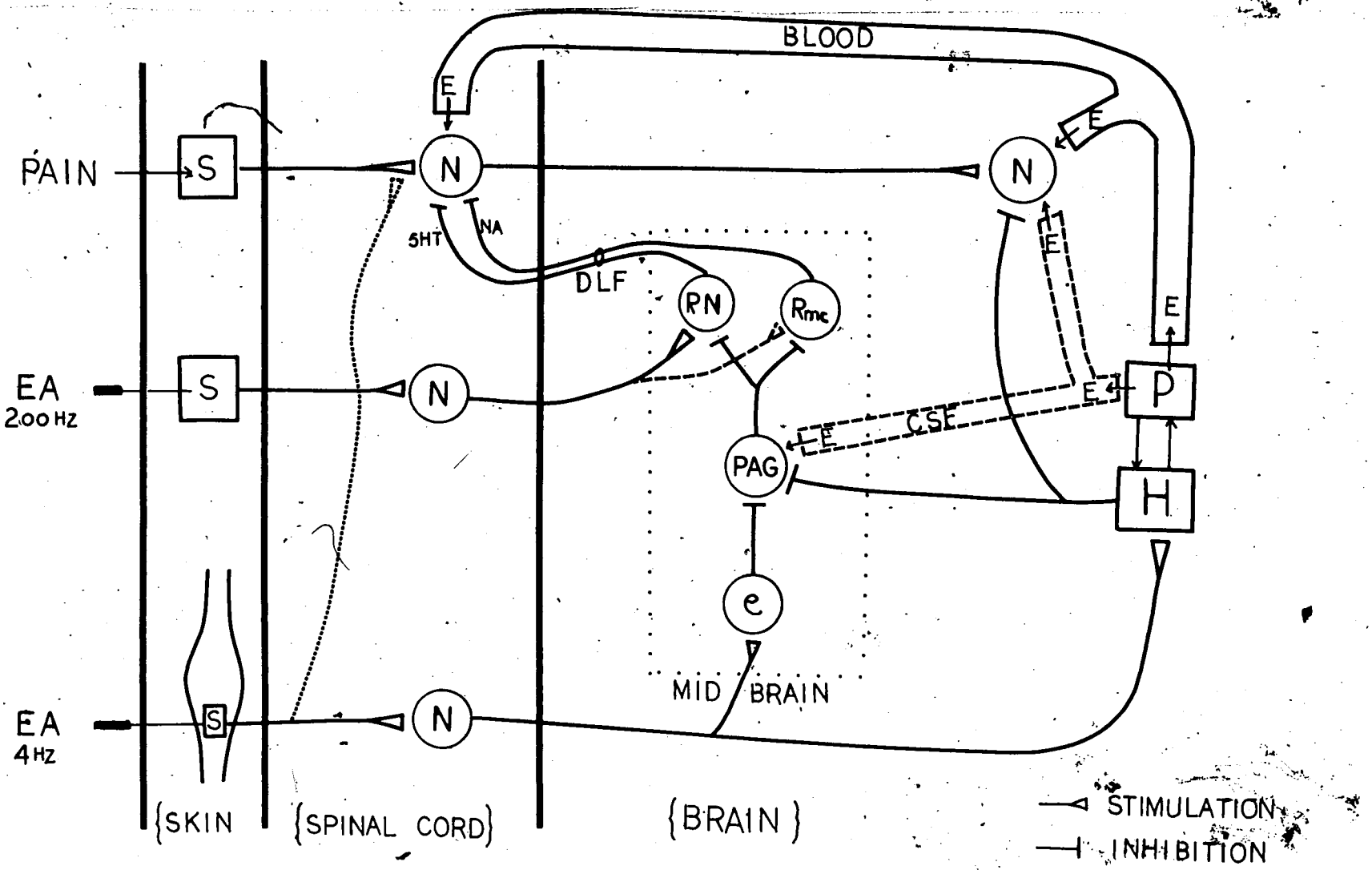
In summary, low frequency (4 Hz) EA may stimulate the release of endorphins in the spinal cord, midbrain, hypothalamus and pituitary for pain-relief, while high frequency (200 Hz) EA may directly stimulate the DLF-serotonin inhibitory systems, thus avoiding the endorphin links.

Figure A

At low frequency(4Hz), 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(4Hz) 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 (200Hz) EA appears to stimulate directly the RN and Rmc descending inhibitory systems, bypassing the endorphin system.
(Details of these systems are described in the text.)

FIG A



EA : ELECTROACUPUNCTURE
S : SENSORY RECEPTOR
N : INTERNEURON
e : ENKEPHALINERGIC NEURON
E : ENDORPHIN (β-ENDORPHIN or DYNORPHIN)
NA : NORADRENALINE

H : HYPOTHALAMUS
P : PITUITARY
PAG : PERIAQUEDUCTAL GRAY
RN : RAPHE NUCLEUS
R_{MC} : RETICULAR MEGNOCELLAR NUCLEUS
DLF : DORSOLATERAL FUNICULUS.

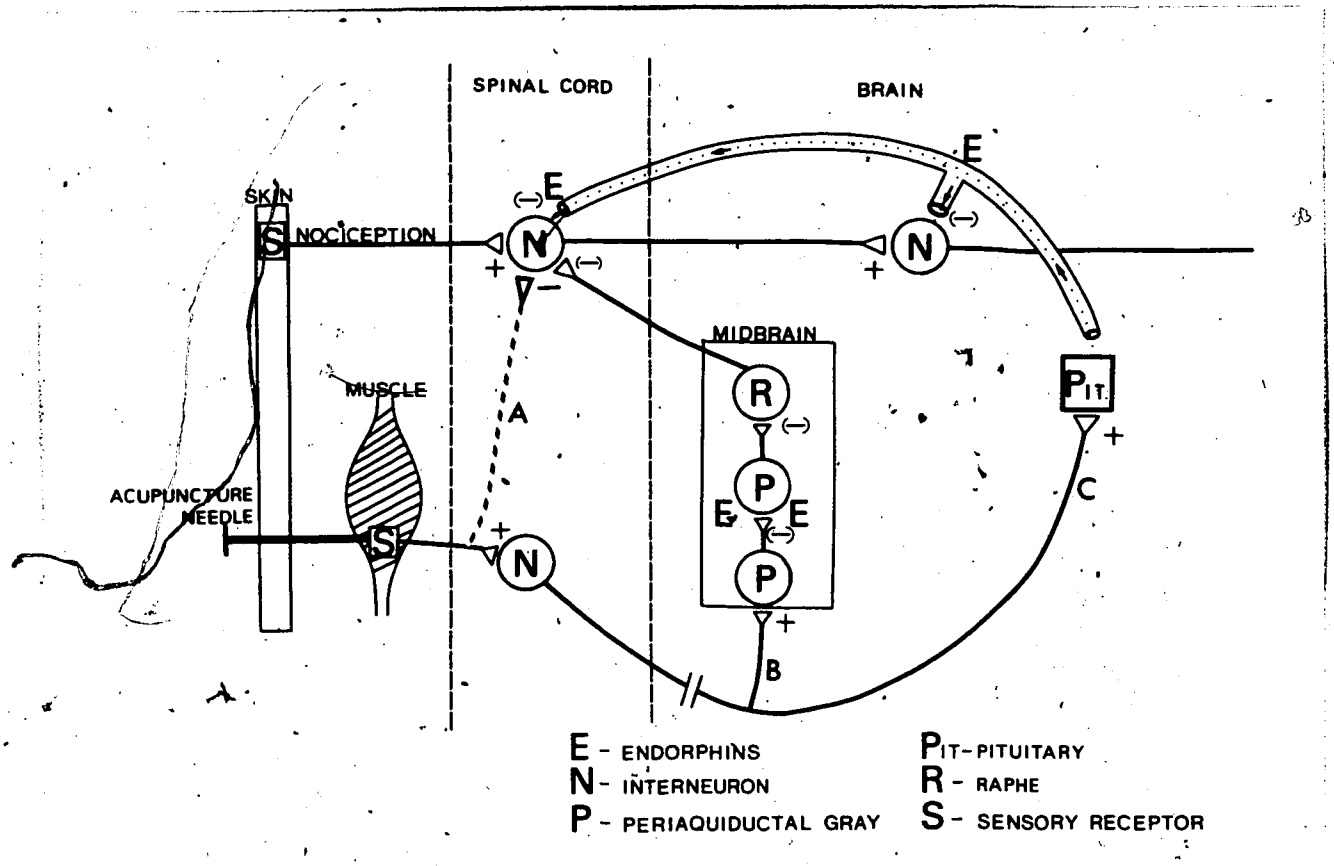
INTRODUCTION

(A) GENERAL INTRODUCTION

In a previous study (Cheng, M.S. thesis, 1977, Pomeranz and Cheng, 1979) it was hypothesized that electroacupuncture analgesia (EAA) might be mediated by endorphins, the endogenous morphine-like peptides. This is based on the observation that naloxone, an opiate antagonist, blocked electroacupuncture (EA) effects in cat spinal cord neurons and hypophysectomy abolished EAA in mice (the pituitary contains beta-endorphin). Figure 1 is a schematic diagram summarizing the hypothesis proposed in my master's thesis. It was postulated that EA may stimulate the periaqueductal gray in the midbrain to release enkephalins which will activate the raphe-DLF (dorsolateral fasiculus) descending inhibitory system to block the pain messages (nociception) at the spinal cord level via serotonin. In parallel, EA may stimulate the pituitary to release endorphins into the blood circulation; these pituitary endorphins may go through the blood-brain barrier and bind to the opiate receptors in the brain and spinal cord to produce analgesia. In the past few years, numerous studies have indicated that this endorphin-acupuncture hypothesis may be valid (Mayer et al, 1977; Pomeranz and Chiu, 1976; Sjolund et al, 1977; Pomeranz and Cheng, 1979; Chapman and Benedetti, 1977; Han, et al 1979; Tsou, et al 1979).

Figure B

Acupuncture to a sensory receptor in the deep muscle causes the brain to stimulate the pituitary gland (and/or midbrain) to release endorphin which in turn bind with the opiate receptors in the pain cells to block pain stimuli. The Master's thesis included: (i) injecting naloxone which prevented endorphins from binding with the pain cells. (ii) cutting central nervous processes and preventing pituitary and mid-brain stimulation. (iii) removal of the pituitary.



To further explore the mechanism of acupuncture and its relation to endorphins and other neurochemicals, investigations were carried out on the mechanisms and the functions of electroacupuncture. In order to do so, I attempted to block EAA by suppressing endorphins, serotonin, noradrenaline and dopamine. I also examined the enhancement of EAA by increasing these neurochemicals. Consistent results were obtained only for endorphins and serotonin. The general objectives of this thesis can be summarized as follows:-

(i) To investigate whether EAA is mediated by stereospecific opiate receptors and to determine what type of opiate receptors might be involved.

(ii) To give evidence that pituitary endorphin, ACTH and cortisol are involved in EAA.

(v) To enhance EAA by using D-amino acids that may protect endorphins from enzymatic degradation.

(iii) To differentiate the role played by endorphins and the DLF-serotonin inhibitory system in EAA.

(iv) To test the monoaminergic mechanisms of EAA by using drugs that can affect either serotonin, dopamine or norepinephrine levels in the CNS.

(vi) To see if there is cross-tolerance between EAA and morphine.

(B) LITERATURE SURVEY

(i) PAIN

In human experience, pain is one of the most debilitating symptoms known to man. Pain can be roughly categorized into two classes - acute and chronic. In acute stages, pain serves as an alarm system warning us that something biologically harmful is happening to our body, and in chronic stages, pain often causes drastic emotional and physical stress. It has been estimated that chronic pain costs the American people alone between 40 to 50 billion dollars per year (Bonica, 1979). This economic burden and physiological disorder induced by pain creates a serious national and worldwide health problem. The mechanism of pain, therefore, has been investigated by numerous professionals in the field of psychology, biology and medicine. The contributions made by these people toward understanding the mechanisms of pain have often given rise to conflicting observations - the result being an overabundance of interpretations of pain mechanisms. In spite of this, a general understanding of pain preception and transmission has prompted a great deal of research.

Psychologically and physiologically, scientists try to classify pain and its pathways into three aspects (Melzack, 1973):-

(i) The sensory-discriminative dimension -- this is a sensory type of pain and is subserved, at least in part, by the neospinothalamic projection to the ventrobasal thalamus and the somatosensory cortex, (via anterolateral thalamic tract, ALT).

(ii) The motivational-affective dimension -- this is the emotional type of pain and is believed that this involves the brainstem reticular formation and the limbic system which receive projections from the spinoreticular and paleospinothalamic components of the anterolaleral somatosensory pathway (via ALT).

(iii) The cognitive-evaluative dimension -- it is known that cultural values, anxiety, attention and suggestion have a profound effect on pain experience which may involve the cortical control on the sensory-discriminative and motivational-affective dimension of pain.

However, pain often arises from intense stimuli that cause tissue damage. It is believed that this tissue damage leads to an accumulation of certain chemicals, such as histamine, bradykinin, prostaglandin E etc. which induce the discharge of afferent nerve impulses. Lindahl (1974), with the use of a pH microelectrode in the tissues, has found that pH is very acid in the tissues where the bare nerve endings are situated. Thus he has proposed that a pH change is the final chemical change that causes pain. However pain is also elicited by mechanical deformation of simple free nerve endings (Basbaum and Fields, 1978).

Many hypotheses have been proposed to explain the mechanisms of pain. Some of them are still considered to be useful in explaining certain physiological phenomena:-

(1) The specific modality theory hypothesizes that pain is detected by high threshold free nerve endings and transmitted through small afferent fibres (like A-delta or C fibres) and the spinothalamic tracts to the focal pain centres in the brain.

(2) The pattern theory proposes that all sensory inputs are transmitted through the same pathway but are interpreted differentially according to the spatiotemporal pattern of the sensory impulses at the higher nervous centre.

(3) The gate control theory which was proposed by Melzack and Wall (1965) suggests that a gate for the control of pain exists at the spinal cord level. Pain can be facilitated or inhibited according to the quantity of small afferent and large afferent inputs. However, the facilitation of pain by primary afferent hyperpolarization (PAH) does not exist; only primary afferent depolarization (PAD) exists in large fibres (Zimmerman, 1977). Thus the gate control theory as described by Melzack and Wall (1965) requires a neurophysiological revision. Despite the controversy and conflicting evidence, the gate control theory gave a stimulating idea on the mechanism of pain and widened the field of pain research. Melzack (1973) later stressed that this gate is also subjected to the influence of higher brain centres.

I. Primary Afferent Neurons -

There is convincing physiological evidence that specialization exists within the somaesthetic system. The skin receptors have specialized physiological properties, yet, free nerve-endings may all look alike despite their highly specialized properties. Recently, Hensel and Andres (1974) found a correlation between the structure of the receptor and the afferent discharge. They marked the sensitive spot on the skin, and excised it and examined it with the electron microscope. They claimed to have found distinctive structure for the free nerve endings.

The special properties of different primary afferent neurons have been extensively studied. They were briefly summarized as follows (Price and Dubner, 1977):-

A. Mechano-sensitive neurons

(i) High threshold A-delta mechanoreceptive afferents -

These neurons respond only to intense mechanical stimuli and have been found in the skin of cat and monkey extremities (Beck et al, 1974; Burgess and Perl, 1967; Burgess and Perl, 1973; Dykes, 1971; Georgopoulos, 1976; Perl, 1968) and monkey facial skin (Dubner et al, 1974). Conduction velocities usually range from 15 - 25 m/sec and receptive fields on limbs are from 1 to 8 sq cm, with more than one sensitive spot.

(ii) Low sensitivity and moderate pressure A-delta mechanoreceptive afferents -

These neurons are found in the same area as (i) but respond to tissue - threatening and tissue - damaging stimuli (Burgess, 1974; Burgess and Perl, 1973).

(iii) High threshold C mechanoreceptive afferents -

These are superficial unmyelinated neurons innervating the extremities of cats and monkey. They have small receptive fields of small C fibre population (Bessou and Perl, 1969; Fields, et al, 1975). Some of these neurons in cats also respond to cold temperature (Bessou and Perl, 1969; Burgess and Perl, 1973).

(iv) Low threshold myelinated (AB and A-delta) mechanoreceptive afferents -

These neurons are sensitive to weak mechanical stimuli and show no differential response to noxious mechanical stimuli (Burgess, 1974; Burgess and Perl, 1973; Perl, 1968). Most of these neurons function as position and/or velocity detectors on the skin (Burgess and Perl, 1973). They synapse with the spinal cord interneurons that project to the dorsal column, spinocervicothalamic, spinothalamic and spinoreticular pathways. Electrical stimulation of AB fibres in the periphery or dorsal columns does not produce pain. However, some low threshold, slowly adapting AB mechanosensitive afferents converge on spinothalamic and trigeminothalamic neurons that most likely participate in the sensory - discriminative aspect of pain (Dubner et al, 1976; Price et al, 1976; Price and Mayer, 1974; Price and

Mayer, 1975) and their activity may summate with nociceptive input terminating on these central neurons. They may play a role in pain modulation as their major effects are to suppress spontaneous activity and nociceptive responses of various types of spinal cord neurons (Cervero et al, 1976; Foreman et al, 1976; Handwerker et al, 1975; Hillman and Wall, 1969). Electroacupuncture or transcutaneous-electrical-stimulation effects may be mediated by these slowly adapting low threshold AB fibres and/or A-delta fibres (Lu et al, 1979).

(v) Low threshold unmyelinated c mechanoreceptive afferents -

These neurons respond to weak, slowly moving mechanical stimuli (Bessou and Perl, 1969; Burgess and Perl, 1973). They comprise about 50% of the C fibres in cat, 10% in monkey (Beitel and Dubner, 1976; Burgess and Perl, 1973; Georgopoulos, 1976) and none in humans (Torebjork, 1973; Van Hees and Gybels, 1972).

B. Thermosensitive Neurons

(i) "Cold" A-delta thermoreceptive afferents -

These respond to small decreases in temperature (≤1 C) with receptive fields (single spots) less than 500 um in diameter. They may respond to steady-state temperature in the noxious heat range (45-52 C)(Boring, 1942; Dodt and Zotterman, 1952) and exhibit spontaneous activity at 20 - 30 C range.

(ii) "Warm" A-delta and C thermoreceptive afferents -

These neurons are similar to (i) and are sensitive to <1 C changes in temperature, but warming increases and cooling suppresses their discharges (Hensel, 1971). Regular neuronal discharges are seen at steady-state temperature in the range 30 - 43 C. They comprise 50% of A-delta fibres in the monkeys face (Dubner et al, 1975; Sumino et al, 1973).

(iii) High threshold A-delta and C thermoreceptive afferents -

These fibres receive wide-dynamic range of stimuli and very few of them respond only to noxious heat or cold (Beck, 1974; Georgopoulos, 1976; Iggo, 1959).

C. Mechanical-thermosensitive neurons

(i) A-delta heat nociceptive neurons

These neurons can be found in limb and facial skin of monkey (Burgess and Perl, 1973; Dubner and Beitel, 1976; Dubner et al, 1976; Georgopoulos, 1976; Iggo and Ogawa, 1971) and limb skin of cat (Beck, 1974). The receptive fields are usually less than 5 sq. mm and respond to a temperature range of 45 - 53 C as well as to non-noxious mechanical stimuli.

(ii) C polymodal nociceptive afferents -

These fibres constitute 80 - 90% of the primate C fibre population (Beitel and Dubner, 1976; Burgess and Perl, 1973) and respond to high threshold mechanical and

thermal stimuli as well as irritant chemical stimuli. Non-noxious mechanical (<1g) and heat (>38-40 C) stimuli also activate these neurons. Strong evidence indicates that C polymodal nociceptors are the important afferents that signal the presence of tissue damage.

(iii) Mechanical cold A-delta and C nociceptive afferents

Several reports (Burgess and Perl, 1973; Georgopoulos, 1976; Iggo, 1959; Iriuchyima and Zotterman, 1960) indicate that these primary afferents respond only to noxious cold and high threshold mechanical stimuli.

II. Spinal Cord

By using different techniques such as suppressive silver staining and axonal transport, LaMotte (1977) has demonstrated that small fibres end in laminae I, II and III whereas large fibres are believed to end in laminae IV, V and VI. The primary afferent inputs to dorsal horn have been studied by many researchers and are described in a review by Kerr and Casey (1978). Evidence has also indicated that the medial aspect of the tract of Lissauer exerts a facilitatory effect and that the lateral part is inhibitory (Denny Brown et al, 1973).

Rexed (1952) divided the dorsal gray into nine layers (laminae) according to the different sizes of the neurons. In general, Cervero et al (1976) have classified the dorsal horn neurons into 3 types:-

(i) Class I - excited by cutaneous mechanoreceptors, these

neurons are located in lamina IV and some in V.

(ii) Class II - excited by both mechano and nociceptors; these neurons are the wide-dynamic range neurons described by Price and Mayer (1975), Iggo (1974) and Cervero et al (1976). These neurons are located in lamina V.

(iii) Class III - respond only to noxious input, some of them receive A-delta input and the others may be excited by both A-delta and C fibres; these neurons are located mostly in lamina I and occassionally in lamina II; thus they may represent another type of nociceptive processing as compared to class II neurons.

Kumazawa and Perl (1978) have reported that A-delta input is localized to neurons in the marginal zone while the C-fiber inputs synapse mostly in the substantia gelatinosa. Visceral and somatic activities usually converge on single neurons in deeper layers such as lamina V, VI and VII of the dorsal horn (Pomeranz et al, 1968). In conclusion, it appears that two populations of nociceptive neurons exist in the dorsal horn; the marginal neurons, which are mostly excited by high threshold polymodal nociceptors, and the lamina V cells which respond to wide dynamic range of thresholds. By means of retrograde axoplasmic transport studies and recordings of the antidromic spikes, neurons from layers I,IV,V,VI and VII show projections to the thalamus (Trevino et al, 1973; Trevino and Carstens, 1975), and layer VIII cells show projection to reticular formation (Albe- Fessard et al, 1974). The existence of different types of neurons in

lamina I to V has been extensively reviewed in my M.Sc. Thesis (Cheng, 1977).

III. Ascending pain-signalling pathways

Experimental evidence indicates that nociceptive transmition is by many pathways. A review (Dennis and Melzack, 1977) shows that there are six pain-signalling systems: three lateral and three medial. The lateral group is comprised of:- (1) the neo-spinothalamic tract, (2) the spino cervical tract and (3) the postsynaptic tract of dorsal column. The medial group is formed by (4) the paleospinothalamic tract, (5) the spinoreticular tract and (6) the diffuse polysynaptic- propriospinal tract. The medial groups differ from the lateral groups by having slower conduction velocity, cell bodies located more deeply in the spinal gray and different patterns of termination. Obviously, the two groups would exert different functions in pain-signalling systems. Moreover, all six pain-signalling pathways (either lateral or medial) have different anatomical routes ascending from the spinal cord to the brain and are controlled by different pain-inhibitory systems from the brain.

The detailed studies of these pain- transmitting pathways are thoroughly reviewed by Dennis and Melzack (1977). They are briefly discussed as follows:-

(1) Neo-spinothalamic tract (nSTT) - it ascends from ventral and ventrolateral regions of the spinal cord to the

thalamus. In primates, 30% of the tract fibres respond to intense mechanical or thermal stimuli, 38% to hair movement, 21% to light pressure and 11% to deep, subcutaneous stimulation (Trevino et al, 1974). It is estimated (Price and Mayer, 1974) that 12.2% of ventrolateral cord fibres respond only to noxious stimuli, 26.8% to gentle tactile stimuli and 61% to both light and noxious stimuli. Thus both light tactile and pain-related information are carried by the neospinothalamic tract in primates (Albe-Fessard et al, 1974; Foreman et al, 1975; Price and Mayer, 1974; Willis et al, 1974) and cats (McCreery and Bloedel, 1975; Manfredi and Castelluci, 1969; Pomeranz, 1973).

(2) The spinocervical tract (SCT) - postsynaptic fibres ascend the dorsolateral spinal funiculus to the lateral cervical nucleus which projects to the somatosensory thalamus and adjacent areas and to the reticular formation through the medial lemniscus (Morin, 1955). Bryan et al (1974) showed that recording from 25 SCT cells which respond to light tactile stimuli, 11 (44%) increased discharge to intense mechanical stimulation; 6 of 16 cells were sensitive to noxious heat while one responded only to noxious stimulation. About 75 % of the fibres ascend ipsilaterally to the lateral cervical nucleus and 25% go to the rostral dorsal column nucleus (Dart and Gordon, 1973; Gordon and Grant, 1972; Nijensohn and Kerr, 1975; Rustioni, 1973; Rustioni and Molenaar, 1975; Tomasulo and Emmers, 1972). The SCT is vestigial in some people as the lateral cervical

nucleus was detected only in 9 out of 16 human spinal cords (Kerr, 1975). In conclusion, a high percentage of the SCT fibres respond to peripheral A-delta and C fibre stimulation (Brown et al, 1974; Brown et al, 1973; Mendell, 1966), showing the "wind-up" effect which may be related to slow pain (Mendell, 1966; Mendell and Wall, 1965).

(3) The dorsal column system - It has been known for decades that the primary afferent fibres ascending to the dorsal column nuclei (DCN) carry only innocuous tactile and proprioceptive sensation. However Uddenberg (1968 a &b) showed that the dorsal column postsynaptic (DCPS) fibres transmit noxious messages. Recording from the cervical dorsal column of cats, he found that 79 out of 295 axons responded to small peripheral fibre (A-delta and C fibres) stimulation. Angaut-Petit (1975 a & b), and Petit (1972) confirmed Uddenberg's finding by showing that 92 units (9.3% of the DC fibres) were DCPS axons of which 77.2% were sensitive to both gentle and noxious stimuli, 6.5% only to noxious mechanical stimuli and the rest only to light mechanical stimuli. Thus pain-signalling information may be carried by the DCPS to the dorsal column nuclei which project to various thalamic and collicular regions and to the zona incerta by the medial lemniscus, and eventually relay to cortex (Boivie, 1971; Hand and Liu, 1966; Lund and Webster, 1967).

These three lateral systems have several similar dimensions of pain signalling. Each has rapid conduction velocity, carries noxious, thermal and light tactile sensations. They all originate from the dorsal horn of the spinal gray and project mainly to the lateral thalamus (ventrobasal, posterior and subthalamic complexes). However, qualitative and quantitative differences exist; for example about 6.7% (Angaut-Petit, 1975) of the feline DCPS cells respond exclusively to noxious stimulation, while 20% of the SCT do so (Brown and Franz, 1969; Willis et al, 1974). The inhibitory controls on these three system can be achieved by stimulating many spinal cord and brain areas (Brown, 1970; Brown, 1971; Brown et al, 1974; Brown et al, 1973; Brown et al, 1972) such as the contralateral dorsalateral and ventromedial cord, the dorsal column, the mesencephalic tegmentum, central pontobulbar core and several cerebellar regions (Taub, 1964) and specific cortical regions (Brown, 1974; Brown et al, 1975). The inhibitory control of SCT appears to be both cortical and subcortical because decerebration does not abolish SCT inhibition (Brown, 1971). The inhibitory effects are mostly exerted on noxious signals in the SCT system leaving the light tactile responses unaltered (Brown, 1971; Brown, 1970; Zimmerman and Handwerker, 1974). In contrast, cortical stimulation only inhibits the light tactile inputs, leaving the nociceptive responses (especially from lamina I) intact in the nSTT units (Coulter et al, 1974; Coulter et al, 1975). Inhibition of the noxious

input in the nSTT cells can be triggered by stimulating the nucleus raphe magnus (Beall and Martin, 1976) and the caudal part of the nucleus gigantocellularis (McCreery and Bloedel, 1975). Recently, J.Dostrovsky found that stimulating nucleus raphe magnus caused some inhibition of non-noxious inputs (personal communication).

The three medial pain-signalling systems:-

(4) The paleo-spinothalamic tract - also ascends from the ventral and ventrolateral regions of the spinal cord but projects to the midline and intralaminar thalamic nuclei (Meher, 1969; Mehler, 1957). Nociceptive signals have been detected in the midline-intralaminar nuclei (Albe-Fessard and Besson, 1973; Albe-Fessard and Kruger, 1962; Casey, 1966; Kruger and Albe-Fessard, 1960; Perl and Whitlock, 1961).

(5) The spinoreticular tract - it ascends from the ventral and ventrolateral regions of the spinal cord and projects to various sites of reticular formation and brain stem central grey (Mehler, 1962; Pompeiano, 1973). The spinal input to the reticular formation is diffuse, multisynaptic and inter-related to other sensory modalities (Bell et al, 1964).

(6) Propriospinal tract - nociceptions may be carried up and down the spinal cord along the ventral and lateral region of the spinal grey, forming synapses with other sensory systems or pathways. This tract is listed here for completeness.

IV. Endogenous pain control mechanisms

The recent advances that indicate several types of endogenous pain control systems have been extensively reviewed by Basbaum and Fields (1978). Evidence shows that there are several pain-suppression systems (Basbaum and Fields, 1978) which can be summarized as follows:-

(i) Segmental system - pain transmission neurons can be inhibited at the spinal cord level by stimulating the large peripheral fibres (e.g. AB) or the dorsal column fibres. This agrees with the Gate control theory proposed by Melzack and Wall (1965). (ii) Medial system - ascending pain-transmission by the medial (paleospinothalamic and spinoreticular) tracts may activate the feedback loop which induce descending inhibitions on the pain-transmission cells. The major sites for this medial descending inhibition are the periaqueductal gray (PAG), the dorsal raphe nucleus, the nucleus raphe magnus (NRM) and the nuclus reticularis magnocellularis (Rmc). Stimulation-produced-analgesia (SPA) by implanting electrodes at PAG is partially blocked by naloxone (an opiate antagonist) (Akil et al, 1976) or is not blocked by naloxone in cats (Carstens et al, 1979) while SPA produced by stimulating NRM is completely blocked by naloxone (Oliveras et al, 1977). The PAG projects to both NRM and Rmc which have projections to identical zones of the dorsal horn via the dorsolateral funiculus (Basbaum and Fields, 1977). Since the NRM is serotonergic and has an enkephalin link, and the Rmc is not serotonergic, it is hypothesized that the medial descending inhibitions have two

mechanisms:- enkephalin-serotonergic and non-endorphinergic systems. By using the method of retrograde horseradish peroxidase transport and midthoracic cord lesions, Basbaum and Fields (1977) were able to show that neurons in other brain regions, expecially the locus coerulus and the hypothalamus also have projections in the DLF. Since locus coerulus is rich in catecholamine and opiate receptors (Basbaum, 1973), endogenous opiate (endorphin), serotonin and catecholamines are important in mediating this descending inhibitory system. Recently, evidence suggests that noradrenaline could be one of the neurotransmitters that is involved in the descending pain inhibition (Yaksh, 1979).

(iii) Lateral system - Mesencephalic tractotomy, which isolates the medial system, results in spontaneous diffuse, burning pain; while electrical stimulation of the central postero-lateral and ventral posteromedial (VPL-VPM) thalamic nuclei inhibits spontaneous pain (Hosobuchi et al, 1973). This pain-relieving system is not antagonized by naloxone (Hosobuchi et al, 1977) and is not endorphinergic.

The detection of stereospecific opiate receptors (Pert and Snyder, 1973) led to the discovery of endogenous opioid peptides - endorphins (Hughes et al, 1975; Li et al, 1976; Terenius and Wahlstrom, 1975). Numerous studies demonstrated that endorphins are major endogenous neurosubstances that mediate pain-suppression. The recent advances in endorphin research will be reviewed later.

Substance P is a potent hypotensive peptide first discovered by Von Euler and Gaddum (1931). It is identified to be an undecapeptide by Chang and Leeman (1970) and is concentrated in synaptosomal fractions of the substantia nigra, hypothalamus and dorsal horns of the spinal cord (Chang et al, 1971). Microiontophoretic application of substance P excited neurons in the spinal cord and the brain (Davis and Pray, 1976). Recent evidence indicates that substance P is a neurotransmitter at the terminals of the small primary afferent (A-delta and C) fibres and coexists with other putative neurotransmitters e.g. serotonin in some specific areas (Leeman, 1979). Immunohistochemical analysis revealed that enkephalin and substance P neurons have intimate spatial relation in the spinal cord and brain stem (Hokfelt et al, 1977). Met-enkephalin and substance P are found to localize in subcellular organelles in axon terminals in the locus coeruleus and A2 region of rat brain; these axon terminals form synapses with dendrites of the catecholaminergic neurons (Pickel et al, 1979). Evidence also shows that acetylcholine, met-enkephalin and substance P are three separate independent inputs on the noradrenaline - containing cells (Guyenet and Aghajanian, 1979).

Primary sensory neuron culture shows that substance P is synthesized by some of these neurons (Leeman, 1979). Depolarization with high K+ releases this peptide from the neurons and the release can be inhibited by enkephalin, serotonin, GABA or norepinephrine (Leeman, 1979; Mudge et

al, 1979). If substance P is the neurotransmitter that plays a role in the perception of pain, the closely associated enkephalin neurons could be the antagonist that inhibits the noxious input. Intraperitoneal or intracerebroventricular administration of substance P produces analgesia which is naloxone reversible (Stewart et al, 1976). Intraventricular application of small doses of substance P (1.25 to 5 ng/mouse) produces analgesia which is naloxone reversible, but at higher doses (50 ng/mouse), substance P produces hyperalgesia when combined with naloxone, and analgesia when combined with baclofen (4 chlorophenyl - gamma-aminobutyric acid); thus it is postulated that substance P releases endorphins at very low doses and directly excites neuronal activity in nociceptive pathways at higher doses (Frederickson et al, 1978).

Thus the endogenous pain control systems can be divided into two main classes:-

(A) The endorphin system - this can be subdivided into two levels of analgesia:-

(i) The enkephalin-serotonergic mediation - electrical stimulation of PAG which releases enkephalin (Akil et al, 1978) or microinjection of opiates into PAG will send down a descending inhibition along the DLF into the spinal cord and this descending inhibition is mediated by serotonin. Recently, Yaksh and Tyce (1979) showed that serotonin was released in the spinal cord by microinjecting morphine into PAG. Also serotonin blockers decreased SPA (Yaksh, 1979).

I have also studied the relationship of serotonin system to EAA.

(ii) Beta-endorphin mediation - this 31 amino-acid peptide is mainly found in the pituitary and hypothalamus. Intraventricular (Hosobuchi and Li, 1978) or intravenous injection (Loh et al, 1976) of beta-endorphin cause profound analgesia in human and animals. Stress analgesia elevates blood beta-endorphin levels (Rossier et al, 1977) and SPA increases ventricular beta-endorphin (Hosobuchi et al, 1979).

(B) Non-endorphin systems - SPA produced by stimulating the dorsal column (Hosobuchi, 1979), ventral posteromedial thalamic nuclei (Hosobuchi et al, 1973) or possibly locus coerulus (Basbaum and Fields, 1978) are not antagonized by naloxone (Hosobuchi et al, 1977). Catecholamine may be one of the mediators for these inhibitory system. Yaksh (1978) observed that the inhibition of the spinal sensory system by the PAG opiate complex associated with antinociception appeared to be mediated by the combined excitation of serotonin and noradrenaline terminals in the spinal cord. Thus serotonin and noradrenaline may be the chief mediators of SPA. Whether catecholamine and endorphins are closely related or exist in parallel systems in endogenous pain-control is still not clear. However further research is necessary to determine their mechanisms.

(ii) ENDORPHINS

I. Opiate Receptors

Biochemical evidence indicates the existence of multiple opiate receptors (Chang et al, 1979). It has been suggested (Martin et al, 1976) and demonstrated (Della Bella et al, 1978) that there are three types of opiate receptors: μ-receptors, k-receptors and d-receptors.

Each of these receptors possesses a specific ligand:- morphine activates μ-receptors for analgesia and euphoria; ketocyclazocine activates k-receptors for feelings of sedation and benzomorphan derivative (SKF10047) activates d-receptors for dysphoric and hallucinatory syndrome (Martin et al, 1976). Recently Pert et al (1979) classified opiate receptors into two groups: Type I and Type II. The following table summarizes the properties of these two types of receptors (Pert et al, 1979):

Type I	Type II
(i) Adenylate cyclase - coupled and GTP sensitive (receptor occupancy produces cAMP production)	(i) Does not or indirectly couple to cyclase
(ii) Located in the analgesic area (e.g.paleospinothalamic pathway, midbrain), pig ileum, adrenal medulla.	(ii) Located in amygdala, hypothalamus,frontal cortex, limbic system, pituitary, vas deferens
(iii) Antagonists: naloxone, Naltrexone, diprenorphine, cyclazocine, N-Allyl-normetazocine	(iii) Naloxone resistant (no available antagonists)
(iv) Agonist Potency: Morphine> leu-enkephalin>benzomorphin	(iv) Leu-enkephalin> D-ala-enkephalin> met-enkephalin
(v) Only found in vertebrates	(v) Found in both vertebrates and invertebrates

Recently, Pasternak et al, (1979) reported that there are high and low affinity opiate binding sites. The high affinity binding sites are responsible for analgesia and the low bindings are for others. Nalozaxone, a newly discovered antagonist, binds irreversible (destroys) high affinity sites and inhibits morphine analgesia in mice (at 250mg/mouse) (Pasternak et al, 1979).

The high affinity binding sites, Type I receptors and u-receptors are probably the same type of opiate receptors which mediates analgesia (G.Pasternak and C.Pert - Personal communication).

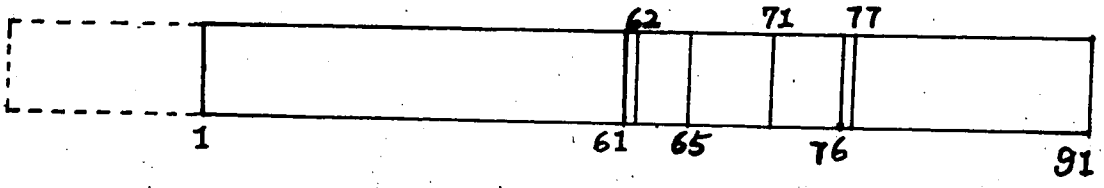
II. Types of Endorphins

The detection of the opiate receptors aroused the search for endogenous opiates. It was predicted that opiate receptors and the "endogenous opiates" served as a lock-and-key system. The hypothesis was: since there is a lock, there must be a key to fit this lock. Successful results were obtained by several groups of workers; several endogenous ligands of the opiate receptors were detected in the brain (Hughes et al, 1975; Terenius and Wahlstrom, 1975a), pituitary (Teschamacher et al, 1975), CSF (Terenius and Wahlstrom, 1975 b) and peripheral tissues (Hughes et al, 1977). These endogenous ligands, called endorphins (endogenous morphine) are peptides which have a similar effect to morphine in inducing analgesia, euphoria, relieving depression and affecting other important metabolic func-

tions (Snyder, 1977). Brain endorphin levels are independent of pituitary endorphin levels (Watson et al, 1978); biochemical and anatomical evidence indicates the existence of two separate opioid systems in brain: beta-endorphin containing neurons and enkephalin containing neurons, which constitute two separate groups of brain cells (Watson et al, 1978; Rossier et al, 1977). Different types of endorphins have been detected in the pituitary, brain spinal cord, CSF, blood plasma and peripheral tissues. The following table summarizes the number of endorphins that have been detected:-

Table 1:- Types of Endorphins

1. Methionine-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH)
2. Leucine-enkephalin (H-Tyr-Gly-Gly-Phe-Len-OH)
3. Pro-opiocortin 31,000 or 37,000 daltons
   (Precursor for ACTH and beta-LPH)



   Beta-Lipotropin (1-91)
   Beta-endorphin (61-91)
   Alpha-endorphin (61-76)
   Gamma-endorphin (61-77)
   Des-tyrl-gamma-endorphin (62-71)
   Met-enkephalin (61-65) - (Fraction II)
4. Morphine-like compound (non-peptide)
5. Beta-Leu5-endorphin (31 amino acids)
6. Dynorphin (17 amino-acids)
7. Fraction I (CSF), Plasma Factor (Plasma), unidentified brain opioid peptide (brain)
8. Hexapeptides and Heptapeptides (unnamed)
9. Alpha-neo-endorphin (12 amino acids)

Other Related Substances

1. Endorphin releasing factor (H-Tyr-Arg-OH)
2. Endogenous anti-opiate substance

III. Location of Endorphins

1. Enkephalins

Met-enkephalin and leu-enkephalin are the two pentapeptides first identified in the brain extracts by Hughes and colleagues (1975). They appear to be neurotransmitters in nerve terminals and mimic the effect of morphine by binding to opiate receptors. By comparing the localization of opiate receptors and enkephalins, it is found that they occur in similar places and correspond closely to the known bodily functions affected by opiate drugs. Met-enkephalin and leu-enkephalin are detected in similar areas. In every region studied, levels of met-enkephalin are higher than levels of leu-enkephalin. Although the concentration of met-enkephalin is generally 3 times higher than leu-enkephalin in the whole brain, the ratio is different in various brain areas and peripheral tissues (Yang et al, 1977). Recently, a 12 amino acid alpha-neo-endorphin which may be the precursor of leu-enkephalin was found in porcine hypothalami (Kangawa et al, 1979).

Immunofluorescence shows that enkephalins in the brain are confined to neurons and are mostly located in the nerve endings, while 85% of the brain supporting cells (glia) show no trace of enkephalin fluorescence (Snyder, 1977). By using colchicine to block the movement of enkephalins from cell body to nerve endings, enkephalin concentration

increases in the cell bodies and can be detected by immunofluorescence. It was found that brain areas with a high density of enkephalinergic nerve terminals also have a large number of enkephalin-containing cell bodies. It is thus suggested that enkephalins are contained in small neurons whose cell bodies, axons and nerve terminals are confined to circumscribed brain areas and spinal cord (Snyder, 1977). Both the regional distribution of high affinity binding sites for leu-enkephalin in striatum and their decreased number after chemical or mechanical interruption of the nigrostriatal bundle, support the assumption that enkephalinergic neurons terminate presynaptically on dopaminergic terminals in the striatum. This also suggests that enkephalinergic neurons do not project from mesencephalon to striatum. Ten days after intra-striatal injection of kainic acid (1 ug) (a more potent depolarizing agent then glutamate), met-enkephalin levels in the striatum decreased more than 50%. The results suggest that either neostriatum contains met-enkephalin neurons or that cell bodies of enkephalinergic neurons of striatum may be located in globus pallidus (Yang et al, 1977).

Indirect immunofluorescence revealed met-enkephalin immunoactive cell bodies in the tel-, di-, mes- and rhombenecephalon (Yang et al, 1977). In general, high concentration of enkephalins were found in the brain regions in the order of:-

Striatum>Hypothalamus>Thalamus>Hippocampus>
Pons and medulla>Cortex>>Cerebellum

Enkephalins are also found in rat pituitary (by radioimmunoassay). The levels of met-enkephalin and leu-enkephalin are highest in pars intermedia (7 and 4 p mole/mg respectively), medium in pars nervosa (2.2 and 2.2 p mole/mg respectively) and the least in pars anterior (0.51 and 0.36 p mole/mg respectively) (Duka et al, 1978). In human CSF, enkephalin concentration is about 3.12 to 3.25 p moles/ml (Akil et al, 1978).

In the spinal cord, enkephalins are located in the grey matter especially laminae I, II and III which are packed with small neurons that interact with each other and impinge on the nerve endings of the sensory neurons. Chronic lesion of the primary afferents decreases the number of opiate receptors in the dorsal horn with no loss of enkephalin in cell bodies in the spinal cord (Hokfelt et al, 1977). It is also shown that electrical stimulation (Otsuka and Konishi, 1976) or K+ provoke the release of substance P from slices of rat trigeminal nucleus and of rat substantia nigra (Jessell and Iversen, 1977), and that morphine and endorphins cause a concentration-dependent, stereospecific, naloxone antagonizable reduction of its release (Jessell and Iversen, 1977). This suggests that met-enkephalins are in neurons in the dorsal horn and that enkephalins act as presynaptic inhibitors in the spinal cord.

Enkephalins also appear in the peripheral tissues and digestive tract (Hughes et al, 1977). Large amounts of enkephalins were found in the small intestine with the highest concentration occurring in the longitudinal muscle-myenteric plexus layer and small quantities in the circular mucosal layer. Both met-enkephalin and leu-enkephalin are also found in the coeliac and superior cervical ganglia of rats (Di Giulio et al, 1978), and in adrenal medullary gland cells (Schultzberg et al, 1978).

2. Beta-endorphin

A "big" beta-lipotropin (LPH) or "big-big" beta-endorphin (pro-opiocortin) is found to be the precursor for ACTH and beta-LPH (beta-endorphin) with molecular weight of 37,000 daltons in human and 31,000 daltons in rat pituitaries (Yoshimi et al, 1978) and brain (Graf et al, 1977). The pro-opiocortin is broken down by peptidase to ACTH and beta-lipotropin and beta-lipotropin is further cleaved into beta-endorphin (Graf et al, 1977). It is believed the small amount of alpha-endorphin, gamma-endorphin, des-Tyr1-gamma-endorphin are derived from beta-lipotropin. ACTH and beta-endorphins are stored in the same secretory granules of anterior pituitary corticotrophs (Weber et al, 1978) and are secreted concomitantly by the adenohypophysis (Guillemin et al, 1977). Structural analysis of beta-LPH indicates that some of its sequence portions are related to secretin, human growth hormone (HGH), ACTH and proinsulin (Graf et al, 1977). These local sequence homologies, from an

evolutionary point of view, reveal that the genome of beta-LPH may have arisen from the fusions of primordial genes for various small peptides, like secretin-glucagon, connecting peptides of proinsulin.

Large amounts of beta-endorphin and alpha-endorphin are found in the pars intermedia of hypophysis, and in discrete cells of adenohypophysis (pars distalis) (Bloom et al, 1977). These adenohypophysial cells, containing the beta-endorphin and alpha-endorphin, often appear to be adjacent to blood vessels. The pars nervosa (neurohypophysis, posterior lobe) and the interlobular stroma of pars intermedia contain no trace of these endorphins (Bloom et al, 1977). Beta-endorphin is also present in the brain and is not derived from the pituitary because hypophysectomy does not alter the level of brain endorphins including beta-endorphin (Cheung and Goldstein, 1976). In monkey brain, highest beta-endorphin levels are found in the interpeduncular, followed by the habenula and the hypothalamic subareas. High amounts of beta-endorphins are also detected in the preoptic areas, the substantia nigra, the pallidum and the superior and inferior colliculi. Low values are found in limbic cortex and cerebral cortical area while the cerebellar cortex contains the lowest value (Matsukura et al, 1978). In human plasma, the level of beta-endorphin is about 21+7.3 pg/ml or 6.2+2.2 f mole/ml (Wardlaw and Frantz, 1979).

Similarly, beta-LPH in the rat brain is detected in the hypothalamus, periventricular, nucleus of the thalamus, ansa lenticularis, zona compacta of the substantia nigra, medial amygdaloid nucleus, zona incerta, PAG, locus coeruleus and a few fibres in the reticular formation (Watson et al, 1977). In human plasma, beta-LPH is from <20 to 150 pg/ml (Wiedemann et al, 1977).

Another peptide, Des-Tyr-gamma-endorphin, may be derived from beta-LPH, beta-endorphin or gamma-endorphin. It has been detected in the pituitary gland (Loeber et al, 1979) and brain (Van Ree et al, 1978). It is shown that this peptide has no opioid activity but can interact with neuroleptic binding sites in various areas of rat brain (Van Ree et al, 1978).

3. Morphine-like compound (MLC)

In 1976, Gintzler and Levy found an endogenous non-peptide morphine-like compound which cross-reacts with morphine-specific antibodies. This MLC was localized in neuronal perikarya and/or processes in nuclei related to vestibular, cerebellar and raphe systems by means of immunocytochemistry (Gintzler et al, 1978). MLC can also be found in rat pituitary (Rubinstein et al, 1977) and in human ventricular CSF (Shorr et al, 1978). MLC is shown to bind to opiate receptors of the mouse neuroblastoma X glioma hybrid cells (Blume et al, 1977).

4. Leucine-endorphin

It has been reported that leu(5)-beta-endorphin is only detected in kidney dialysis of schizophrenic patients and may play a role in the chemical etiology of schizophrenia (Palmour and Ervin, 1977; Palmour et al, 1979; Lewis et al, 1979). Schizophrenics have been successfully treated by hemodialysis (Wagemaker and Cade, 1977). Despite the high cross reactivity of leu-enkephalin antibodies with beta-endorphin, no beta-endorphin immunoreactivity was detected in dialyzates of nonpsychotic renal patients and schizophrenic patients (Ross et al, 1979). These results show no compatible agreement with the idea of extremely high concentrations of leu(5)-beta-endorphin in hemodialyzates from schizophrenic patients. Perhaps, a novel endorphin may exist in these dialyzates.

5. Dynorphin

Recently, Goldstein and colleagues (1979) have successfully identified a novel pituitary endorphin - dynorphin. It is a tridecapeptide containing [Leu]-enkephalin and is 700 times more potent than leu-enkephalin in inhibiting the guinea pig ileum longitudinal muscle contraction. This effect is only partially reversed by naloxone.

6. Other unidentified endogenous opioid peptides are found in human CSF (Fraction I elution - Terenius et al, 1976), human plasma (Ho et al, 1979) and brain (Hughes et al, 1979). The properties, molecular weight and characterisa-

tion of these peptides indicate that they are not enkephalins nor beta-endorphin. Further research is necessary to look for the functions and similarity of these unidentified peptides.

6. Other related substances

1. Endorphin Releasing Factor - A dipeptide: H-Tyr-Arg-OH, is found to be an endorphin releasing factor in hypothalamus and has analgesic potency of 4.2 times higher than morphine (Beaumont et la, 1979; Takagi et al, 1979)

2. Endogenous anti-opiate substance - This appears to be a physiological induced substance either by chronic morphine (Ungar, 1976; Han et al, 1979) or chronic acupuncture treatment (Han et al, 1979).

Intraventricular injection of this anti-opiate-like substance, the crude extracts from the brain of morphine addicted or acupuncture tolerant animals, reverses morphine or acupuncture analgesia in naive animals (Han et al, 1979).

IV. Function of Endorphins

In general, endogenous opioid peptides act similarly to morphine which has a profound effect on the bodily functions including physiological and behavioral in human and animals. There are different types of opiate receptors and endorphins which are located in numerous areas of the CNS and other parts of the body. The functions of endorphins are numerous and variable. The physiological or behavioral

variability and characteristics of endorphin actions depend on the location, action and types of endorphin-opiate receptor activities. In the past few years, met-enkephalin, leu-enkephalin, beta-enkephalin and their analogues as well as other endorphins have been extensively studied. Their effects on animals can be summarized in the following list (to be amplified later in the text):-

(1) Analgesia - relieve pain and modulate stress

(2) Homeostasis:-

(a) Releasing regulator of other hormones

Prolactin ↑ LH ↓

Growth hormone ↑ FSH ↓

Thyrotrophin ↑ Release oxytocin

Corticosterone synthesis ↑ and vasopressin

(Beta-endorphin and ACTH secreted concomitantly)

(b) Thermoregulation

Hypothermia (Alpha-endorphin)

Hyperthermia (Beta-endorphin)

(c) Digestive system - Inhibit pancreatic secretions and increase insulin and glucagon release. Suppress intestinal motility in small intestine.

(d) Cardiovascular system - Depress heart and blood pressure

(e) Respiratory system - Depress respiration.

(3) Mental illness:-

(a) Schizophrenia (abnormal high level of CSF endorphins, Leu(5)-beta-endorphin found in kidney dialysis).

(b) Small dose of beta-endorphin causes catatonia (akinesis),

epilepsy and limbic seizures.

(c) High doses of naloxone reduces hallucination of schizophrenic patients.

(d) des-Tyr -gamma-endorphin - a neuroleptic substance.

(4) Behaviour:-

(a) Interact with other neurotransmitters - acetylcholine, dopamine, GABA, 5HT, noradrenaline

(b) Maintain normal behaviour -

alpha-endorphin (tranquilization)

gamma-endorphin (agitation and violent behavior)

beta-endorphin (catatonia)

Morphine and Endorphins (Euphoria)

Leu-enkephalin (Reward-Pleasure-Learning)

Abnormally high pituitary beta-endorphin (Obesity)

des-tyr-gamma-endorphin (tranquilizer)

(c) Sexual regulation -

High dose (6ug) suppressed copulatory behavior of rats. Low dose (3ug) increased mounting and intromission latencies.

(d) Naloxone was found to enhance memory and morphine antagonized this effect (Messing et al, 1979)

(5) Addiction:- Morphine and endorphin are addictive.

(1) Analgesia - It has been verified that endorphins mimic the effect of opiates. Opiate receptors appear most densely in the paleospinothalamic pathway that transmits diffuse pain, and application of endorphins either in the CSF or in various nociceptive centres of the brain causes

analgesia. Presently Pert and colleagues (1979) have reported that Type I opiate receptors are located in the brain areas that mediate analgesia. Type I receptors are probably responsible for mediating endogenous pain-relief while Type II opiate receptors are responsible for the feeling of sedation, dysphoric and hallucinatory syndromes. Experimental evidence indicates that analgesia is observed after microinjection of met-enkephalin (120 ug/injection) into or near the ventral, caudal midbrain, PAG (Belluzzi et al, 1976; Bradley et al, 1976; Buscher et al, 1976; Frederickson and Norris, 1976), while seizures and other pathological EEG changes are seen with injections into or near the forebrain dorsomedial nucleus of the thalamus (Frenk et al, 1978). The noxious response of thalamic nociceptive neurons is also depressed by systemic or iontophoretic injections of D-ala(2) or D-Leu(5)-enkephalins (Hill and Pepper, 1978). Met-enkephalin (0.2 - 20 ug/rat) and leu-enkephalin (1-20 ug/rat) produced a dose-related and naloxone antagonizable analgesia when microinjected into the nucleus reticularis gigantocellularis and nucleus reticularis paragigantocellularis of the medulla oblongata (Takagi et al, 1978). Microiontophoretical application of met-enkephalin into laminae I, II and III selectively suppresses the excitability of nociceptive dorsal horn neurons located in laminae V (Duggan et al, 1977).

Enkephalin analgesia is one type of the endogenous pain-control system which functions at the brainstem and spinal cord levels. Met-enkephalin acts like neurotransmitters that are released through the synapses of nerve terminals (Snyder, 1977). Enkephalin-degradating enzymes (enkephalinase) and opiate receptors have markedly heterogenous and parallel distribution between regions of mouse brain (Malfroy et al, 1979). Extremely rapid degradation of met-enkephalin is observed in vivo and vitro (Dupont et al, 1977). Thus a enkephalin pain-control system tends to be localized at the segmental or regional areas of the body and the analgesic effect will be short-lasting.

The total brain enkephalin levels (by radioimmunoassay, RIA) represent only 2-13% of the total endorphins radioreceptor assay, RRA (Gros et al, 1978; Llorens-Cortes et al, 1977). Perhaps the other endorphins may play a role for more profound, generalized and long-lasting pain-relief (e.g. beta-endorphin and dynorphin).

Evidence indicates that intraventricular (Moroni et al, 1978; Hosobuchi and Li, 1978) and intravenous (Loh et al, 1976) administration of beta-endorphin can induce analgesia in human and animals. Stimulation-produced analgesia by implanting electrodes at PAG (Hosobuchi et al, 1979) or periventricular brain areas (Akil et al, 1978) increases beta-endorphin in human ventricular CSF by 50 to 300% while stimulating the posterior limb of the internal capsule has

no such effect (Hosobuchi et al, 1979). Foot-shock induced analgesia is reversed by naloxone and is cross-tolerant with morphine in mice (Chesher and Chan, 1977). However this is contrast to the results observed by Akil et al (1978) in rats in which stress-induced analgesia shows no cross-tolerance to morphine. This foot-shock induced stress produces naloxone-reversible hyperthermia (Blasic et al, 1978) and increases plasma beta-endorphin levels by six fold (Rossier et al, 1977). beta-LPH, beta-MSH and beta-endorphin can pass from the circulation to the CSF and circulating beta-LPH can be cleaved to beta-endorphin (Pezalla et al, 1978). Beta-endorphin has a half-life of 9.3+0.75 minutes (Chang et al, 1978), but the pain-relief induced by systemic injection of beta-endorphin will be long-lasting and throughout the whole body.

Naloxone reverses endotoxin hypotension (Holaday and Faden, 1978) and has great potential therapeutic value for shock (like severe blood loss) (Faden and Holaday, 1979). These results suggest that endorphins play a role in shock but the true mechanism still has to be explored.

Recently synthetic analogues of enkephalins which have been made by changing the amino acid sequence can resist enzymatic degradation and can pass through the blood-brain barrier. Thus they can be taken orally to cause analgesia and their analgesic potency can be tremendously increased (even up to 30,000 fold as compared to met-enkephalin)

(Snyder, 1977). Unfortunately they are highly addictive drugs.

(2) Homeostasis - High concentrations of endorphins in the hypothalamus and adenohypophysis suggest that they play a role in pituitary endocrinological functions. Enkephalin neurons project from the hypothalamus to the pars nervosa in the pituitary and may be involved in the regulation of neurohypophyseal neurosecretion (Rossier et al, 1979). Enkephalins (May et al, 1979; Stubbs et al, 1978) and beta-endorphins (Kato et al, 1978; Guillemin, 1978) have been shown to stimulate prolactin, growth hormone, thyrotrophin but depress luteinizing hormone, thyroid stimulating hormone and FSH secretions. It has also been demonstrated that beta-endorphin releases oxytocin (Haldar et al, 1979) and D-ala(2)-enkephalinamide releases vasopressin (Bisset et al, 1978). Beta-endorphin stimulates corticosterone synthesis in isolated rat adrenal cells by binding to the adrenocorticotropic hormone receptors (Shanker and Sharma, 1979). ACTH and beta-endorphin are stored in the same secretory granules of anterior pituitary (Weber et al, 1978) and are secreted concomitantly (Guillemin et al, 1977). There is a feedback loop between the beta-endorphin (or ACTH) and corticosterone (or cortisol); as dehydration (Mata et al, 1977; Cox et al, 1978) or dexamethasone administration (French et al, 1978) reduces beta-endorphin and ACTH secretion, while adrenalectomy or metyrapone administration elevates plasma beta-endorphin levels by 7

fold (Hollt et al, 1978; Tseng and Li, 1979). Endorphins in the pituitary seem to be releasing factors or precursors to releasing factors of corticosterone. Furthermore, in normal animals, naloxone alone produced opposite effects in modulating the hormonal release (Meites et al, 1979). This suggests that endorphins participate in regulating normal secretions of the pituitary. Enkephalinergic cells are found in the islets of Langerhans and beta-endorphin and enkephalins have been shown to inhibit pancreatic secretions and increase insulin and glucagon release (Meites et al; Konturek et al, 1978). Large amount of opiate receptors are found in the intestine and stimulation of enkephalinergic neurons in the small intestine suppresses intestinal motility. Beta-endorphin produces hypothermia and alpha-endorphin produces hyperthermia (Guillemin et al, 1977). Finally met-enkephalin has been shown to depress heart rate, blood pressure and respiration when applied to brain stem (Laubie et al, 1977). All the above results indicate that endorphins are involved in the physiological regulation of the cardiovascular system, the respiratory system, the digestive system and body temperature.

(3) Mental illness - Endorphins have been shown to produce profound physiological effects when administered directly into the brain. They cause "wet-dog" shakes, sedation, catatonia and catalepsy (Guillemin et al, 1977; Frenk et al, 1978; Elazar et al, 1979). Seizures and long lasting epileptiform changes in EEG are observed with injections of

met-enkephalin (170 ug/injection) into or near the forebrain dorsomedial nucleus of the thalamus (Frenk et al, 1978) or leu-enkephalin (1-20 ug) into the dorsal hippocampus (Elazar et al, 1979). Low (non-analgesic) doses of beta-endorphin induces non-convulsive limbic seizures (Henrikson et al, 1978) and catalepsy (Moroni et al, 1978).

Beta-endorphin has been used to induce akinesia in animals and a significant increase in concentration of 5-hydroxytryptamine is observed in the midbrain (Izumi et al, 1977); the induced akinesia can be reversed by methylmorphine, L-dopa and partially by L-amphetamines. It has been demonstrated that beta-endorphin or d-ala(2)-enkephalin analogue increase serotonin metabolism in hypothalamus and dopamine turnover in neostriatum, tuberculum olfactorium and nucleus accumbens (Fuxe et al, 1977; Van Loon et al, 1978; Biggio et al, 1978). It is proposed that there is a feedback relationship between endorphin neurons and dopaminergic neurons.

The potent and divergent behavioral responses of animals to naturally occurring substances (especially beta-endorphin which has a more marked, prolonged effect even at very minute doses) indicate that alteration in their homeostatic levels might have etiological significance in mental illness. CSF endorphin levels were found to be higher than normal in depressed or schizophrenic patients (Terenius et al, 1976) and lower than normal in chronic pain patients

(Terenius, 1977). This might explain how electric shock therapy has therapeutic effect on schizophrenic patients as it might reduce their endorphin levels. Chronic schizophrenic patients under neuroleptic medication show rapid inactivation of C.S.F. endorphin (Dupont et al, 1978). Type II opiate receptors (or K and d receptors) may be the ones that mediate sedation, dysphoric and hallucinatory syndromes (Pert et al, 1979 and Martin et al, 1977). High doses of naloxone, have been reported to eliminate hallucinations in schizophrenic patients (Gunne et al, 1977; Watson et al, 1978). Leu(5)-beta-endorphin are only detected in kidney dialysis of schizophrenic patients and it has been suggested that this may play a role in the chemical etiology of schizophrenia (Govoni et al, 1979)

Recently, Van Ree and colleagues (1978) reported that des-Tyr(1)-gamma-endorphin is a neuroleptic substance; it contains no opioid activity but can interact with neuroleptic binding sites in various areas of rat brain. Beta-endorphin and other opiates have no affinity for neuroleptic binding sites (Czhonkowski et al, 1978). Des-Tyr(1)-gamma-endorphin is found to be very effective in treating schizophrenic patients (Verhoeven et al, 1979; Van Ree et al, 1979). They postulate a dual system that endorphin reduces stress and des-tyr-gamma-endorphin produces sedation.

(4) Behaviour - Endorphinergic neurons are shown to have extensive interactions with acetylcholine, dopamine, 5HT, noradrenaline and GABA neuronal systems in the brain (Costa et al, 1978). Beta-endorphin increases the turnover rate of GABA in the substantia nigra and lobus but decreases the turnover rate in nucleus caudatus (Moroni et al, 1978). The turnover rate of acetylcholine is decreased by beta-endorphin in cortex, hippocampus, nucleus accumbens and globus pallidus but not in nucleus caudatus (Moroni et al, 1977). Met-enkephalin and leu-enkephalin also inhibit the spontaneous release of acetylcholine from the cerebral cortex in vivo (Thamandas et al, 1977). This indicates that endorphins may act as presynaptic inhibitors of cholinergic neurons. The interactions of endorphins with other neurosubstances may preserve a homeostatic state of bodily functioning which helps to maintain normal behaviour. Evidence has shown that alpha-endorphin induces tranquilization, gamma-endorphin produces agitation and sometimes violent behaviour, beta-endorphin causes catatonia (Costa et al, 1978) and hypotension which is reduced by PCPA (Lemaire et al, 1978), and des-Tyr(1)-gamma-endorphin improves schizophrenia (Van Ree et al, 1979). All these effects suggest that the endorphinergic systems are probably involved in maintaining normal behaviour and that a malfunction of this system may result in psychiatric illnesses or abnormal behaviour. For example, evidence indicates that overeating is probably caused by the abnormally high pituitary beta-

endorphin level (5 times higher than normal animals) in obese mice (ob/ob) and rats (fa/fa) (Margules et al, 1978). Naloxone selectively abolishes overeating in these genetically obese mice and rats (Margules et al, 1978).

Endorphins, like morphine, produce euphoria. It has been found that leu-enkephalin induces higher rates of self-administration in the self-administered bar-pressing behaviour as compared to met-enkephalin when they are injected into the limbic areas in rats (Belluzzi et al, 1977). It is possible that leu-enkephalin is a major intrinsic substance that elicits pleasure and reward-system.

Social and sexual behaviours are also affected by endorphins. Beta-endorphin increases grooming activity, decreases mounting and increases the length of time before ejaculation in rats (Meyerson and Berg, 1977). A high dose (6 ug) of D-Ala(2)-met-enkephalinamide (analogue) also suppresses the copulatory behaviour of rats but a low dose (3 ug) increases mounting and intromission latencies (Quarantotti et al, 1978).

(5) Addiction - Endorphins, like morphine, produce tolerance, cross-tolerance and withdrawal effects on long-term treatment, i.e. they are also addictive (Tseng et al, 1976). Endorphin can modify the relative activities of adenylate cyclase and guanylate cyclase which are two enzymes floating freely in the double layer of lipid molecules that make up the cell membrane. These two enzymes

will be activated to synthesize cyclic AMP and cyclic GMP when appropriate neurotransmitters bind to the membrane receptors. Often, these two enzymes display antagonistic action in mediating the same hormonal effect inside the cell. In neuroblastoma-glioma hybrid cell cultures, long-term exposure to opiates or endorphins will induce an abnormal production of adenylate cyclase which enhances cyclic AMP synthesis to compensate for the opiate-inhibited level. As a result, more opiates or endorphins are required to produce decreases in cyclic AMP levels (Klee et al, 1975). On withdrawal of opiate or endorphin, cyclic AMP levels increase markedly owing to the abnormally high quantity of adenylate cyclase. This increase in cyclic AMP level correlates biochemically to the withdrawal symptoms. The increase in the cyclases during and after long-term opiate or endorphin treatment may act as a bio-feedback mechanism which will change the firing rate of endorphin neurons, inducing the development of tolerance and physical dependence. It has been shown that SPA of PAG or chronic beta-endorphin administration cause tolerance which can be reversed by 5-hydroxytryptophan (Hosobuchi et al, 1977; Hosobuchi et al, 1978). Thus serotonin may play an important role in addiction and analgesia.

V. Enkephalinase:-

Met-enkephalin and leu-enkephalin are sensitive to trypsin and chymotrypsin (Kastin et al, 1976) and carboxypeptidase A and leu-aminopeptidase (Hughes et al, 1975). It is suggested that rapid inactivation of enkephalins may be due to at least two different enzymes localized in different structures of cell, a peptidyl dipeptidase which is a component of plasma membrane may degrade enkephalins by liberating a C-terminal dipeptide while an aminopeptidase in endothelial cells may cleave peptides that are taken up as blood flows past the endothelial surface (Erdos et al, 1978). Aminopeptidases are also found in the brain homogenates (Shaw and Cook, 1978) and recently a high affinity enkephalin-dipeptidyl-carboxypeptidase (enkephalinase) is found to have markedly heterogenous and parallel distribution to opiate receptors in different regions of mouse brain (Malfroy et al, 1979). The concentration of enkephalinase in mouse brain is in the order of:-

Striatum>Hypothalamus>Cortex>Brainstem>
Hippocampus>Cerebellum

(Malfroy et al, 1978; Gorenstein and Snyder, 1979). Enkephalinase in brain is increased after morphine administration (Malfroy et al, 1978) and kainic acid or 6-hydroxydopamine lesions of the nigrostriatal dopaminergic pathways lead to similar decreases in this peptidase and opiate receptors.

Peptidase-inhibitors such as puromycin inhibit the degradation of leu-enkephalin by enzymatic activities present in rat brain homogenates and guinea pig ileum (Vogel and Altstein, 1979) while bacitracin potientiated and prolonged the in vivo analgesic activity of beta-endorphin (Patthy et al, 1977) and acupuncture analgesia (Han et al, 1979). Inhibitors of carboxypeptidase A and leu-aminopeptidase, D-phenylalamine and D-leucine (respectively), also induce naloxone reversible analgesia in human and mice (Ehrenphreis et al, 1978; Cheng and Pomeranz, 1979). Thus drugs that protect endogenous endorphins from enzymatic degradation may be very useful for clinical pain-relief. Recent biochemical evidence supports this hypothesis; D-phenylalanine and D-leucine can cross the blood-brain barrier (Okafor et al., 1980) and inhibit enkephalinases in guinea pig ileum assay (Greenberg et al., 1980). Combining the D-amino acids and EA treatments produced a higher analgesia in larger numbers of mice as compared to either treatment alone (Cheng and Pomeranz, 1980). It is postulated that EA releases endorphins which are protected by the D-amino acids to produce a higher analgesia.

(C) SPECIAL OBJECTIVES

This thesis is composed of ten chapters. Each of which was written a paper for publication. The object of each chapter is summarized as follows:-

Chapter 1: Since the methodology depended on a novel technique of measuring pain, the purpose of this paper is to compare my methods with a more established technique, which uses radiant heat.

Chapter 2: Since the discovery that naloxone reverses acupuncture analgesia in man and animals (Mayer et al., 1976; Pomeranz and Chiu, 1976; Pomeranz and Cheng, 1979; Chapman and Benedetti, 1977), it has been suggested that naloxone may have other unknown pharmacological actions unrelated to its opiate antagonist role; thus, inhibition of acupuncture analgesia by naloxone does not necessarily prove that this activity is mediated by opiate receptors. To solve this ambiguity, I used three different approaches to test the EAA-opiate receptor phenomenon. (i) Firstly, dextronaloxone, the enantiomer (+)naloxone, was used in parallel experiments with (-) naloxone to test their effect on EAA in mice. The (+) naloxone, which was recently synthesized by Iijima et al., (1978), showed very poor binding activity to opiate receptors. Thus it is predicted that dextro-naloxone should have a much smaller effect than the levoisomer in reversing EAA and would thus implicate stereospecific opiate receptors in EAA. (ii) Secondly, I studied the blocking

effects of levo-naloxone on EAA at doses as low as 0.025 to 0.05 mg/kg and plotted a dose-response curve to see if naloxone works in a dose-dependent manner. (iii) Thirdly, other opiate receptor antagonists such as naltrexone, cyclazocine and diprenorphine were used to test EAA. These substances are the antagonists of Type 1 opiate receptors (Pert et al, 1979). Type 1 opiate receptors are located in the brain areas which mediate analgesia (Pert et al, 1979).

Chapter 3: It has been shown that removing the pituitary abolishes EAA in mice (Cheng, Master thesis, 1977): I extended this study with other methods of depleting pituitary endorphins to see if they also reduce EAA (Cheng et al, 1979). This is done by treating the animal with dexamethasone, a cortisol analogue, which has been shown in a negative feedback system (high serum cortisol level inhibits the release of corticotrophic releasing factor) to inhibit the release of ACTH and beta-endorphin (Schapiro et al, 1958). Another method I used was to feed the animals a 2% NaCl solution for three days; this had been shown to deplete the pituitary endorphins (Cox et al, 1978; Mata et al, 1977). If both methods of chemically suppressing the pituitary endorphins reduced EAA they would confirm the results of hypophysectomy that EAA may be partially mediated by pituitary endorphins.

Chapter 4: Moreover, recent evidence suggests that pituitary endorphins and ACTH are released together from the pituitary (Guillemin et al, 1977). I thus predicted that EA should release ACTH which should enhance cortisol release in the adrenal gland. I studied plasma cortisol levels in awake horses before and after EA.

Chapter 5: Evidence indicated that acupuncture analgesia was mediated by endorphins (Cheng, M.Sc. thesis, 1977; Mayer et al, 1977; Pomeranz and Chiu, 1976; Pomeranz and Cheng, 1979; Sjolund et al, 1977; Takeshige et al, 1978). This acupuncture analgesia induced by low frequency stimulation (2 - 6 Hz) was reversed by naloxone in human (Mayer et al, 1977), cats (Pomeranz and Cheng, 1979), rats (Takeshige et al, 1978) and mice (Pomeranz and Chiu, 1976). Recently, we demonstrated that pituitary endorphin is involved in electroacupuncture (EA) analgesia (Cheng et al, 1979) while McLenna et al, (1977) showed that electrical or chemical lesions of raphe serotonin output abolishes EA analgesia in rabbits. Thus more than one pain-relieving mechanism may be involved in EA analgesia. Furthermore Chapman and Benedetti (1977) showed that analgesia induced by high frequency (100 - 200 Hz) transcutaneous nerve stimulation (TNS), was only partially reversed by naloxone; moreover, Sjolund and Eriksson (1978) reported that naloxone blocked low frequency TNS analgesia but had no effect on the high-frequency TNS analgesia in humans. This implies that different mechanisms of

stimulation during TNS or acupuncture. I wondered whether EAA induced by low frequency stimulation is mediated by endorphin while high frequency stimulation may be mediated by serotonin. The present study undertook to compare the effect of naloxone (an opiate antagonist) and/or parachlorophenylalanine (a serotonin synthesis inhibitor) on EA analgesia at high or low frequency stimulation.

Chapter 6: Akil and Liebeskind (1975) had demonstrated the role played by the three monoamines (dopamine, noradrenaline and serotonin) in SPA. In order to compare SPA and EAA and to look for monoaminergic mechanisms of EAA, I used various drugs capable of depleting or enhancing serotonin, dopamine ~~and/or~~ noradrenaline to test effects on EAA.

Chapter 7: Recent reports suggest that systemic treatment with D-amino acids (DAA) can cause analgesia in man and mice (Ehrenpreis et al, 1978). D-leucine and D-phenylalanine were postulated to produce analgesia through the endorphinergic system, since the analgesia they produced was naloxone-reversible. To further test the D-amino acid-endorphinergic hypothesis, three strains of mice were compared. One strain (CXBK) is low in opiate receptors (Baran et al, 1975) and exhibits poor EA (Peets and Pomeranz, 1978) and morphine analgesia (Baran et al, 1975); another strain (ob/ob) was found to have abnormally high levels of pituitary endorphins (Margules et al, 1978). A third strain

B6AF1/J, was used as control as it exhibits "normal" EA which is naloxone-reversible; all three strains are related as they are descended from C57BL. It is predicted that the DAA analgesia should have the following rank order: : ob/ob > B6AF1/J > CXBK based on differences in endorphin systems. If true, this would give further support to the ideas that DAA analgesia is mediated by endorphins.

Chapter 8: DAA, D-phenylalanine and D-leucine produce naloxone reversible analgesia. Electroacupuncture (EA) also produces analgesia which is blocked by naloxone. Combining the two treatments may produce a greater analgesic effect than that produced by either treatment alone. Thus this experiment is carried out to test the idea that the combination of EA with DAA will provide a more potent method for the treatment of clinical pain.

Chapter 9: In clinical practice, it has been observed that EAA has an accumulative effect after repeated treatments. However, this observation has never been studied in animal experimentation. Thus an experiment was set up to test whether the second EA treatment can produce a more profound analgesia then the first when mice receive two EA treatments 3 hours apart.

Chapter 10: Acupuncture was reported to show successful result in treating human addicts (Wen, 1977) and reducing morphine withdrawal syndromes in animal experimentation (Ng et al, 1977). Endorphins have been shown to be

addictive (Tséng et al., 1976). If acupuncture is mediated by endorphin (Mayer et al, 1978; Pomeranz and Chiu, 1976; Pomeranz and Cheng, 1979; Sjolund et al, 1977), it is important to know whether acupuncture is addictive or not. Thus the present experiment tried to find whether EAA will show cross-tolerance to morphine in morphine addicted mice.

MATERIALS AND METHODS OF ELECTROACUPUNCTURE IN MICE

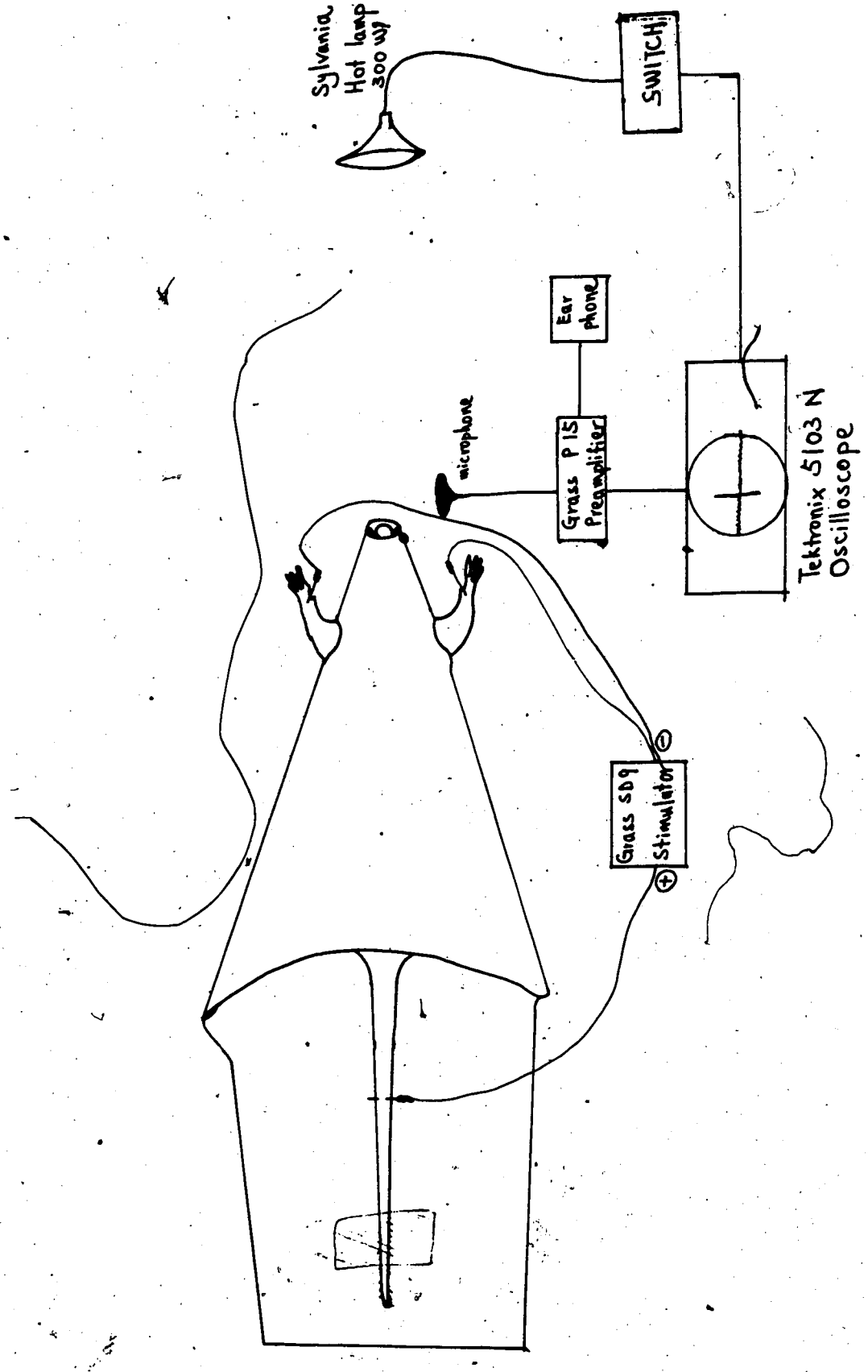
Animals: B6AF1/J female mice (mus musculus) were used for the EA experiments because they squeaked reliably to noxious heat stimulation. The B6AF1/J mice (8 weeks old) were purchased from Jackson Laboratories and were housed in the animal rooms of the Zoology Department, University of Toronto for at least one week before the experiment. Five mice were stored in each cage with a 12 hours light cycle from 6 a.m. to 6 p.m. The time of EA treatments ranged from 11 a.m. to 6 p.m.

Methods: Figure 1 depicts a schematic diagram for the experimental set-up. The mouse was placed in a paper receptacle, the forelimbs and the tail were immobilized by masking-tapes. Noxious heat stimuli were delivered by a sylvania ELH, 300 watt, 120 volt light bulb positioned at about 9 cm from the animal's nose. A switch would activate simultaneously the heat source and the sweep control of the Tektronix 5103N storage oscilloscope. The squeak latencies were recorded by a small microphone (8 ohm, 0.2 watt) connected to the oscillospe through a Grass P15 preamplifier. Thus an audiogram was displayed on the oscilloscope and was confirmed and identified by ear-phones connected to the preamplifier. The cut-off point for the heat stimulation was 9 seconds in order to avoid tissue damage. Four squeak latencies (pain threshold) were measured four times at three

FIG 1

A schematic digram depicting the set-up of EA experiments on the mouse restrainted in a paper recepticle. 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 a osscilloscope 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 interosseus muscles and positive pole was connected to the needle that was inserted through the middle of the tail.

FIG 1


Sylvania
Hot lamp
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Preamplifier
Tektronix 5103 N
Oscilloscope
Grass SD9
Stimulator
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minutes apart in the control period. Only those mice giving reproducible responses (with latencies between 3 to 5.5 seconds) were used for subsequent EA tests. The mean of the last three vocalization measurements determined the control squeak (pain) latency for each animal.

EA was applied by inserting two stainless steel needles (34 gauge) into the first dorsal interosseus muscles (Hoku or L.I.4 point). Electrical stimulation was applied for 20 minutes at 4 Hz 0.1 ms duration by a Grass SD9 stimulator. The negative pole was connected to the needle inserted into the Hoku point and the positive pole connected to a needle inserted in the middle of the tail. Voltage was adjusted to be above the threshold for muscle vibration but below the threshold of vocalization.

The electrical currents were measured to range from 0.1 to 0.4 mA. An opening underneath the paper recepticle allowed intraperitoneal injection of drugs. Squeak latency was again measured 20 minutes after EA before removing the needles. The the post EA responses were measured at 10 minute apart for a total of 50 (or 120) minutes.

Other details about materials and mathods are presented in the respective chapters which were written as papers for publication.

CHAPTER 1: COMPARISON OF TWO TECHNIQUES OF BEHAVIORAL MEASUREMENTS IN MICE USING NOXIOUS RADIANT HEAT STIMULATION

SUMMARY

Two different noxious responses were measured and compared in the same mice by shining a hot lamp on the heel of the mice. Two kinds of noxious responses were obtained by measuring the latencies for leg withdrawal and vocalization (squeak). In 58 mice, the average leg-withdrawal latency is 2.47±0.02 sec. and average squeak latency is 3.64±0.02 sec. These two responses showed a high coefficient correlation ($r = 0.73$).

INTRODUCTION

Behavioral nociceptive measurements in experimental animals provide an important tool in medical research. There are different methods of producing pain and quantifying the nociceptive response thus produced. Although pain can be aroused in a variety of ways, it is not easy to find a stimulating device which will perm[illegible] quantification of painful effects. For example mechanical stimulators damage tissues or create radical deformations, chemical stimuli are practically impossible to control in that they cannot be specifically confined. Electrical stimuli are not considered a natural type of painful stimulus. However there

is general agreement that the production of pain by thermal stimuli currently offers the best possibility of experimental control of the noxious input (Kerr and Casey, 1978). Furthermore radiant heat avoids touching the skin which might confuse pain and touch stimuli. There are different ways of measuring the painful response elicited in animals by the radiant heat and these may give varying results. The different types of pain measurements may emphasize different aspects of pain perception such as sharp (fast) pain, dull (slow) pain or emotional pain. The present experiment was carried out to compare two ways of measuring noxious response - reflex withdrawal and vocalization in the same animal receiving the same painful stimulus (i.e. radiant heat).

METHODS

Female B6AF1/J mice (Jackson's Lab.) are used because they squeak reliably to painful stimulation. These mice were immobilized in cardboard holders, leaving both forelegs, left hindleg, and tail accessible. The holders and the forlegs were taped to a thread which led to a force transducer (Grass transducer FT03C) which is connected to a preamplifier and storage oscilloscope. Noxious thermal stimulation was applied by shining a hot lamp (GE. Quartzline ELH, 300 watts) on the exposed heel blackened with a black marking pen. This blackening gave a consistent area

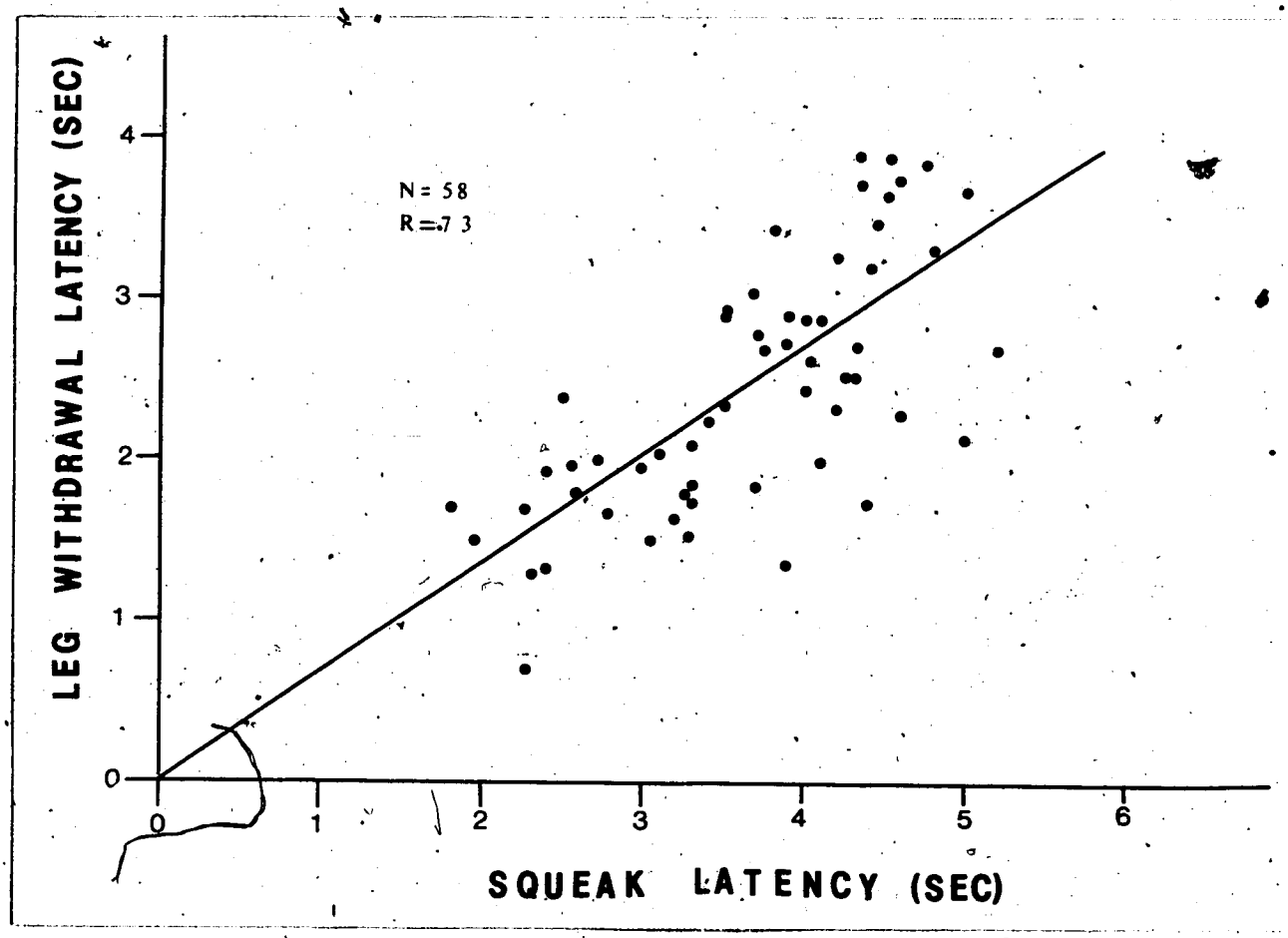
for stimulation. Vocalization by the mice was monitored by an audiogram transmitted by a small mircrophone connected to an amplifier and oscilloscope. The distance of the hot lamp to the exposed heel was kept constant at 9 cm. The apparatus consisting of a restrained mouse, force transducer, hot lamp and microphone was contained in a large sound-proof box. Pain threshold of each mouse was tested four times at three minutes apart. For each measurement the hot lamp was turned on until the mouse pulled its leg and squeaked. The latencies of leg withdrawal and vocalization were displayed and measured by the storage oscilloscope. Fifty eight mice were tested and the last three tests of each mouse were averaged for statistical analysis.

RESULTS

On the average the mice exhibited leg withdrawal at 2.47 $\pm$ 0.02 sec. and then produced vacalization at 3.64 + 0.02 sec. ($\pm$SE.). The correlation between leg withdrawal and squeak is plotted in figure 1; a straight line is observed and the correlation coefficiency is $r = 0.73$ ($p<0.001$, t-test two tailed) which indicates a highly significant interrelationship between the two responses.

Fig 1

Figure 1 shows the relationships between leg withdrawal latencies and squeak latencies. Ordinate indicates latencies for the leg to withdraw and the abscissa shows the latencies to squeak after the onset of thermal stimulation. 58 mice were used. A straight line is drawn through the middle of the points giving a correlation coefficiency: R= 0.73.



DISCUSSION

The mean difference between leg withdrawal and vocalization is 1.17 sec. It is similar to the phenomenon whereby we withdraw our hand from a hot stove before we cry out from pain. This result is consistent with the idea that noxious withdrawal reflex is mediated by lower central nervous control, possibly a spinal reflex, while vocalization may be mediated through higher brain centres such as cortex. If true, this conclusion suggests that vocalization represents a more cognitive response, while the withdrawal response may be merely a reflex. It is interesting to note that in previous papers from our laboratory it was reported that electroacupuncture caused a naloxone reversible analgesia; this analgesia occurred, whether the behaviour measure was vocalization (Pomeranz and Chiu, 1976) or leg withdrawal (Peets and Pomeranz, 1978). In the human studies, it was also found that nociceptive flexion reflex corresponded to the threshold of pain sensation (Willer, 1977).

Other common or standard behavioral pain threshold tests may include tail flicks and paw licking in hot-plate tests which appear to be similar to leg-withdrawal response. It was reported that naloxone faciliated the nociceptive response of jumping off the hot-plate but had no effect on the latency of paw-lick in mice (Grevert and Goldstein, 1977). By using three types of pain tests, Dennis and co-

workers (1979) also observed that the type of pain test is crucial in determining the pattern of drug influences. Similarly, nociceptive responses like jumping off the hot-plate, vocalization and formalin-induced nociceptive response may be elicited or controlled by different central nervous systems as compared to tail-flick, paw-lick and withdrawal- reflex. Further experiment is necessary to differentiate which nociceptive responses are more subjective to the endogenous pain control system mediated by endorphins.

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CHAPTER 2: ELECTROACUPUNCTURE ANALGESIA IS MEDIATED BY STEREOSPECIFIC OPIATE RECEPTORS AND IS REVERSED BY ANTAGONISTS OF TYPE I RECEPTORS

SUMMARY

Dextro-naloxone, a recently synthesized stereoisomer, which was shown to possess much less opiate receptor affinity than levo-naloxone, produces no reversal of electroacupuncture analgesia (EAA) in mice. Since levo-naloxone completely reverses EAA, this proves that stereospecific opiate receptors are involved. It has been reported that there are two classes of opiate receptors: Type I and Type II. Type I opiate receptors may be responsible for opiate analgesia. Antagonists of Type I receptors, levo-naloxone, naltrexone, cyclazocine and diprenorphine, all block electroacupuncture analgesia at low doses. All together, these results strongly support the hypothesis that electroacupuncture analgesia is mediated by opiate receptors. Possibly Type I receptors are the major component of this system.

INTRODUCTION

Recent results suggest that acupuncture analgesia (antinociceptive response) is mediated by the binding of endorphins to opiate receptors (Chapman and Benedetti, 1977;

Mayer et al.,1977; Pomeranz and Cheng, 1979; Pomeranz and Chiu, 1976). The best evidence is that naloxone (an opiate antagonist) inhibits the following processes: (a) electroacupuncture (EA) - mediated reduction of noxious responses of neurons in cat spinal cord (Pomeranz and Cheng, 1979); (b) electroacupuncture analgesia (EAA) in mice (Pomeranz and Chiu, 1976); and (c) analgesia induced in humans by acupuncture (Mayer et al, 1977) or transcutaneous stimulation (Chapman and Benedetti, 1977). Although naloxone has been verified as a pure antagonist for opiates with no agonist activity (Blumberg et al, 1961; Blumberg et al, 1965), it may have other unknown pharmacological actions unrelated to its opiate antagonist role; thus, inhibition of acupuncture analgesia by naloxone does not necessarily demonstrate that this activity is mediated by opiate receptors. To solve this ambiguity we used several approaches to test the EAA-opiate receptor phenomenon. Firstly, dextronaloxone, the enantiomer, was used in parallel experiments with (-) naloxone to test their effect on electroacupuncture analgesia in mice. The (+) naloxone was recently synthesized by Iijima et al (1978). Inactivity of the dextroisomer relative to the (-) isomer was shown by these authors in 3 different systems: (i) rat brain receptor binding assay; (ii) electrically stimulated guinea pig ileum assay; (iii) neuroblastoma x glioma adenylate-cyclase assay. In all 3 systems (+)naloxone had between 1/1000 to 1/10,000th the activity of (-) naloxone in binding to opiate

receptors (Iijima et al, 1978). These studies were done *in vitro*, but opiate receptors also inhibit stereospecificity *in vivo*: for example, dextrorphan has much less than 1/100th the analgesic potency of levorphanol both *in vivo* (mice) (Goldstein and Sheehan, 1969) and *in vitro* (Goldstein et al, 1971). We thus predicted that dextro-naloxone should have a much smaller effect than the levoisomer in reversing acupuncture effects *in vivo* and would thus implicate stereospecific receptors in acupuncture analgesia; the results in this paper support this prediction. Secondly, we demonstrated that levo-naloxone inhibits EAA at doses as low as 0.025 to 0.05 mg/kg and in a dose dependent manner. Thirdly, we tested other opiate receptor antagonists: Martin et al (1976) and Della Bella et al (1978) have described that there are 3 types of opiate receptors: u, k and d-receptors. Morphine is the agonist for u-receptors and can provoke analgesia and euphoria. Recently Pert and colleagues (1979) reported that there are two types of opiate receptors; Type I and Type II. Type I receptors are located in the brain areas which mediate analgesia. Type I receptors may be u-receptors. To test whether EAA is mediated by the Type I receptors, we measured the effect of four Type I receptor antagonists; levo-naloxone (0.05,0.025, 0.0125 mg/kg), naltrexone (2 mg/kg), cyclazocine (1, 0.1, 0.05, 0.02 mg/kg) and diprenorphine (2, .1, 0.3, 0.05 mg/kg) on EAA in mice.

METHODS

The methods used in the present study were similar to those described in the previous work (Pomeranz and Chiu, 1976). Briefly I used a behavioural pain threshold measurement in female mice of an F1 cross between CB57 BL/6J and A/J from Jackson Laboratories; the latency to squeak was determined in response to noxious heat stimuli applied to the nose. The latency was measured from an audiogram of the squeak. Three control tests were given, 3 minutes apart, to the restrained mice. The mean of these 3 tests gave zero time control value for each mouse (before electroacupuncture began). Only those mice giving 3 reproducible responses in the control period, with latencies between 3 to 5.5 seconds, were used for subsequent acupuncture treatment. Electroacupuncture was then administered to the mice by inserting fine needles (34G stainless steel) into Hoku (L.I.4), a point which is located on the forepaw between the first and second digits. Electrical stimulation was applied for twenty minutes by means of square pulses from a Grass SD9 stimulator at 4Hz, 0.1 msec duration and voltage was adjusted to be above threshold for muscle contraction but below threshold for pain vocalization. Electrical current was measured to range from 0.2 to 0.3 mA.

For the stereospecific experiments, there were five matched groups of mice with 15 mice in each group: I, EA plus saline (0.9%), II, EA plus dextro-naloxone (1 mg/kg), III, EA plus dextro-naloxone (4 mg/kg), IV, EA plus levo-naloxone (1 mg/kg) and V, EA plus levo-naloxone (4 mg/kg). Injections were given intraperitoneally in a "blind" manner (i.e. the experimenter did not know which mouse received (+) naloxone, (-) naloxone or saline). For each mouse the injection was administered twice immediately before and again after electroacupuncture treatment. Squeak (pain) latencies were taken just before the acupuncture needles were removed (i.e. after 20 minutes of EA treatment); next the squeak latencies were again measured at 30 and 40 minutes (i.e. 10 and 20 min. after removing the needles). For statistical analysis, the 20 to 40 minute values for each mouse were averaged, and statistics were done on this averaged value. This time window was preselected on the basis of several previous acupuncture papers in humans (Mayer et al, 1977; Chapman and Benedetti, 1977) and animals (Pomeranz and Chiu, 1976) which showed that analgesia peaks at 20 to 40 minutes after onset of acupuncture. Then a within group Student's paired t-test (two tailed) was used to compare the 20 to 40 minute mean to the acupuncture control value. Statistics were also done by using a between group analysis of variance on the 20 - 40 minute means, and a subsequent Newman-Keul's test to compare the 5 groups.

To test the effect of Type I antagonists on EAA, various doses of levo-naloxone (0.1, 0.05, 0.025 and 0.125 mg/kg), naltrexone (2 mg/kg), cyclazocine (1, 0.1, 0.05 and 0.02 mg/kg) and diprenorphine (2, 1, 0.3 and 0.05 mg/kg) were used. Each dose was tested on 15 mice. Similar EA experiments were carried out as in the stereospecific studies above, except that the injection of these drugs was administered once (immediately before EA). Cyclazocine was disolved in a vehicle containing a few drops of 1 normal NaOH. Vehicle or 0.9% saline and drugs were injected in a blind manner (0.4 ml/injection, I.P.).

RESULTS

(A) Dextronaloxone: Mice that were treated with either saline (0.9%) or dextronaloxone (1 mg/kg and 4 mg/kg) showed significant EAA (Fig. 1, $p<0.001$, t-test two tailed) while those mice that were treated with levo-naloxone (1 mg/kg and 4 mg/kg) showed no EAA (Fig. 1, $p>0.05$). The average percentage change versus time for each group of 15 mice was plotted in Figure 1, showing that EAA peaks at 30 to 40 minutes, while the average squeak latency decreased (hyperalgesia) at 20 minute for the levo-naloxone treated groups, Statistical analysis indicated that there were no significant differences among the saline and dextro-naloxone treated groups (Fig. 1, $p>0.05$, Newman-Keul's test). There was also no difference between the 1 mg/kg and 4 mg/kg levo-

naloxone treated groups (Fig. 1, $p>0.05$, t-test). However, there were significant differences between saline (I) and levo-naloxone (IV or V) treated groups (Fig. 1, $p<0.01$, F-test and Newman-Keul's test). Thus levo-naloxone blocks EAA but dextro- naloxone does not.

(B) Levo-naloxone: At small doses, levo-naloxone also antagonizes EA effect. Figure 2 shows the effect of naloxone, at several small doses (0.1, 0.05, 0.025, 0.0124 mg/kg), on EA analgesia in mice. Statistical analysis shows that all the above doses significantly antagonize the EA effect as compared to the saline (0.9%) control. The average percentage change for each dose at 40 minutes is taken to plot a dose-response curve for the effect of levo-naloxone on EAA (Fig. 3).

(C) Naltrexone: At 2 mg/kg, naltrexone, which is similar to levo-naloxone, also antagonizes EAA and causes hyperalgesia in B6AF1/J mice (Fig. 4).

Fig. 1

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 of 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.

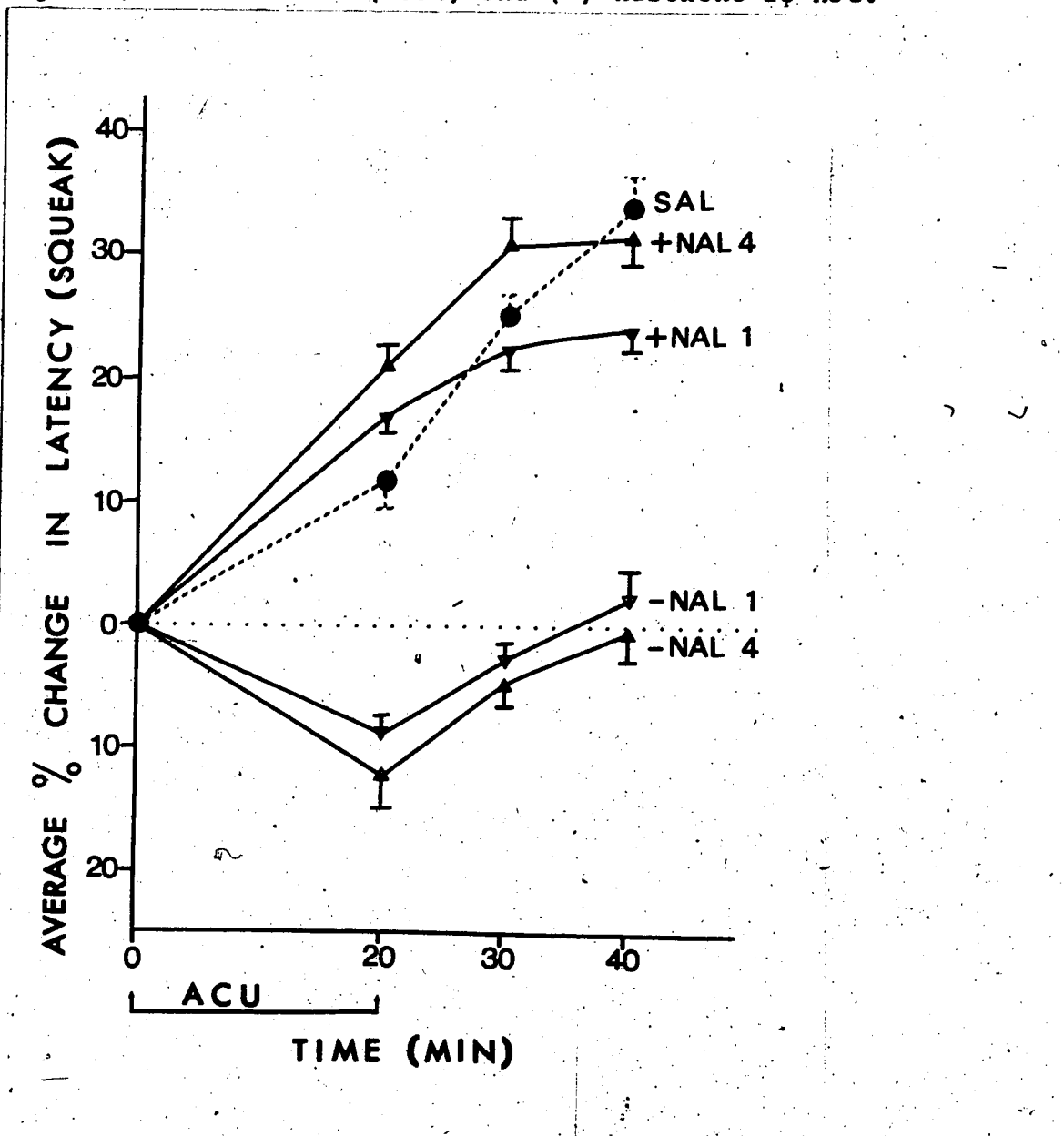


Fig. 2

Effect of four different low doses of levonaloxone on EAA in mice. This figure is similar to figure 1. Levonaloxone doses are indicated by the numerals 0.1, 0.05, 0.025 and 0.0125 in mg/kg. Each curve is the mean of 15 mice.

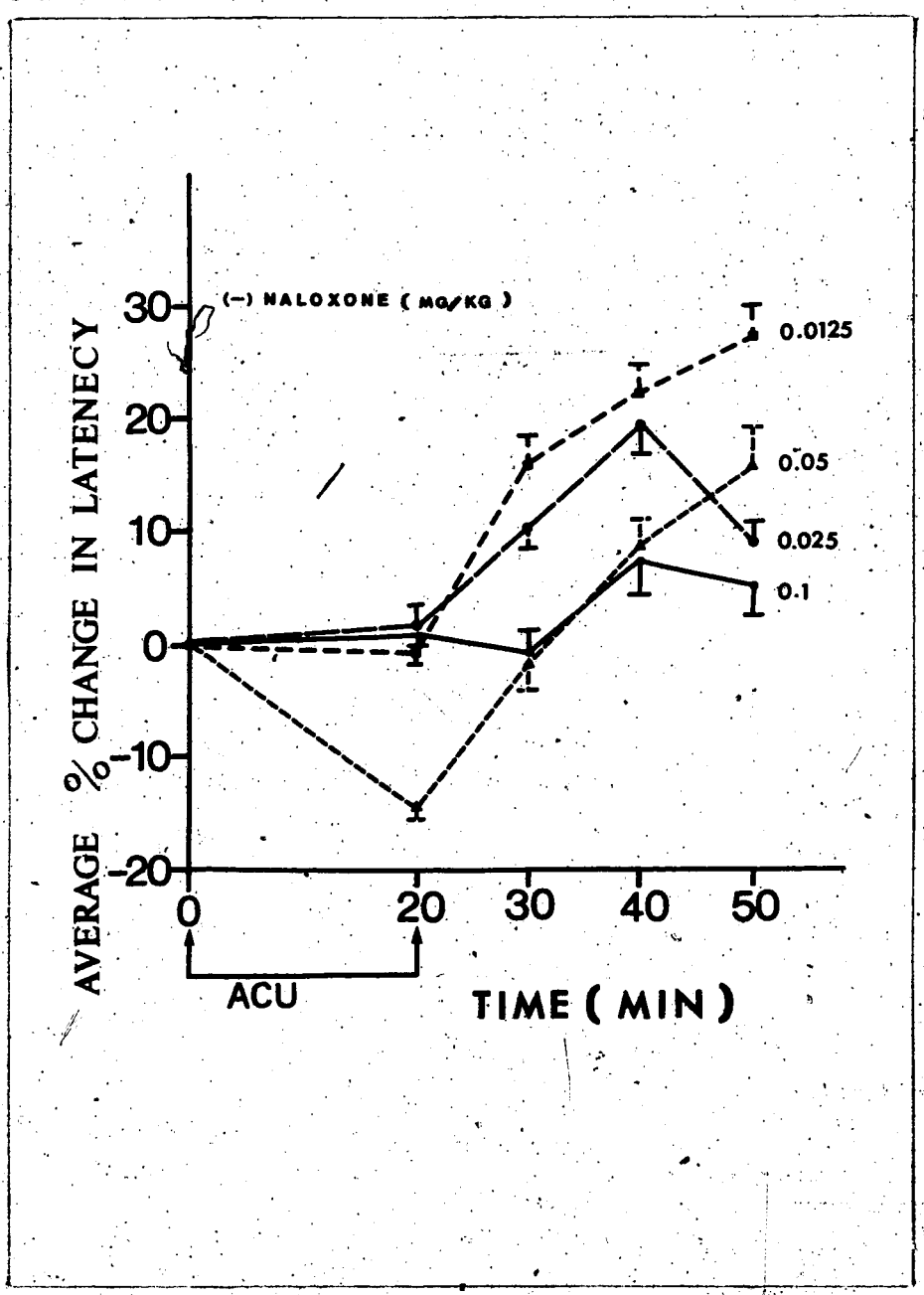


Fig. 3

A dose-response curve derived from Fig. 1 and 2 showing that levonaloxone reverses EAA in a dose-relalted manner. Ordinate represents the average % change in squeak latency taken from the 40 minute values. Abscissa represents the dose of (-) naloxone in mg/kg. Each point is the mean of 15 mice. Dotted line indicates change of scale. Bars show standard error.

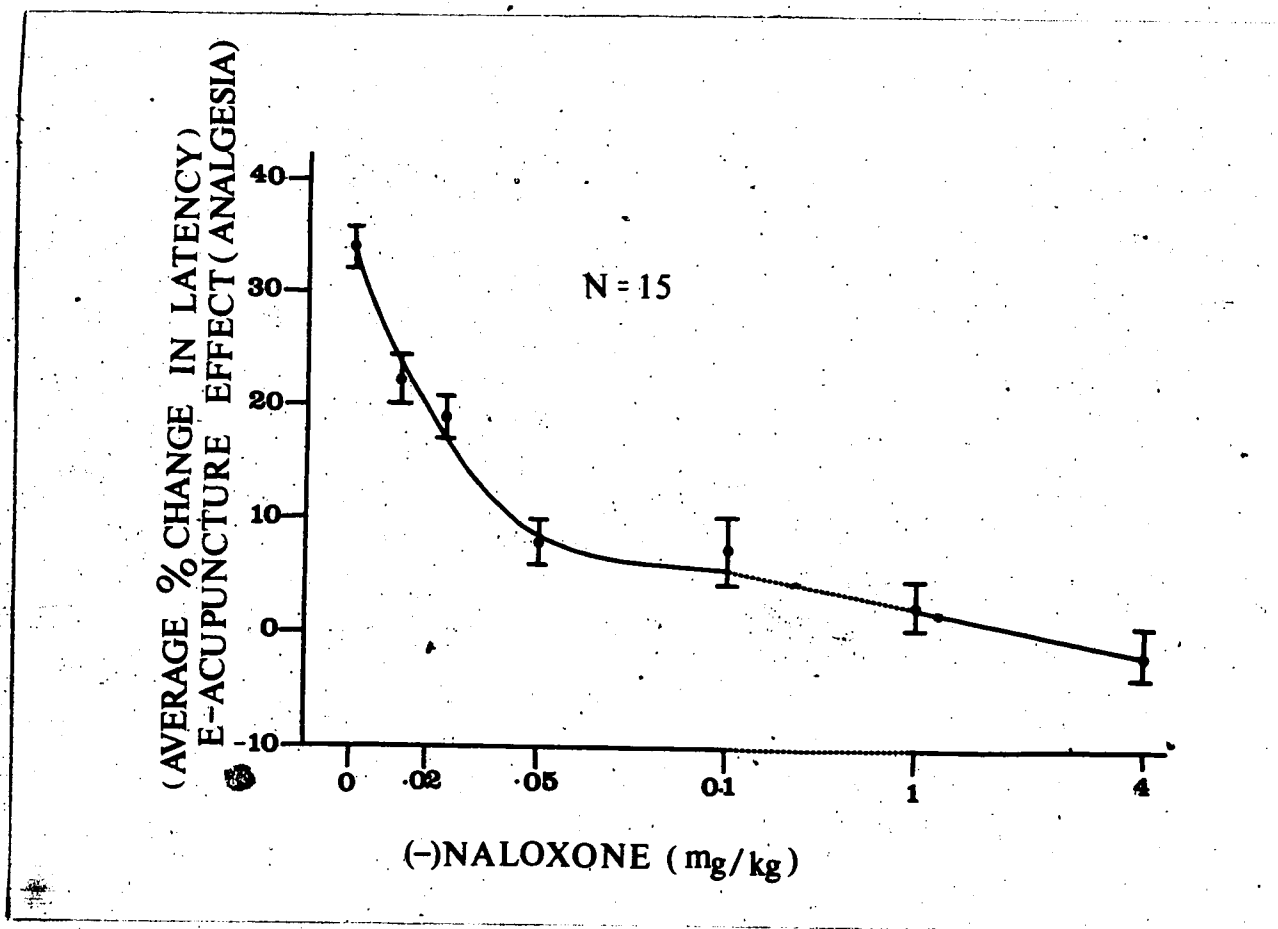


Fig. 4

Effect of naltrexone (2 mg/kg) on EAA in mice. This diagram is similar to Fig.1 and 2 except naltrexone or 0.9% saline (control) is administered once (immediately before EA indicated by the arrow). Naltrexone reverses EAA in mice.

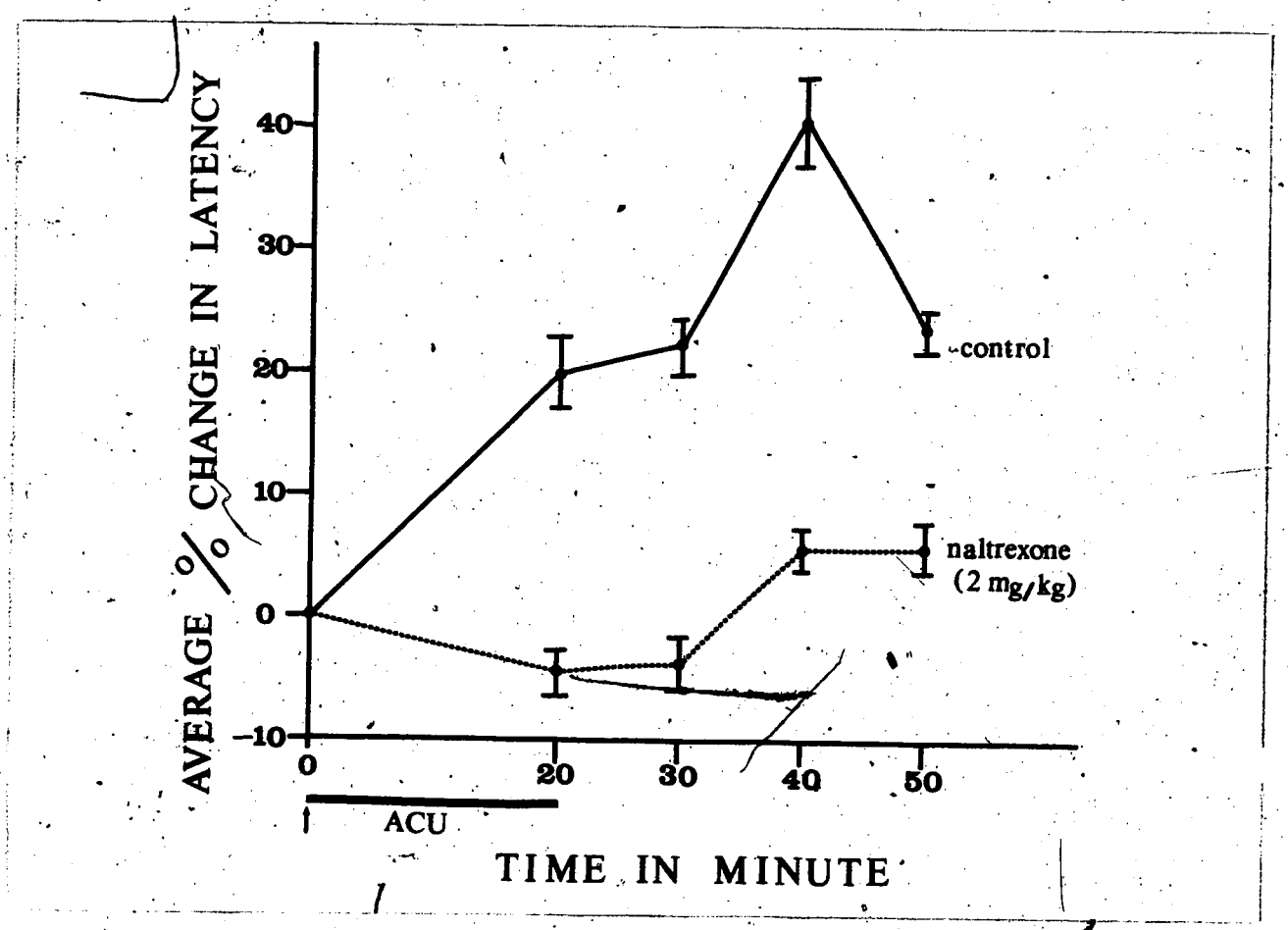


Fig. 5

Effect of cyclazocine on EAA in mice. This diagram is similar to figure 1 and 2 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.

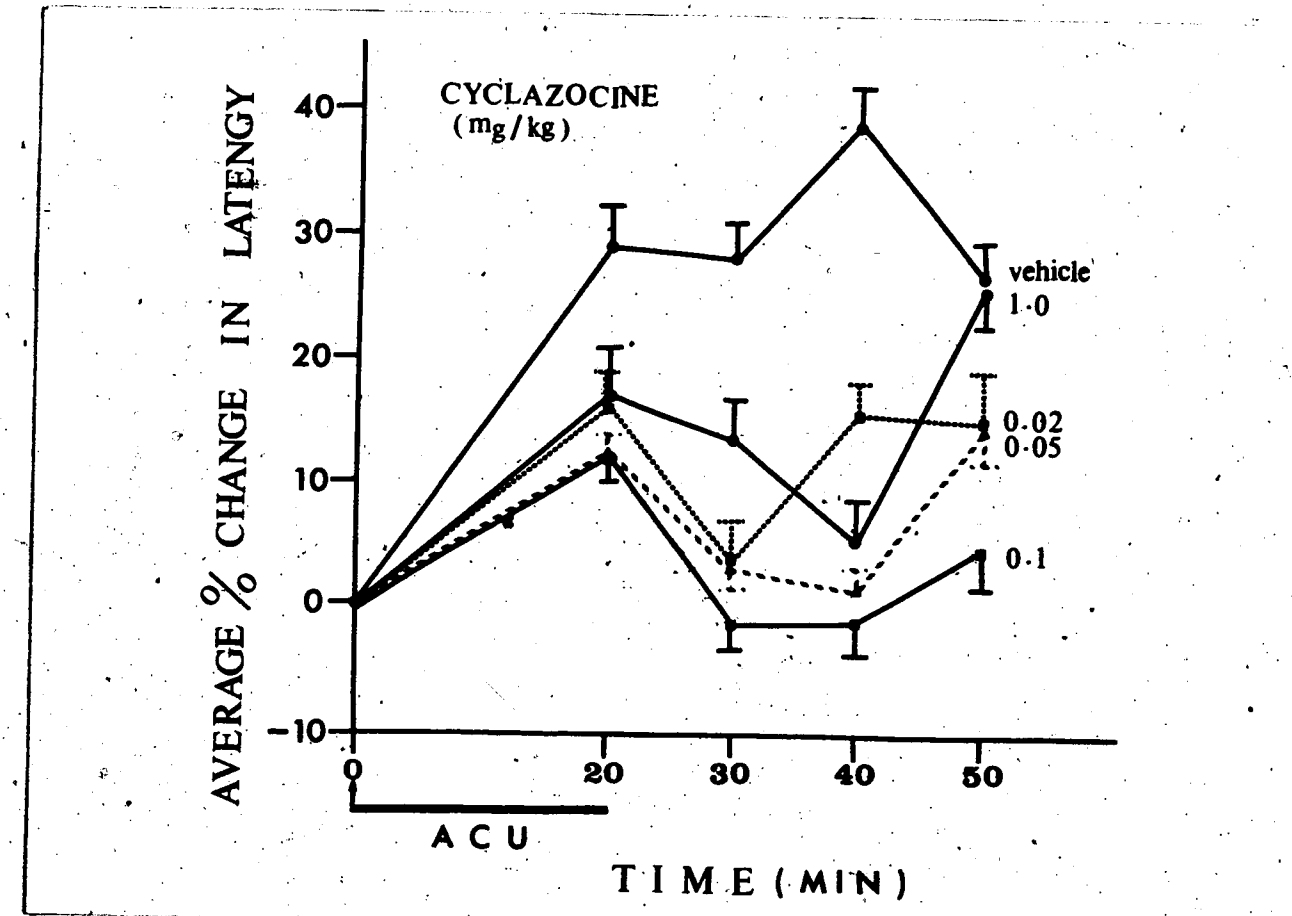


Fig. 6

Effect of diprenorphine on EAA in mice. This diagram is similar to figure 1, 2, 4 and 5. 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.

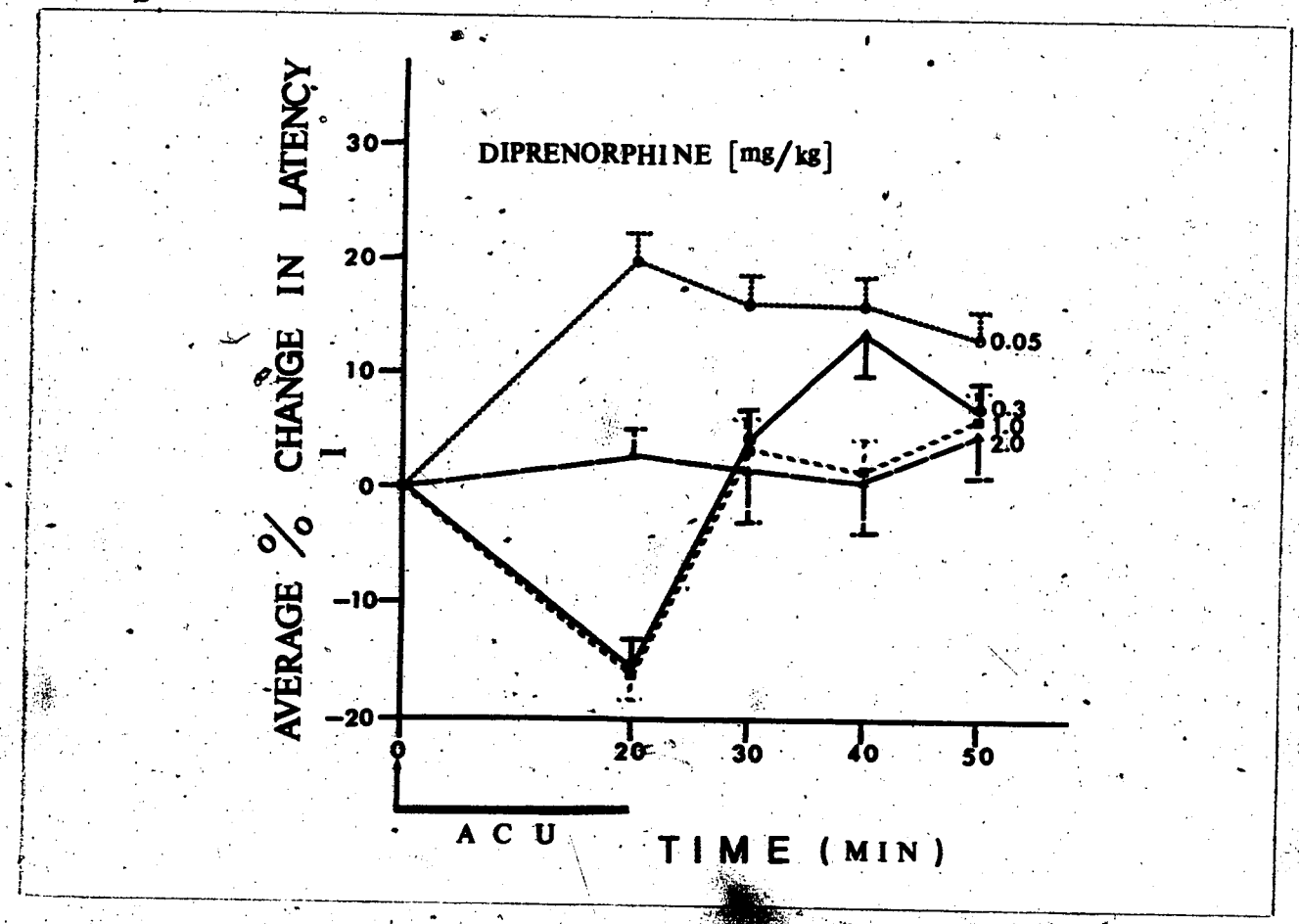
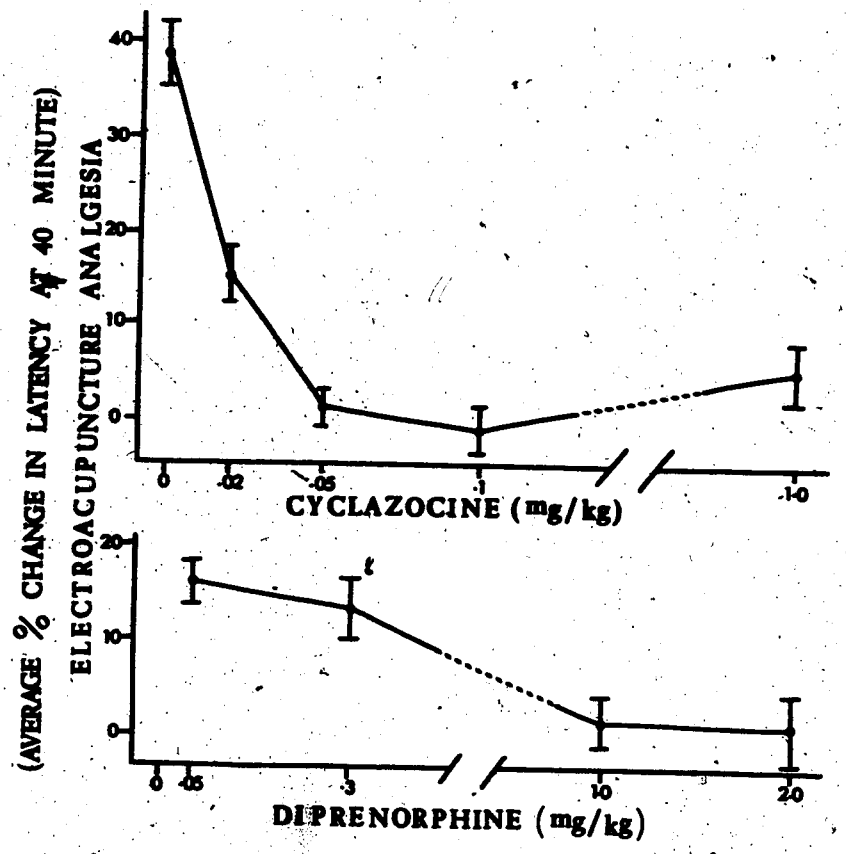


Fig. 7

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.



(D) Cyclazocine and Diprenophine: Fig. 5 shows that cyclazocine inhibits the EAA at doses 1, 0.1, 0.05, and 0.02 mg/kg as compared to the vehicle control ($p<0.05$, F-test and Newman-Keul's test). The maximum inhibitory effect is observed at 0.1 mg/kg (Fig. 7 upper line, the dose-response curve). Mixed agonistic and antagonistic effects began to show at dose higher than 1 mg/kg. At 1 mg/kg, 5 out of 15 mice showed profound EA analgesia.

Similarly diprenorphine also blocks EAA at doses 2, 1, 0.3 and 0.05 mg/kg (Fig. 6). Maximum inhibitory effect occurs at 1 mg/kg; hyperalgesia is observed at 20 minutes after the drug administration (Fig. 6). The bottom line of Fig. 7 shows a dose-response curve for the effect of diprenorphine on EAA.

DISCUSSION

The results of this study show that electroacupuncture analgesia is mediated by stereospecific opiate receptors. When the opiate receptor sites were occupied by (-) naloxone, electroacupuncture analgesia was blocked. On the other hand, the dextroisomer (+) naloxone (having 1/1000 - 1/10,000th the affinity of (-) naloxone for opiate receptors) did not block electroacupuncture analgesia. Levonaloxone, naltrexone and diprenorphine are all antagonists of Type I opiate receptors and they all inhibit EAA. All

these results converge to the same conclusion that EAA is mediated by Type I receptors.

Type I opiate receptors are found mostly in the brain areas which mediate analgesia (Pert et al, 1979). They are most likely the receptors responsible for exogenous or endogenous opiate analgesia. Cyclazocine at doses of 0.02 and 0.03 will antagonize meperidine analgesia in mice (Pearl and Harris, 1966) and morphine analgesia in rats (Machne et al, 1974) respectively. EAA is significantly inhibited by all these antagonists at doses ranging from 4 to 0.0124 mg/kg. Type II opiate receptors are probably responsible for feelings of sedation, dysphoria and the hallucinatory syndrome. Unfortunately, no antagonist has yet been found for Type II receptors. However, it is highly unlikely that Type II receptors are involved in EAA since Type I antagonists completely reverse EAA. These results (the stereospecificity data, and the effects of Type I blockers) strongly support the hypothesis that opiate receptors are involved in electroacupuncture analgesia.

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CHAPTER 3: DEXAMETHASONE PARTIALLY REDUCES AND 2% SALINE-TREATMENT ABOLISHES ELECTROACUPUNCTURE ANALGESIA: THESE FINDINGS IMPLICATE PITUITARY ENDORPHINS

SUMMARY

Dexamethasone, a cortisol analogue which inhibits ACTH and endorphin release in a negative feedback system, partially reduces electroacupuncture analgesia (EAA) in mice. In addition, mice forced to drink 2% saline for 3 days (this reduces pituitary endorphin levels) had a complete loss of EAA. These two experiments support our previous finding that hypophysectomy abolishes EAA. Altogether, these results implicate pituitary endorphins in EAA.

INTRODUCTION

Several reports have indicated that electroacupuncture analgesia (EAA) may be mediated by endorphin (Pomeranz, 1977; Pomeranz et al, 1977; Pomeranz and Chiu, 1976; Cheng, Master Thesis, 1977; Mayer et al, 1977). Endorphin levels are elevated in human CSF after transcutaneous EAA but the fraction I involved is not beta-endorphin (Sjolund et al, 1977; Akil et al, 1978). However, applying electrical foot-shock to the rats elevated their blood beta-endorphin levels (Rossier et al, 1977). Recently Hosbuchi

et al (1978) reported that beta-endorphin levels increase in human ventricular CSF after brain stimulation (SPA). However, little evidence has yet been published to show that beta-endorphin is elevated in EAA. The previous results showed that hypophysectomy abolished EAA in mice (Cheng, 1977), but removing the pituitary is a very drastic procedure, so more subtle means of reducing pituitary endorphin were used for this experiment.

The present studies demonstrated the effect of dexamethasone injections and 2% saline treatment on EAA in mice. It has been found that ACTH and beta-endorphin are made from the same prohormone and are released concomitantly (Guillemin et al, 1977). ACTH causes the adrenal gland to release corticosterone and cortisol which will, in a negative feedback system, reduce the further release of ACTH (Schapiro et al, 1958). Dexamethasone is a potent cortisol analogue which can strongly inhibit ACTH (Mangili et al, 1966; De Wied, 1964) and beta-endorphin release from the pituitary (Santagostino et al, 1978). Recently it was shown that morphine or stress-induced increase of plasma beta-endorphin and prolactin are prevented by dexamethasone pretreatment (French et al, 1978). If beta-endorphin is involved in EAA, dexamethasone should block EAA by inhibiting release of beta-endorphin.

B. M. Cox et al (1978) and Mata et al (1977) reported that 50% of the rats drinking 2% saline for one to seven days showed a significant reduction of the pituitary endorphins. The above reduction should also reduce the effect of EAA, if pituitary endorphins are involved.

METHODS

The methods used in this study were similar to those described in the first paper. Briefly, a behavioural pain threshold measurement was used in female mice of an F1 cross between C57 BL6J and A/J from Jackson Laboratory; the latency to squeak (vocalize) was determined in response to noxious stimuli applied to the nose. The squeak latency was measured from noxious stimuli applied to the nose. The squeak latency was measured from an audiogram. Four control tests were given, 3 minutes apart, to the restrained mice. The mean of the last 3 tests gave the zero time control value for each mouse (before electroacupuncture began). Only those mice giving 3 reproducible responses in the control period, were used for subsequent acupuncture treatment.

(i) In the first study, mice were injected with either dexamethasone (500 ug/kg, I.M.) or vehicle control (0.9% saline) four hours before the testing began in a "blind" manner. Eighty mice were used in this experiment with 40 randomly assigned to each substance. Electrical stimulation was applied for 20 minutes by means of diphasic square

pulses from a Grass SD9 stimulator at 4 Hz, 0.1 msec. duration and with voltages ranging from 6 - 18 volts. Voltage was again adjusted to maximum (just below the squeak threshold) at 10 minutes after the onset of EA. The acupuncture needles were removed after a 20-minute treatment period and recovery was monitored for another 30 minutes. (ii) In the other study, 40 mice were given only 2% saline to drink and 40 mice were given ordinary tap-water for 72 hours before the electroacupuncture treatment began. In both of the above studies, mice were randomly assigned to either the control or experimental group.

Statistics were done with Student's t-test (2 tailed) on the average of 20 to 40 minute values. This time window was preselected on the basis of several previous acupuncture papers in humans (Mayer et al, 1977; Chapman and Benedetti, 1977) and animals (Pomeranz and Chiu, 1976) which showed that analgesia peaks at 20 to 40 minutes after onset of acupuncture.

RESULTS

(i) In the first experiment, there is a significant difference between the 20 to 40 minute means and preacupuncture control values in both dexamethasone-treated mice (Fig. 1, bottom line) ($p<0.001$) and vehicle-treated mice (Fig.1, top line) ($p<0.001$).

Fig. 1

Effect of dexamethasone on electroacupuncture in mice. Percentage change in latency to squeak as compared to zero time preacupuncture control value. Positive values denote analgesia. Mice were pretreated with either dexamethasone (500 ug/kg, I.M.) or vehicle (0.9% saline) 4 hours before the experiment. Average baseline pain latencies just prior to EAA were 4.37 + 0.23 sec. for vehicle-treated mice and 4.02 + 0.24 sec. for dexamethasone-treated mice. Top line shows the acupuncture effect on vehicle-treated mice. Each point is the mean for 40 mice. Lower line shows the acupuncture effect on dexamethasone-treated mice. Bars show S.E. Arrows indicate time of electroacupuncture which starts after zero time and stops at 20 minutes. Dexamethasone partially blocks acupuncture analgesia.

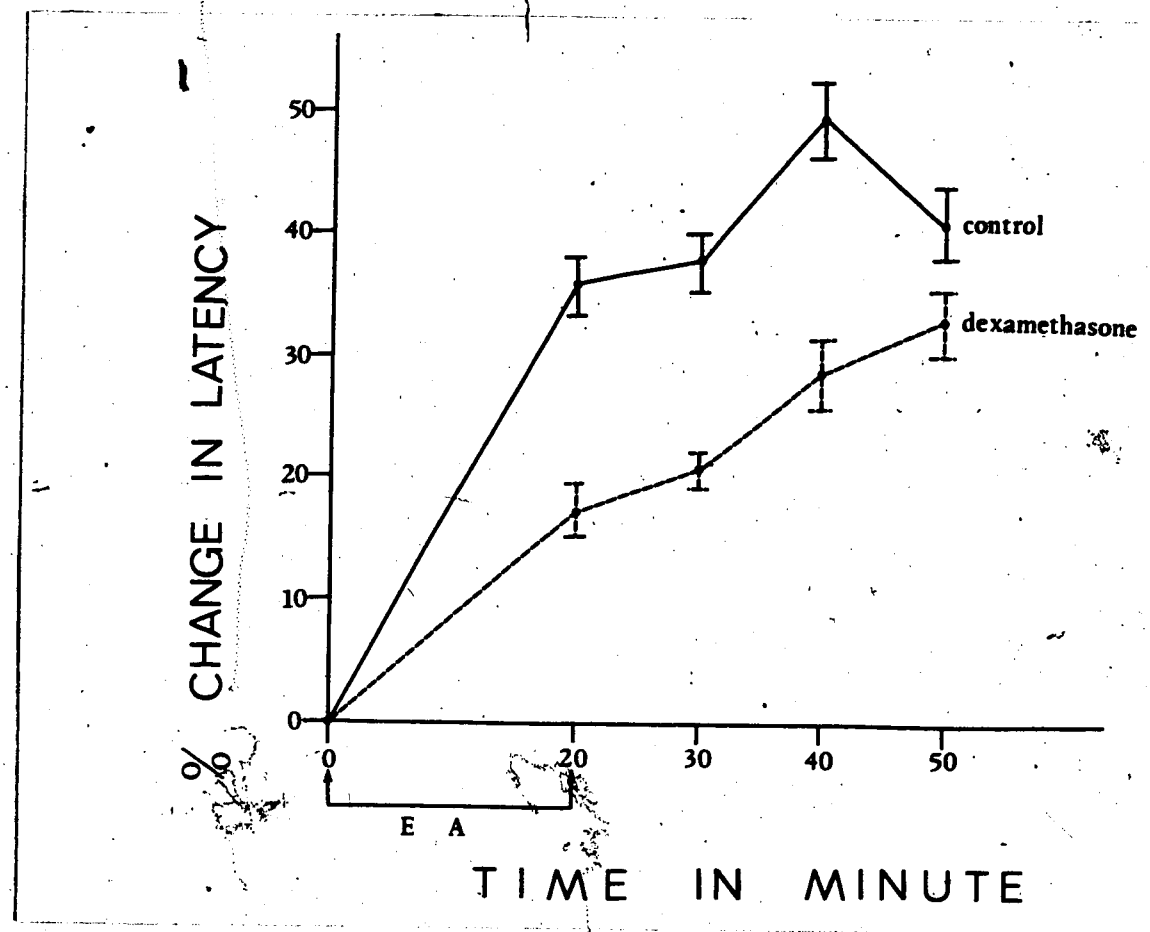


Fig. 2

Effect of 2% saline-treatment on electroacupuncture in mice. Percentage change in latency to squeak as compared to zero time preacupuncture control value. Positive values denote analgesia. Mice were fed with either 2% saline or ordinary tap-water for 3 days before the experiment. Average baseline pain thresholds just prior to EAA were 4.14 + 0.26 sec. for 2% saline-treated mice and 4.23 + 0.15 sec. for tap-water treated mice. Top line shows the acupuncture effect on tap-water fed mice. Lower line shows the acupuncture effect on 2% saline-fed mice. Each point is the mean for 40 mice. Bars show S.E. Arrows indicate time of electroacupuncture which starts after zero and stops at 20 minutes. Long term feeding with 2% saline abolishes electroacupuncture.

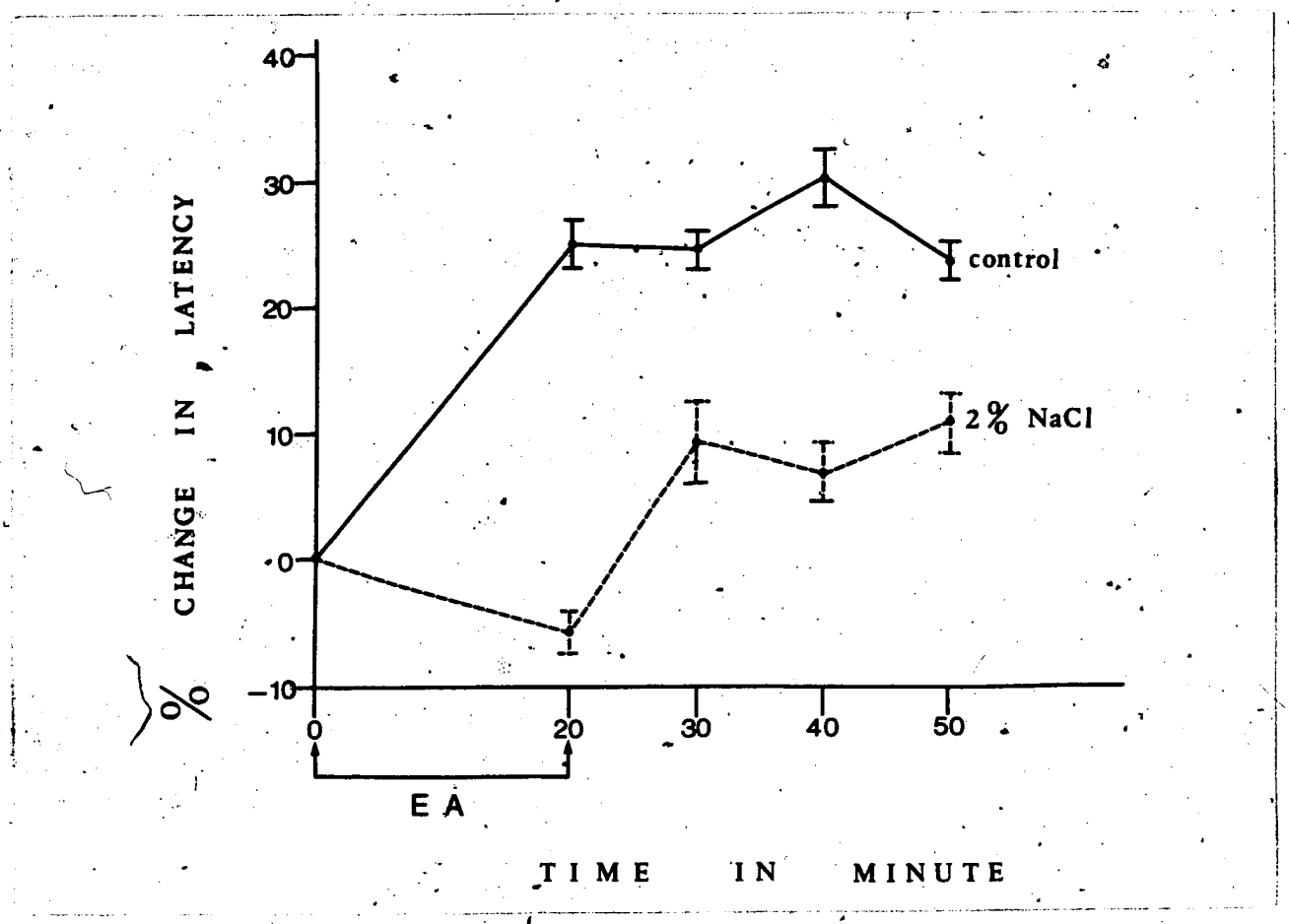
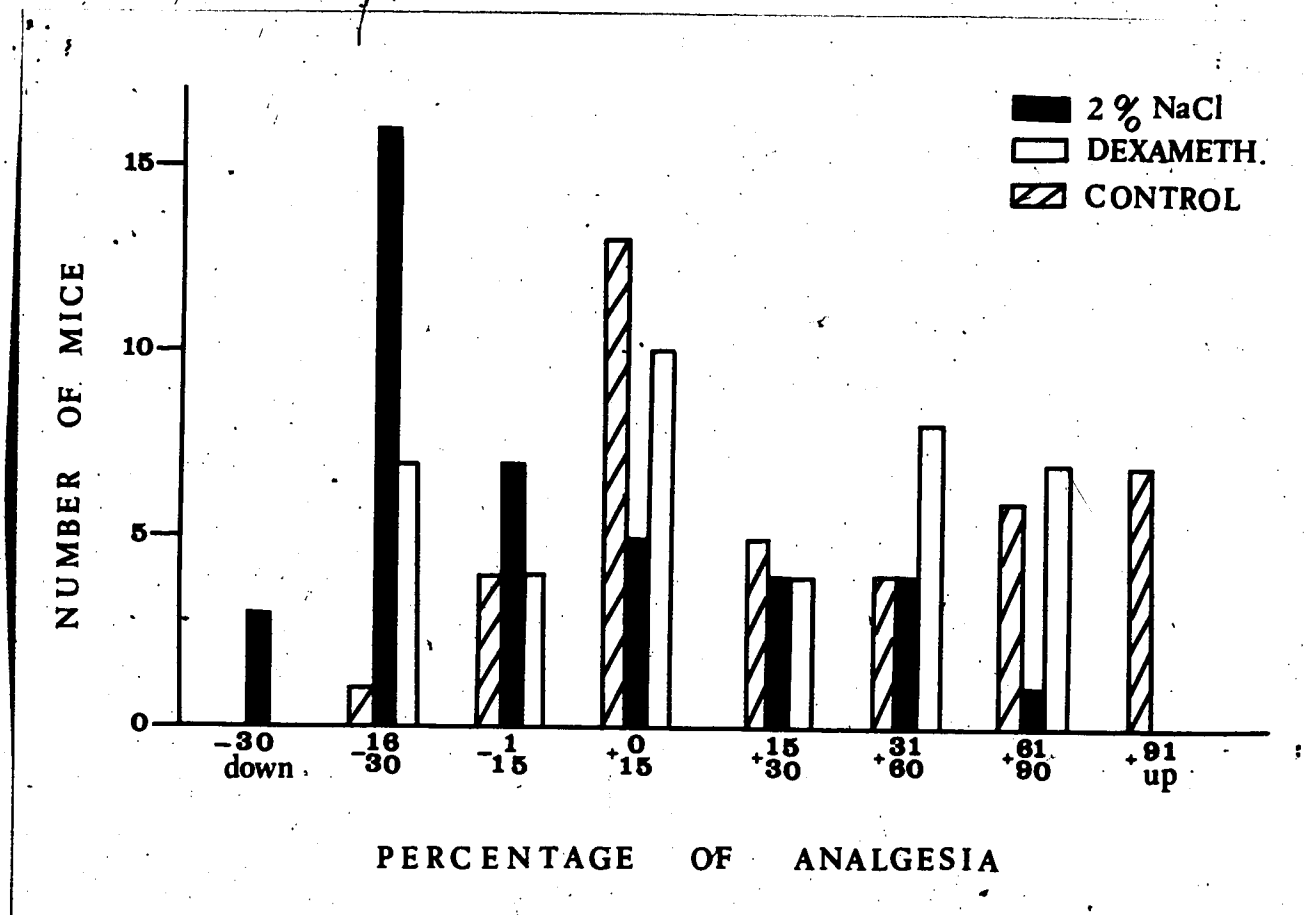


Fig. 3

This is a histogram of EAA on the mice that were treated with 2% NaCl solution, dexamethasone and water (40 per group). Ordinate shows the number of mice. Abscissa indicates EAA which is averaged from the 20, 30 and 40 minute measurements for each mouse. Significant EAA (>15%) were observed in 22 control mice, 19 dexamethasone treated mice and 9 (2%)-saline-treated mice.



However, a between-group significant difference exists in the 20 to 40 minute means in both groups ($p<0.05$) indicating that dexamethasone partially blocks EAA.

(ii) In the second experiment, the 20 to 40 minute mean shows no significant difference as compared to the preacupuncture control value ($p>0.1$) in the 2% saline-treated mice (Fig. 2, bottom line); while the control group (Fig. 2, top line) shows significant EAA ($p<0.001$). Moreover, there is a significant difference between the 20 to 40 minute means in the 2% saline-fed mice and the normal water-fed mice ($p<0.001$). This result indicates that the depletion of beta-endorphin (by 2% saline) blocks acupuncture analgesia.

(iii) Figure 3 showed a histogram of EAA on three groups of mice(40 per group) that were treated with 2% saline, dexamethasone, and water(control).

DISCUSSION

Recently it was shown that dexamethasone inhibits the release of beta-endorphin (Santagostino et al, 1978; French et al, 1978) and ACTH (Mangili et al, 1966; De Wied, 1964). The present results have shown that it also reduces EAA in mice. From this it may be concluded that EAA requires beta-endorphin.

Pituitary endorphins are significantly depleted by 2% saline-treatment in 50% of the animals (Cox et al, 1978; Mata et al, 1977). This depletion of endorphin completely abolishes EAA in mice and shows that pituitary endorphin may be involved in EAA.

In the experiments of both Cox et al (1978) and Mata et al (1977), pituitary endorphin levels were measured by bioassay. Since other opioid peptides, dynorphin (Goldstein et al, 1979) and enkephalin (Rossier et al, 1978) are reported to be present in pituitary, it is possible that 2% saline treatment may affect endorphins other than beta-endorphin. In addition, both dexamethasone and 2% saline treatments may affect the endorphins in the brain as well as pituitary. Although the present results and the previous hypophysectomy data strongly support the pituitary endorphin mediation of EAA, they cannot exclude a role for brain endorphins in EAA.

In conclusion, the present results show that EAA may be mediated by pituitary endorphins (possibly beta-endorphin). This supports the previous results using hypophysectomized mice (Pomeranz et al, 1977). An interesting clinical correlation might be the concomitant release of ACTH and endorphin by acupuncture: one prevents inflammation and the other causes analgesia, both of which would benefit diseases like arthritis.

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CHAPTER 4: ELECTROACUPUNCTURE ELEVATES BLOOD CORTISOL LEVELS IN NAIVE HORSES; SHAM TREATMENT HAS NO EFFECT

SUMMARY

It was hypothesized that electroacupuncture releases beta-endorphin and ACTH from the pituitary. Since ACTH induces the release of cortisol from the adrenal glands, blood cortisol level should be enhances by electroacupuncture. The present result shows that the blood cortisol levels of horses are significantly increased after 30 min. of electroacupuncture treatment while the sham treatment (control) shows an insignificant effect.

INTRODUCTION

Numerous experiments suggest that electroacupuncture (EA) induces pain reduction (analgesia) which may be mediated by endorphins (Pomeranz & Chiu, 1976; Mayer et al., 1977; Pomeranz et al., 1977; Cheng, 1979; Cheng et al., 1977). Sjolund et al. (1977) reported that transcutaneous electroupuncture elevates human CSF endorphin which is not beta-endorphin. However, foot-shock raises blood beta-endorphin levels in rats (Rossier et al., 1977) and brain stimulation (SPA) increases beta-endorphin in human ventricular CSF (Hosobuchi et al., 1979). Beta-endorphin is mainly found in the pituitary (Goldstein, 1976) and our previous

results show that reduction of pituitary endorphins abolish electroacupuncture analgesia (EAA) in mice (Cheng et al., 1979). It has been reported that ACTH and beta-endorphin are made from the same prohormone and are released concomitantly (Guillemin et al., 1977). ACTH induces the adrenal gland to release cortisol which will, in a negative feedback system, reduce the further release of ACTH (Schapiro et al., 1958). Dexamethasone is a potent cortisol analogue which can strongly inhibit ACTH (Mangili & Martini, 1966; De Wied, 1964) and beta-endorphin release from the pituitary (Santagostino et al., 1978). Recently we have shown that dexamethasone partially blocks EAA in mice (Cheng et al., 1979): this shows that EAA is at least partially mediated by beta-endorphin (and ACTH). However, it is possible that EA could release beta-endorphin (and/or other endorphins) for pain relief as well as ACTH for therapeutic functions. For example, ACTH is used to prevent inflammation in the treatment of arthritis. Thus, it is hypothesized that if EA releases ACTH it will eventually enhance blood cortisol level. The present paper shows that blood cortisol levels are greatly elevated after EA in awake, naive horses.

METHOD

Experiments were carried out in awake, naive horses (at Dr. L. McKibbin's Wheatley Hall Farm, Ontario, Canada). For each horse blood samples (5 ml/sample from the right jugular vein) were taken twice immediately before and 30 min. after

EA or sham EA. The blood samples were heparinized and immediately centrifuged to obtain the plasma which was frozen for later cortisol assay by radioimmunoassay (RIA). The RIA assay for cortisol was recently developed and reported by Wong et al. (1979). EA was done by inserting 4 or 5 sterilized needles (14 gauge, 10 cm long) to 3 to 5 cm deep at 4 or 5 of the following acupuncture points: B13, B65, GB26, PC9, Ki 1 or Ki 2. These points are generally used to treat pain and arthritis in the hind limbs and fore limbs (Shanghai Institute, 1973). All the needles were connected to a five channel neuro- stimulator (MRL Medical Research Lab. Corp., Chicago). Electrical stimulation was applied at 5 Hz, voltage was adjusted to be above threshold for muscle contraction, 0.25 msec duration and biphasic pulses for a period of 30 min. Similar treatment was carried out during sham EA, except that 4 or 5 needles were inserted subcutaneously at nonacupuncture points located at about 5 cm below the points GB30, G29, B13, B18 and B47. Similar electrical parameters were used for sham EA. Horses were randomly assigned either for EA or sham treatment.

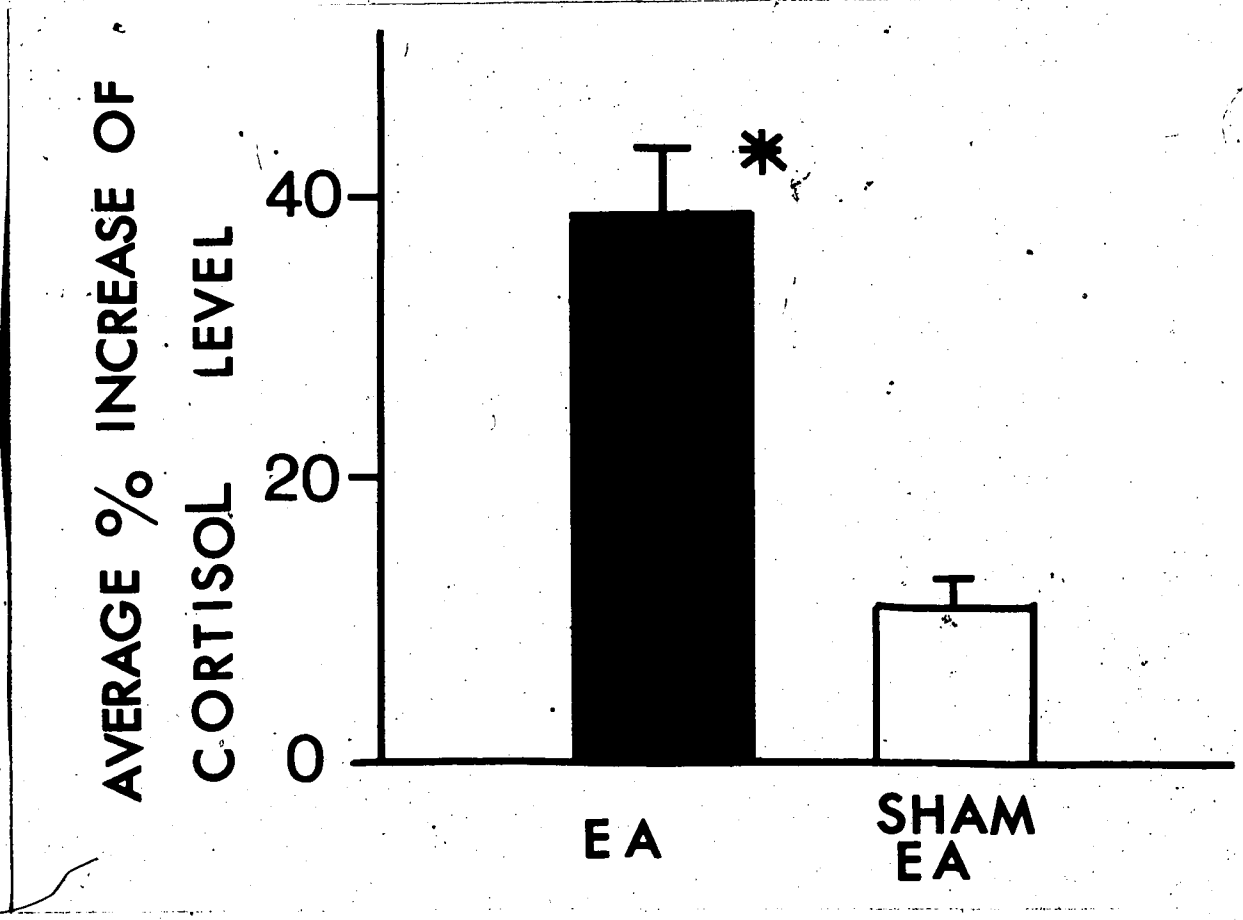
RESULTS

The results are shown in Figure 1. In 15 horses, EA produced a significant increase in cortisol levels ($p<0.01$, t-test, two tailed), while sham treatment in another 15 horses showed a small but non-significant elevation ($p>0.05$, t-

test, two tailed). Those two results were significantly different ($p<0.01$, t-test, two tailed). Since the sham EA controlled for such variables as stress and learning, we are confident that the results reflect a genuine EA effect.

Fig 1

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.



DISCUSSION

This experiment showed that electroacupuncture enhances blood cortisol levels in horses. This may indicate that electroacupuncture has a dual effect: first to release endorphins for the relief of pain, and second, the secretion of ACTH which could be anti-inflammatory and help cope with stress. This result may also indicate that electroacupuncture is useful for the clinical treatment of problems like arthritis and allergy which are often helped by ACTH or cortisone injections. Since these drugs have serious side effects when injected, perhaps their natural release by EA would be a safer method for therapy.

Footnote: Dr. McKibbin has treated over 500 racehorses for painful limbs with EA at these points. They raced much better after treatment with EA and limping was noticeably improved.

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CHAPTER 5: ELECTROACUPUNCTURE ANALGESIA COULD BE MEDIATED BY AT LEAST TWO PAIN-RELIEVING MECHANISMS; ENDORPHIN AND NON-ENDORPHIN SYSTEMS

SUMMARY

This present experiment shows different levels of electroacupuncture analgesia (antinociceptive effect) induced by three different frequencies of stimulation (i.e. 0.2, 4 and 200 Hz); highest analgesia is induced at 200 Hz and lowest at 0.2 Hz. Naloxone (1 mg/kg) completely reverses the electroacupuncture effects at low frequency stimulation (4 Hz) but produces no inhibition at high frequency stimulation (200 Hz). Conversely, parachlorophenylalanine (320 mg/kg) partially blocks the high- frequency (200 Hz) analgesia but produces no effect on the low-frequency (4 Hz) electroacupuncture analgesia. This suggests that electroacupuncture analgesia induced by low frequency stimulation may be mediated by endorphins while high frequency stimulation does not activate endorphinergic mechanisms but may exert its effect partly through release of serotonin.

INTRODUCTION

A considerable amount of evidence (Anderson et al, 1973; Anderson et al, 1974; Melzack, 1973; Pomeranz and Cheng, 1979) suggests that acupuncture induced analgesia (antinociceptive effect) initiates a slow onset but long lasting pain relief. The basic technique of acupuncture involves the insertion of stainless steel needles and vibration of the needles manually or by low frequency (2 - 6 Hz) electrical stimulation (Shanghai Acupuncture Group, 1972). Evidence indicated that acupuncture analgesia was mediated by endorphins (Pomeranz and Cheng, 1979; Mayer et al, 1977; Pomeranz and Chiu, 1976; Sjolund et al, 1977; Takeshige et al, 1978). This acupuncture analgesia induced by low frequency stimulation was reversed by naloxone in humans (Mayer et al, 1977), cats (Pomeranz and Cheng, 1979), rats (Takeshige et al, 1978) and mice (Pomeranz and Chiu, 1976). Recently, we demonstrated that pituitary endorphin is involved in electroacupuncture (EA) analgesia (Cheng et al, 1979) while McLenna et al (1977) showed that electrical or chemical lesions of raphe serotonin output abolish EA analgesia in rabbits. Thus more than one pain-relieving mechanism may be involved in EA analgesia. Furthermore Chapman and Benedetti (1977) showed that analgesia induced by high frequency (100 - 200 Hz) transcutaneous nerve stimulation (TNS), was only partially reversed by naloxone; moreover Sjolund and Erikson (1978) reported that naloxone blocked low frequency TNS analgesia but had no effect on the high-frequency TNS analgesia in humans. This implies that dif-

ferent mechanisms of pain relief may be mediated by different frequencies of stimulation during TNS or acupuncture. Perhaps EA analgesia induced by low frequency stimulation is mediated by endorphin while high frequency stimulation is not endorphinergic. The present study undertook to compare the effect of naloxone (an opiate antagonist) and/or para-chlorophenylalanine (a serotonin synthesis inhibitor) on EA analgesia at high or low frequency stimulation.

METHODS

A similar method to the one described in the previous papers, (Pomeranz and Chiu, 1976; Cheng et al, 1979) was used in this study. Briefly, a behavioral pain threshold measurement was used in female B6AF1/J mice from Jackson Laboratories. This was done by shining a hot lamp on the nose of the restrained mice until they squeaked. The latency to squeak was measured from an audiogram displayed on a storage oscilloscope. Three control tests were given, 3 minutes apart, to each mouse. The mean of these 3 tests gave zero time control value for each mouse before EA began. Only those mice giving 3 reproducible responses in the control period, with latencies between 3 to 5.5 seconds, were used for subsequent EA treatment. EA was given by inserting stainless steel needles (34G) in the first dorsal interosseus muscle on each forepaw (this point is called Hoku or L.I.4 in the acupuncture literature, Shanghai Acupuncture

Group, 1972). EA was applied for twenty minutes by means of square pulses from a Grass SD9 stimulator at 0.2 Hz, 4 Hz, or 200 Hz and 0.1 msec. duration. Voltage was adjusted to be above threshold for muscle contraction and just below the threshold for pain vocalization; this required electrical current in the range from 0.3-0.4 mA for 0.2 Hz, 0.2-0.3 mA for 4 Hz, and 0.1-0.2 mA for 200 Hz. Squeak latencies were measured again just before the EA needles were removed (i.e. after 20 minutes of EA treatment); then the squeak latencies were measured at 10 minutes apart for a total of 120 minutes. The average value of the squeak latencies at 20, 30 and 40 minutes was used for statistical analysis. Injection (I.P.) of 0.9% saline or levo-naloxone (1 mg/kg) was given twice in a blind manner, immediately before and again after treatment.

In the first study, there were six groups of mice (15 per group); two groups of mice which received EA with high frequency stimulation were injected either with saline or naloxone; two groups of mice which received EA with low frequency stimulation (4 Hz) were injected either with saline or naloxone; the fifth group received saline injection and EA treatment with electrical stimulation at 0.2 Hz. As a control, a sixth group received naloxone alone without EA treatment.

Naloxone reverses the EA analgesia which is induced by electrical stimulation at 4 Hz; hyperalgesia is observed at 20 minutes (Fig. 2, bottom line). However naloxone produces no inhibitory effect on the high-frequency (200 Hz) EA analgesia (Fig. 3). In the control study, naloxone given alone produces a small hyperalgesia of 9% ($p<0.05$, t-test two tailed), On the other hand, PCPA, a serotonin synthesis inhibitor, partially reverses the high-frequency (200 Hz) EA analgesia (Fig. 4, line 'PCPA-200Hz' and Table I,(iii)B) while PCPA produces no effect on low-frequency (4 Hz) EA analgesia (Fig. 4, Line 'PCPA-4Hz' and Table I,(iii)A). The combined treatment of PCPA and naloxone has a profound reversal effect on the low-frequency (4 Hz) EA treatment (Fig. 4, bottom line); significant hyeralgesia (8%) is observed at 20 - 40 minute interval ($p<0.05$, t-test two tailed). Although PCPA plus naloxone treatment shows no statistical difference as compared to PCPA treatment alone on high-frequency EA analgesia at the 20 - 40 minute interval (Table I,(iv)B) a delay and reduction of high-frequency EA analgesia is observed at 20 minutes (Fig. 4, line 'PCPA+Nal-200Hz'). In the control study, the average pain thresholds are 5.44 + 0.08 sec. and 5.21 + 0.08 sec. for 15 mice before and 3 days after 0.9% saline injection ($p>0.1$, t-test two tailed); and for PCPA (320 mg/kg) treated mice the thresholds are 4.28 + 0.01 sec. and 4.43 + 0.01 sec. respectively ($p>0.1$, t-test two tailed). Thus PCPA alone does not change the pain thresholds of the mice.

Fig. 1

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 EA. Bars indicate standard error. Each point is the mean of 15 mice.

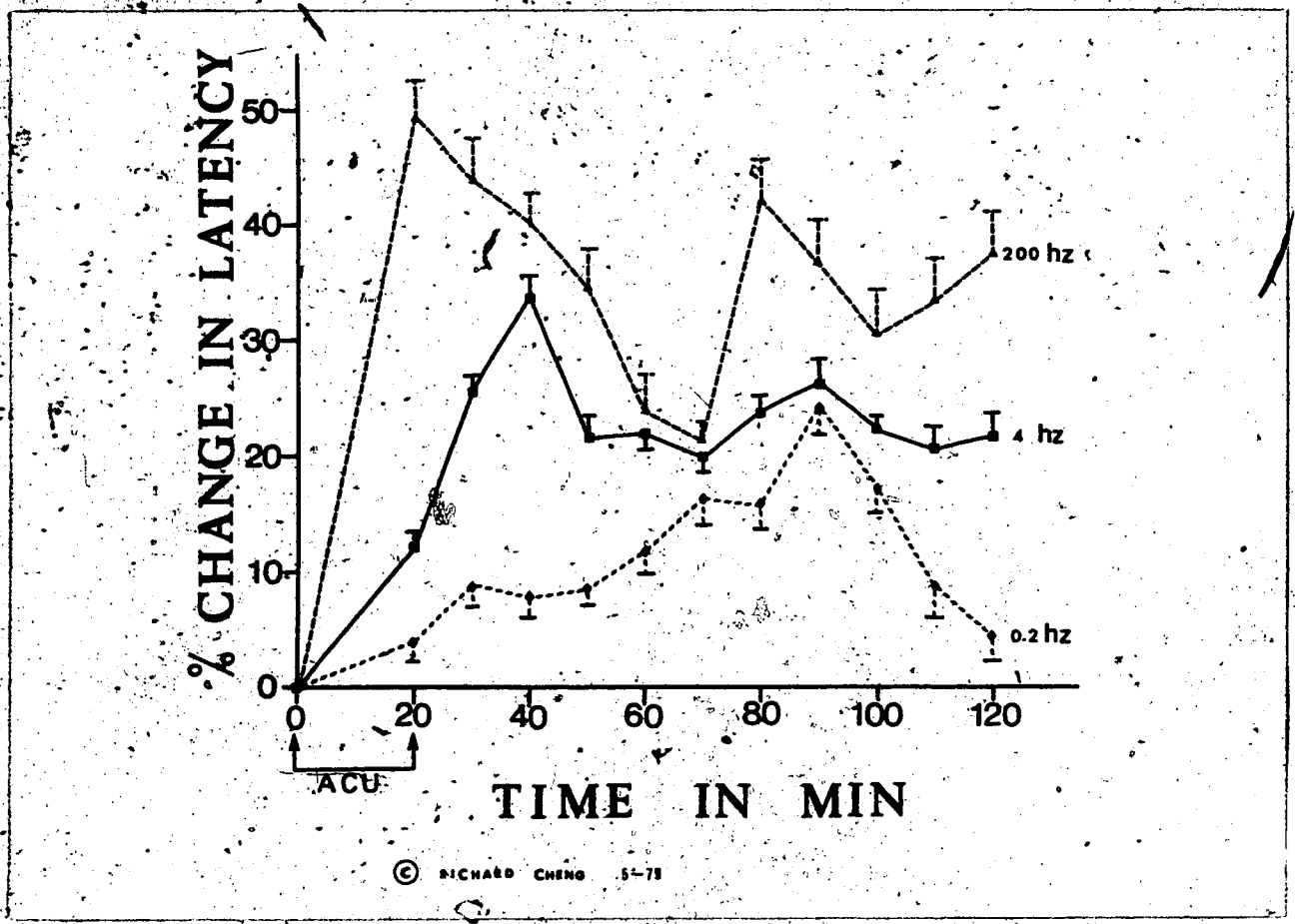


Fig. 2

Naloxone inhibits electroacupuncture (EA) analgesia which is induced by 4 Hz electrical stimulation. Coordinates same as Fig. 1. 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.

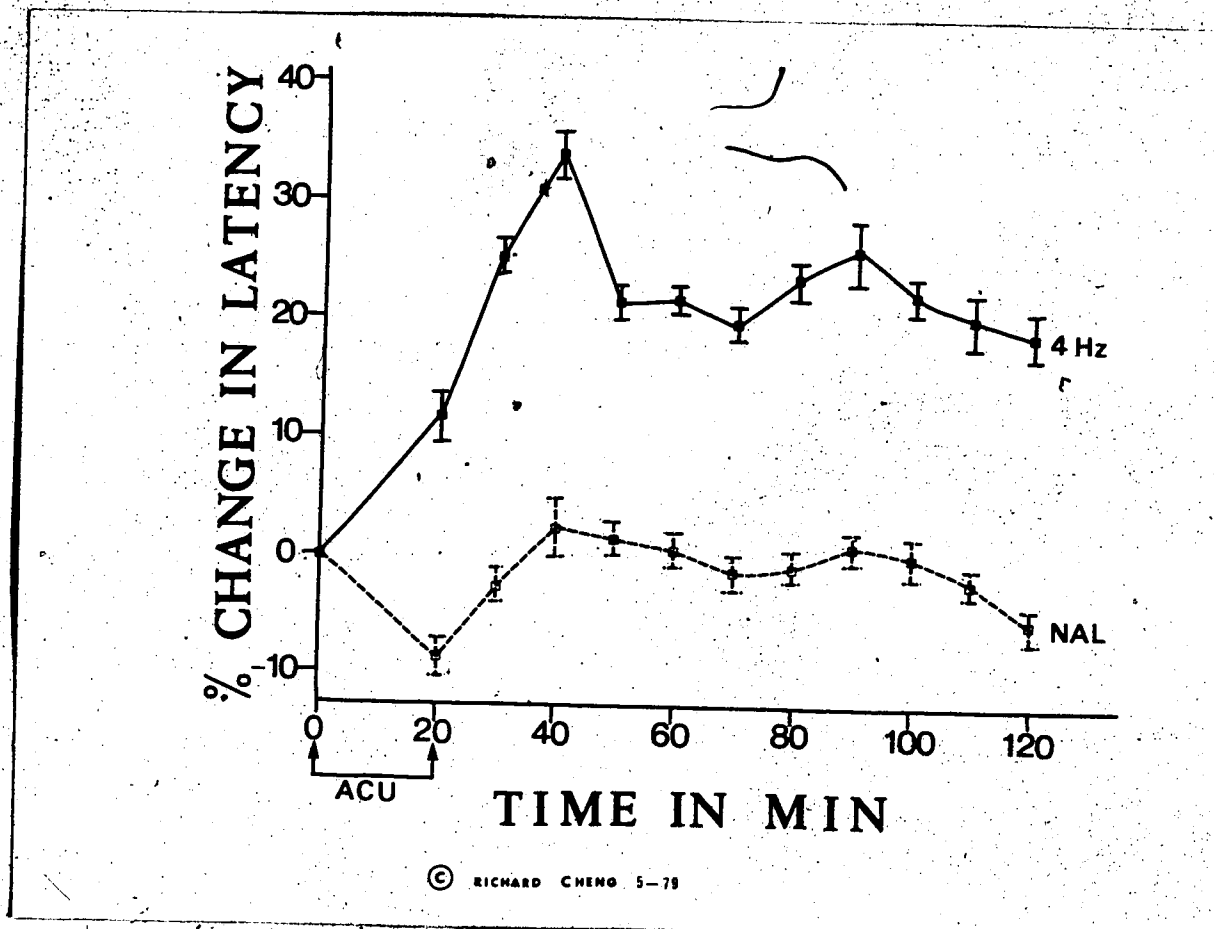


Fig. 3

Naloxone does not reverse the electroacupuncture (EA) effect at high frequency (200 Hz) stimulation. Coordinates same as Fig. 1. 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.

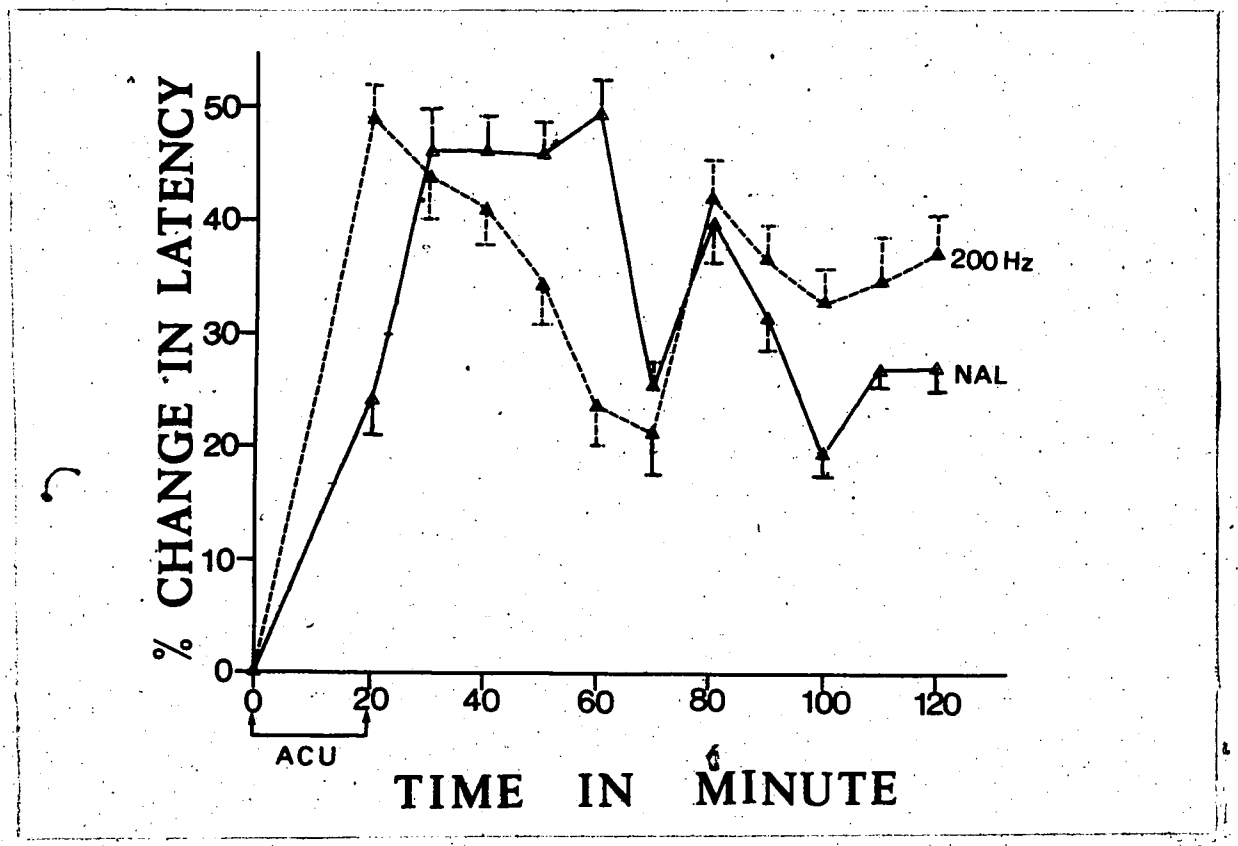
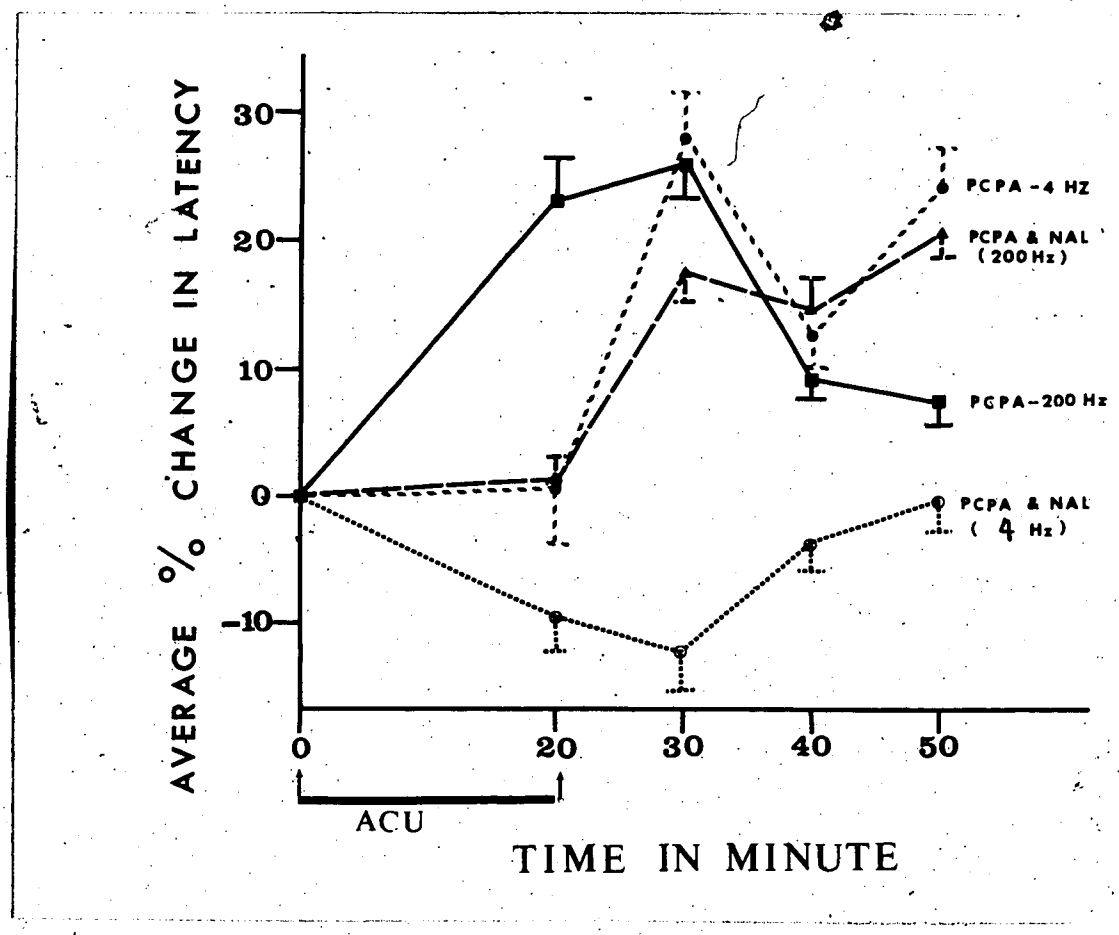


Fig. 4

Parachlorophenylalanine partially blocks high frequency (200 Hz) EA analgesia but not 4 Hz EA effect. Coordinates same as Fig. 1. 'PCPA-200Hz' indicates high frequency EA treatment in PCPA treated mice. 'PCPA+Nal-(200Hz)' indicates high frequency EA treatment in PCPA and naloxone treated mice. treatment in PCPA treated mice. 'PCPA+Nal-(4Hz)' 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.



The above results indicate that low-frequency EA analgesia is mainly mediated by endorphins while high frequency EA analgesia is partially mediated by serotonin.

Table 1

To compare the effect of naloxone and/or PCPA on EA analgesia.

Frequency	(i) Control	(ii) Compared to (i)	(iii) Compare to (i)	(iv) Compared to (iii)
(A) 4 Hz	Saline	Naloxone	PCPA	PCPA+Naloxone
t-test (two-tailed)		$p<0.01$*	$p>0.05$	$p<0.05$*
(B) 200Hz	Saline	Naloxone	PCPA	PCPA+Naloxone
t-test (two-tailed)		$p>0.1$	$p<0.05$*	$P>0.05$

* indicate significant difference : t-test two tailed

DISCUSSION

The present results suggest that EA analgesia may be mediated by more than one pain-relieving mechanism. At low frequency stimulation, EA induces naloxone reversible analgesia but at high frequency stimulation, naloxone does not

reverse the EA effects. This indicates that the former may be mediated by endorphins and the latter is not endorphinergic. Moreover PCPA partially blocks high-frequency EA analgesia; this suggests that analgesia elicited by high-frequency stimulation is at least partially mediated by serotonin. It should be noted that naloxone in the controls produced only a 9% hyperalgesia (refer to Jacob et al, 1974) while reducing 4 Hz EA effects by 25%. This shows that naloxone effects are not a mere subtraction but rather a true reversal of endorphinergic effects. Also PCPA controls showed no threshold changes indicating a true abolition of EA effects by PCPA.

Anderson and his colleagues (1973) observed that higher frequency stimulation (10 and 100 Hz) gave a more rapid onset of the pain-threshold increase for tooth-pulp stimulation in humans. However stimulation at a low frequency (2 Hz) gave a more generalized analgesic effect which appeared to have a hormonal mediation. Thus it can be interpreted that low frequency stimulation is mediated by endorphins, while high frequency stimulation is strictly segmental and interferes with the transmission in the pain pathway in the CNS at the segmental level (Anderson et al, 1973). Perhaps high frequency electrical stimulation is similar to stimulation-produced-analgesia (SPA) by electrodes in the dorsal column as this analgesia is not naloxone reversible (Hosobuchi, 1978), and/or to SPA by electrodes in the raphe nucleus in which the analgesia is

reduced by PCPA (Akil and Mayer, 1972).

Clinically, it has been found that patients who show no pain-relief during high frequency stimulation obtain analgesia from low frequency stimulation during EA treatment (Sjolund and Erikson, 1978). It is possible that pain relief for certain diseases can be accomplished only by triggering a particular pain-relieving mechanism. This may be achieved by varying the frequencies of stimulation during EA or TNS treatment.

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CHAPTER 6: MONOAMINERGIC MECHANISMS OF ELECTROACUPUNCTURE ANALGESIA

SUMMARY

This experiment showed the effects of systemic injections of monoamine depletors, enhancers or receptor blockers on 200 Hz electroacupuncture analgesia (EAA) in mice. The following results emerged:

(i) EAA is reduced by depletors of monoamines (tetrabenazine, TBZ, depletes all monoamines; parachlorophenylalanine, PCPA, depletes serotonin; alpha-methylparatyrosine, AMPT depletes catecholamines). However, depletion of noradrenaline and increase of serotonin by disulfiram enhances EAA.

(ii) Replacement of depleted monoamines after TBZ treatment by their precursors (5HTP or L-DOPA) restores EAA.

(iii) EAA is enhanced by potentiating serotonin and dopamine by probenecid. EAA is also enhanced by the administration of monoamine precursors (L-DOPA for dopamine, 5HTP for serotonin). The dopamine receptor stimulator, apomorphine reduces EAA.

(iv) EAA is also reduced by receptor blockade of dopamine (by haloperidol), or blockade of noradrenaline and dopamine (by yohimbine) or serotonin (by cinanserin). However, blockade of dopamine by pimozide has no significant

effect on EAA.

There are two main conclusions: (a) EAA results are similar to those previously reported for SPA for all drugs except apomorphine and pimozide. (b) EAA shows consistent results only with manipulations of serotonin: the data indicating that EAA at 200 Hz is mediated by serotonin. Since previous studies show that raphe or DLF (dorsolateral fasciculus) lesions abolish EAA, we postulate that descending axons from raphe release serotonin to inhibit spinal cord nociception during EAA.

INTRODUCTION

Recently, numerous results indicate that acupuncture analgesia may be mediated by endorphins (Mayer et al, 1977; Pomeranz and Chiu, 1976; Sjolund et al, 1977; Pomeranz and Cheng, 1979; Cheng and Pomeranz, 1979). Additionally, it has been demonstrated that electroacupuncture analgesia (EAA) induced by low frequency electrical stimulation (4 Hz) may be mediated by endorphins while high frequency electrical stimulation (200 Hz) may cause EAA through serotonin (Cheng and Pomeranz, 1979). Therefore, the 200 Hz electrical stimulation was chosen to study the involvement of all the monoamines on EAA.

Akil and Mayer (1972) reported that stimulation-produced analgesia (SPA) elicited by stimulation of the dorsal raphe nucleus in rats was inhibited by para-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor, while SPA elicited from other midbrain regions is only partially blocked by naloxone (Akil et al, 1976), an endorphin antagonist. Moreover in cats SPA is often not reversed by naloxone (Carstens et al, 1979). Perhaps SPA has two mechanisms depending on the location of the electrodes: by one mechanism it may trigger the release of enkephalins which indirectly stimulate the raphe serotonergic cells to send a descending inhibition to the spinal cord to suppress pain; by another mechanism SPA may stimulate directly the serotonin containing cells of the raphe to activate this descending inhibition (Akil and Mayer, 1972; Carstens et al, 1979; Basbaum and Fields, 1978). It has also been suggested that catecholamines are also involved in SPA (Basbaum and Fields, 1978). Moreover the monoamine-mediated descending inhibition from SPA appears to travel mainly via the dorsolateral fasciculus (DLF) from raphe to spinal cord (Basbaum and Fields, 1978). The role played by the cerebral monoamines (dopamines, serotonin, noradrenaline) in SPA has been extensively studied by Akil and Liebeskind (1975). In order to compare SPA and EAA with respect to the role played by the three monoamines (dopamine, noradrenaline and serotonin), we used similar drugs to the ones that were employed by Akil and Liebeskind (1975), when they investigated the

monoamine mechanisms of SPA. The results showed many similarities between SPA and EAA.

METHODS

A. Pain threshold measurements and EA treatment

A method similar to the one described in our previous papers (Pomeranz and Cheng, 1979; Cheng and Pomeranz, 1979) was used in this study. Briefly, we used a behavioral pain threshold measurement in female B6AF1/J mice from Jackson Laboratories. This was done by projecting radiant heat on the nose of restrained mice until they squeaked. The distance from the hot lamp to the nose was kept constant at 9 cm. The latency to vocalization was measured from an audiogram displayed on a storage oscilloscope. Three control tests were given, 3 minutes apart, to each mouse three days before the EA experiments began: these 3 tests gave control (pre-drug) values for each mouse. Drugs were than administered at different times during those 3 days as indicated in the following 'RESULTS'. After giving these drugs, EAA was studied during the optimum time for the drug effects (see results) in the following manner: three squeak latencies taken just before EA treatment began and the mean gave a measure of the post-drug (baseline) effect. Then EA was given by inserting stainless steel needles (34G) in the first dorsal interosseus muscle on each forepaw (this point is called Hoku or Large Intestine 4 in the acupuncture

literature, Shanghai Institute, 1973). EA was applied for twenty minutes by means of square pulses from a Grass SD9 stimulator at 200 Hz and 0.1 msec duration. Voltage was adjusted to be above threshold for muscle contraction and below the threshold for pain vocalization; this required from 0.1 to 0.2 mA. This electrical current was just above the threshold for stimulating AB fibers (Pomeranz and Paley, 1979) and was a non-noxious stimulus (i.e. the mice did not squeak during EA). Previous results indicated that mice showed no significant EAA if they were treated in a similar way except with 0.2 Hz (Cheng and Pomeranz, 1979); this served as a control for stresses caused by restraint and needle insertions. Squeak threshold was measured again just before the EA needles were removed (i.e. after 20 min of EA treatment); then the squeak threshold was measured at intervals of 10 min for a total of 50 min after the initiation of EA. The average value of the squeak latencies at 20, 30 and 40 min was used for statistical analysis, as numerous previous studies showed this time to be the optimum for EAA (Pomeranz and Chiu, 1976; Mayer et al, 1977; Cheng and Pomeranz, 1979). Percentage analgesia for EAA was determined for each mouse from the formula: 100% x (Post EA latency - Predrug latency) / Predrug latency. Injection of 0.5 ml vehicle or drug was given intraperitoneally in a blind manner to 336 naive mice randomly assigned to 28 groups (12 mice per group). Unpaired Student's t-test (two tailed) was used to compare two groups of animals; for more

than two groups of animals, ANOVA and Newman-Keul's tests were employed. Within group comparisons were done with paired Student's t-test (two tailed). The criterion for significance was $p<0.05$ for all tests.

B. General approach and drugs employed:

Four approaches similar to those described by Akil and Liebeskind (1975) were used to alter transmission in monoamine pathways in order to study their effects on EAA. (this was done to facilitate comparisons with SPA):

(i) Monoamine depletion with non-specific depletors (tetrabenazine methyl sulfate TBZ) and more specific depletors (parachlorophenylalamine methyl ester, PCPA for serotonin, alpha-methyl-paratyrosine, AMPT for catecholamine and disulfiram for norepinephrine) were used.

(ii) Cerebral monoamines depleted by treatments in (i)(above) were replaced by administration of their precursors (DL-5-hydroxytryptophan, 5HTP and L-3,4-dihydroxyphenylalanine methyl ester, L-DOPA). (iii) Serotonin and dopamine were potentiated by probenecid, dopamine was enhanced by administration of precursor L-DOPA and serotonin was augmented by giving the precursor 5HTP or by disulfiram. The effect of apomorphine, a specific dopamine receptor stimulator, on EAA was also tested.

(iv) Finally, studies were done by using receptor blockade of dopamine (by haloperidol or by pimozide) or norepinephrine (by yohimbine), or serotonin (by cinanserin).

RESULTS

Tetrabenazine (TBZ)

TBZ was used to deplete all 3 brain monoamines (Quinn et al, 1959). 5HTP or L-DOPA were then administered to some mice to replace serotonin (Way and Shen, 1971) or dopamine (Bartholini et al, 1967) respectively after TBZ treatments.

Four groups of mice (12 mice per group) were used in this study. Group I was injected with vehicle (0.9% saline). Group II was injected with 10 mg/kg TBZ dissolved in 0.9% saline. Groups III and IV were used for "replacement" experiments: 25 minutes after TBZ treatments, group III and IV were injected with 50 mg/kg of benserazide hydrochloride, a peripheral decarboxylase inhibitor to prevent peripheral L-DOPA or 5HTP degradation; then group III was injected with 5HTP (200 mg/kg) and group IV with L-DOPA (200 mg/kg) 55 minutes after TBZ. As a control group II was given vehicle instead of replacement drug. EAA was then studied 30 min later at the time of optimum drug effect.

Table I shows the effect of TBZ alone or of TBZ followed by 5HTP or L-DOPA replacement on pain baseline thresholds (post-drug) and on EAA.

Table I

Effect of TBZ and (5HTP and L-DOPA) replacement on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	3.99±0.05	4.01±0.05	5.54±0.15
Analgesia (%)		0.71 ±0.53	40.81±3.98*
II. TBZ:			
Latency (sec)	4.66±0.03	4.74±0.04	5.10±0.12
Analgesia (%)		1.68±0.20	9.04±2.18
III. TBZ+5HTP:			
Latency (sec)	3.98±0.05	3.97±0.05	6.12±0.16
Analgesia (%)		-0.34±0.2	53.94±4.18*
IV. TBZ+L-DOPA:			
LATENCY (SEC)	4.56±0.03	4.59±0.03	5.63±0.13
ANALGESIA (%)		0.73±0.15	24.02±2.91*

These are the means of 12 mice ± S.E.

* Indicates that the % analgesia was significantly greater than pre-drug levels at $p<0.05$ using within group t-test. This format is used in tables I-XI.

Fig. 1

Effect of tetrabenazine (TBZ) on EAA. Ordinate indicates the average % change in squeak latency as compared to the control value. Positive denotes analgesia. Abscissa shows the time in minutes. Bars indicate S.E. (Pre) = pain thresholds taken as the zero control value before the administration of drugs. (Post) = average change of post-drug pain threshold. EA was applied from 0 to 20 minutes. Each data point represents the average of 12 mice. TBZ inhibits EAA. Replacement of 5HT (TBZ + 5HTP) enhances EAA as compared to that of vehicle-treated (0.9% saline) mice. Replacing dopamine (TBZ + L-DOPA) partially restores EAA. (Note: a similar legend is applied to fig II to XI.)

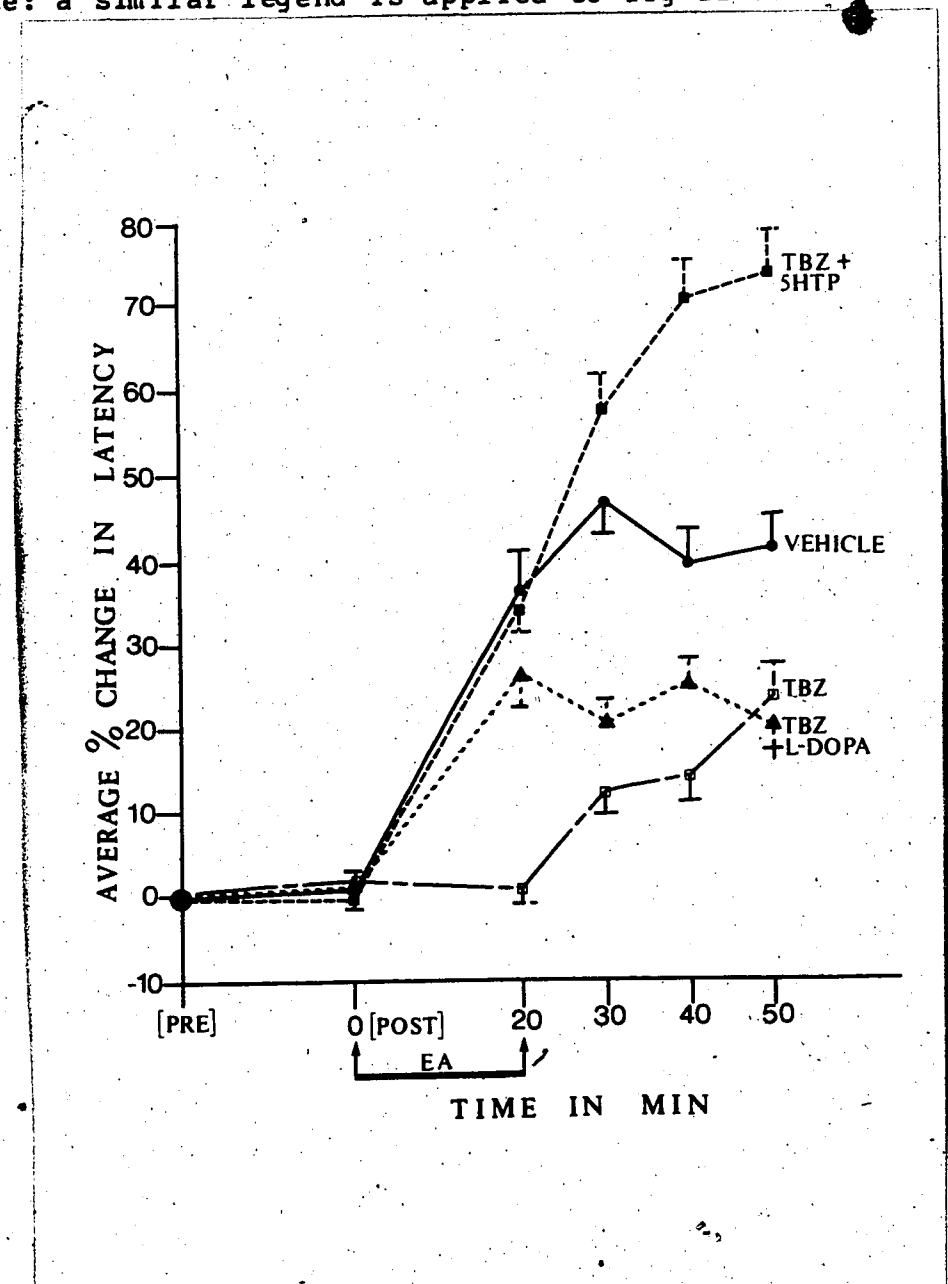


Fig. 1 shows the EAA of group I, II, III and IV at the different times of measurement. Between group comparisons, using Newman-Keul's test showed the following: EAA was decreased by TBZ; 5HTP (fully) and L-DOPA (partially) reversed the blockade of analgesia by TBZ; moreover, 5HTP actually enhanced EAA. Prior to EAA, however, the drugs did not significantly change the baseline pain threshold (within group t-test comparing pre- and post-drug latency to squeak).

PCPA and 5HTP replacement

The effect of PCPA (a serotonin synthesis inhibitor, Koe and Weissman, 1966) and replacement with 5HTP (a serotonin precursor) (Way and Shen, 1971) on EAA were tested on three groups of mice ; I- vehicle (0.9% saline), II-PCPA + vehicle, III- PCPA + 5HTP. In groups II and III, animals were injected with PCPA (320 mg/kg) immediately after predrug tests. Seventy-two hours later, group III animals were injected with 5HTP (200 mg/kg) preceded by 50 mg/kg benserazide hydrochloride to prevent degradation. EAA was studied 30 min later. Group I was injected with vehicle only. Table II summarizes the results and figure 2 shows the time course. PCPA partially blocks EAA while 5HTP reverses this PCPA blockade (Newman-Keul's tests). These drugs themselves caused no change in baseline (within group t-test of predrug versus postdrug data).

Fig. 2

Effect of PCPA and 5HTP on EAA. PCPA partially blocks EAA. Replacing serotonin (PCPA + 5HTP) restores the EAA.

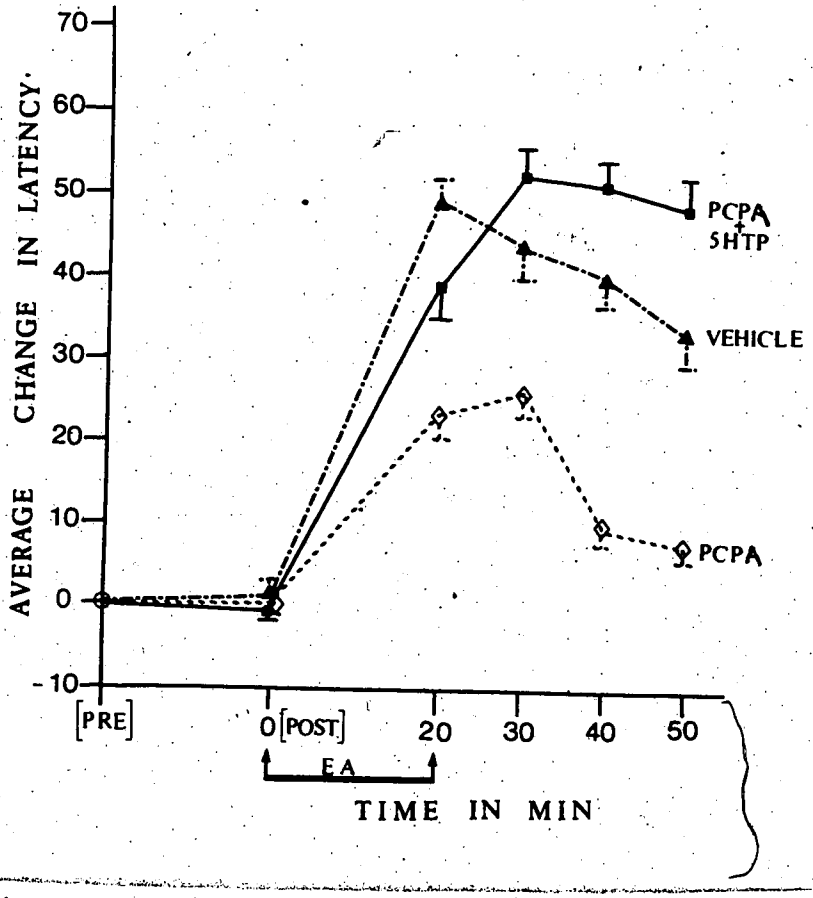


Table II

Effect of PCPA and 5HTP on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	4.37±0.05	4.41±0.06	6.15±0.13
Analgesia (%)		0.53±0.51	44.44±3.22*
II. PCPA:			
Latency (sec)	4.15±0.04	4.00±0.08	4.96±0.11
Analgesia (%)		0.42±0.30	19.71±2.33*
III. PCPA+5HTP:			
Latency (sec)	4.39±0.06	4.39±0.07	6.41±0.14
Analgesia (%)		-0.49±0.22	47.79±3.00*

AMPT and L-DOPA Catecholamine synthesis is blocked by AMPT (Spector et al, 1965). L-DOPA produces higher levels of dopamine within the first hour of administration while the brain noradrenaline levels restore to normal. Two hours after L-DOPA administration, both dopamine and noradrenaline are at normal levels (Corrodi and Hanson, 1966). We made use of this time course of drug effects in our study.

Four groups of mice were designated as follows: I - vehicle (0.9% saline), II - AMPT, III - AMPT + L-DOPA (1 hr), IV - AMPT + L-DOPA (2 Hr). Group II, III and IV were injected with AMPT methyl ester (250 mg/kg) sixteen hours before the experiment. Group I was injected with vehicle (0.9% saline). Sixteen hours after AMPT injection, group III and IV were injected with L-DOPA (100 mg/kg) twenty-five minutes after benserazide hydrochloride (50 mg/kg) administration. EA treatment was done 1 hour after L-DOPA injection in group III and 2 hours after L-DOPA administration in group IV. Similar treatments were done on groups I and II except vehicle instead of L-DOPA was used. Results are summarized in Table III, and in figure 3. AMPT suppresses the analgesic effect of EA (between group and Newman Keul's test) without changing baseline (within group t-test). L-DOPA 1 hour after injection reverses the AMPT inhibitory effect on EAA (Newman Keul's tests) when brain dopamine levels are elevated and brain noradrenaline levels are depressed. However at 2 hours after L-DOPA injection, when the noradrenaline levels increase to normal level and dopamine decreases, there is less effective restoration of EAA in AMPT treated animals (Newman Keul's tests).

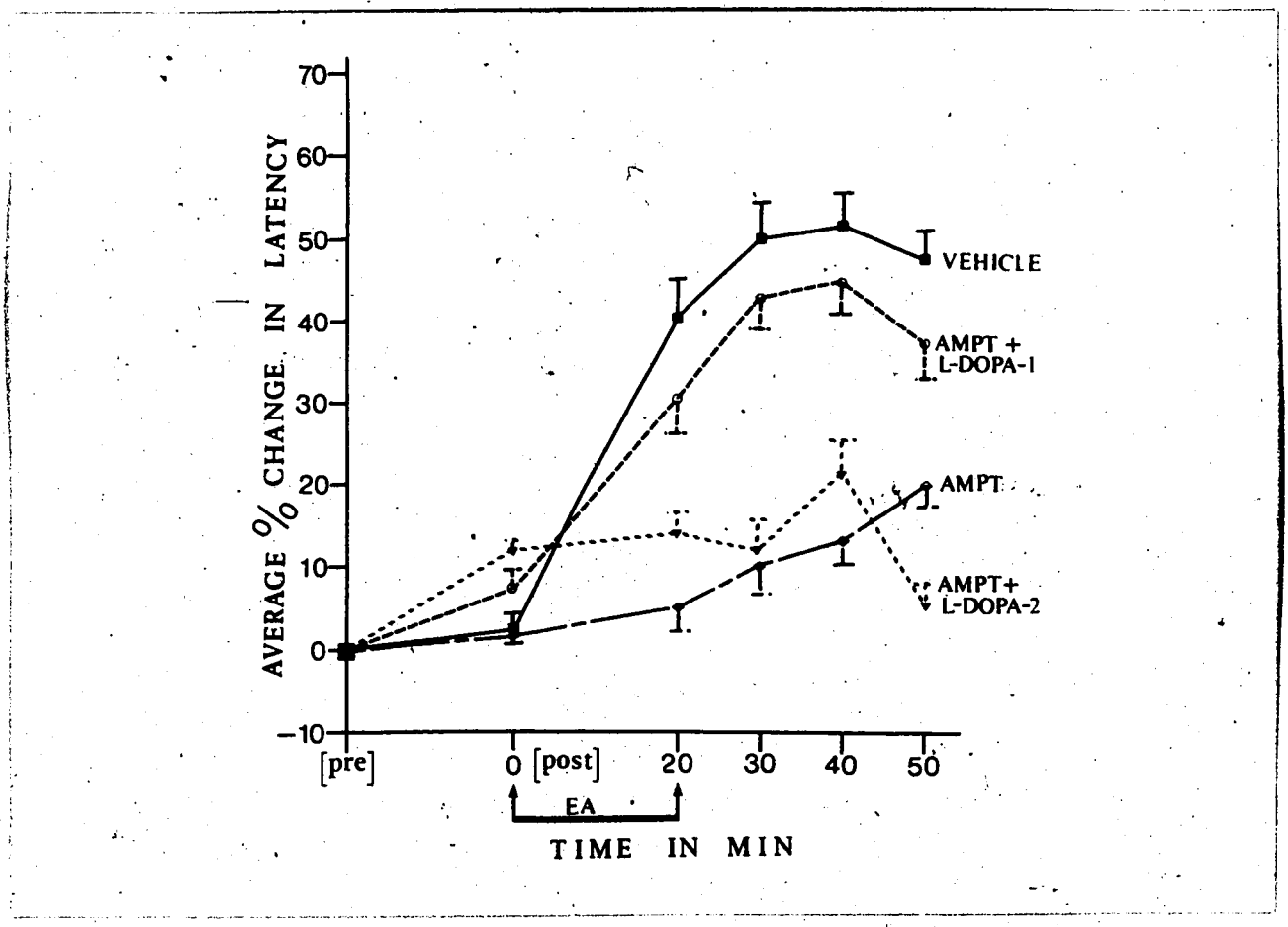
Table III

Effect of AMPT and L-DOPA replacement on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	4.18±0.13	4.1±0.1	5.38±0.18
Analgesia (%)		2.11±1.98	47.67±3.9 *
II. AMPT:			
Latency (sec)	4.6±0.08	4.71±0.09	5.04±0.16
Analgesia (%)		1.84±0.36	9.68±2.79
III. AMPT+L-DOPA (1 hr):			
Latency (sec)	4.75±0.07	5.16±0.1	6.49±0.14
Analgesia (%)		7.87±0.59	39.69±3.18*
IV. AMPT+L-DOPA (2 hrs):			
Latency (sec)	4.32±0.05	4.82±0.08	4.95±0.16
Analgesia (%)		12.34±1.49	16.17±3.72

Fig. 3

AMPT abolishes EAA. Replacing dopamine (AMPT + L-DOPA-1) by L-DOPA in one hour restores EAA. However, replacing noradrenaline (AMPT + L-DOPA-2) by L-DOPA over a 2 hour period only partially restores EAA.



Disulfiram

Disulfiram, a specific dopamine-beta-hydroxylase inhibitor, depletes norepinephrine (Goldstein and Nakajima, 1967) without changing levels of dopamine. Recently, it was found that disulfiram also may increase serotonin in the brain (Fukumori et al, 1980). Group I received 100 mg/kg disulfiram and group II received a matched volume of the vehicle (0.25% methyl cellulose). Six hours after injection, the mice were studied for EAA.

Table IV

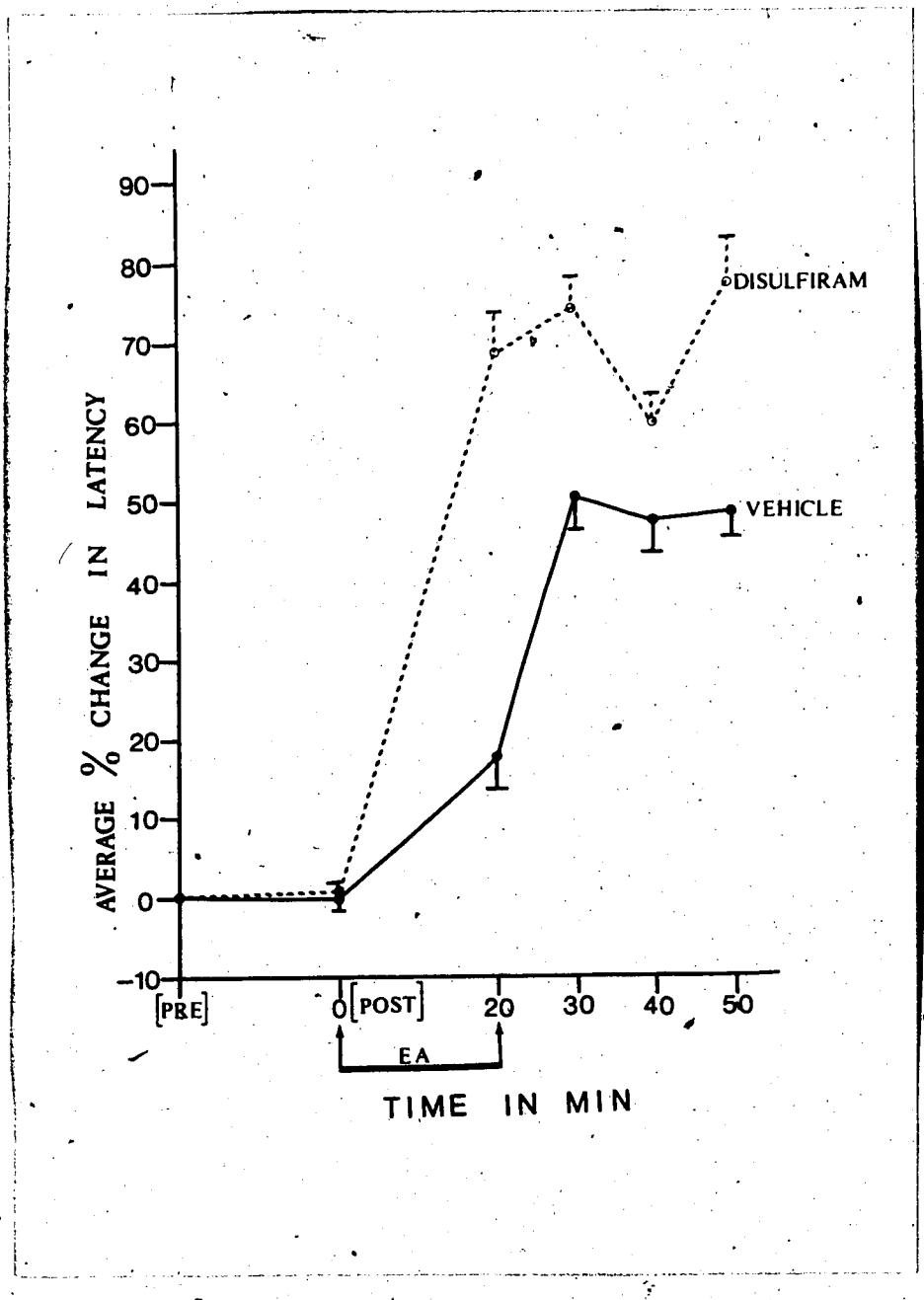
Effect of disulfiram on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	3.96±0.08	3.91±0.09	5.31±0.13
Analgesia (%)		-1.73±0.32	38.65±3.55*
II. Disulfiram:			
Latency (sec)	4.05±0.08	4.07±0.09	6.51±0.11
Analgesia (%)		0.32±0.2	67.3±4.14*

Table IV shows that disulfiram does not affect the baseline pain threshold level (within group t-test) but significantly potentiates EAA (between group t-test). Fig 4 shows the time course of the EA effect of the disulfiram and vehicle treated mice.

Fig. 4

Effect of disulfiram on EAA. Disulfiram, which depletes noradrenaline and may increase serotonin, enhances EAA.



Precursors (5HTP, L-DOPA)

To test the effect of elevated serotonin or dopamine levels on EAA, the precursors 5HTP (Way and Shen, 1971) or L-DOPA (Bartholini et al, 1967) were given respectively to two groups of previously untreated animals. Animals were injected with 200 mg/kg 5HTP or 200 mg/kg L-DOPA 30 minutes after treatment with benserazide hydrochloride (50 mg/kg). 30 minutes later, animals were given EA. Group III animals were treated with the same procedure except that they received the vehicle (0.9% saline).

Table V

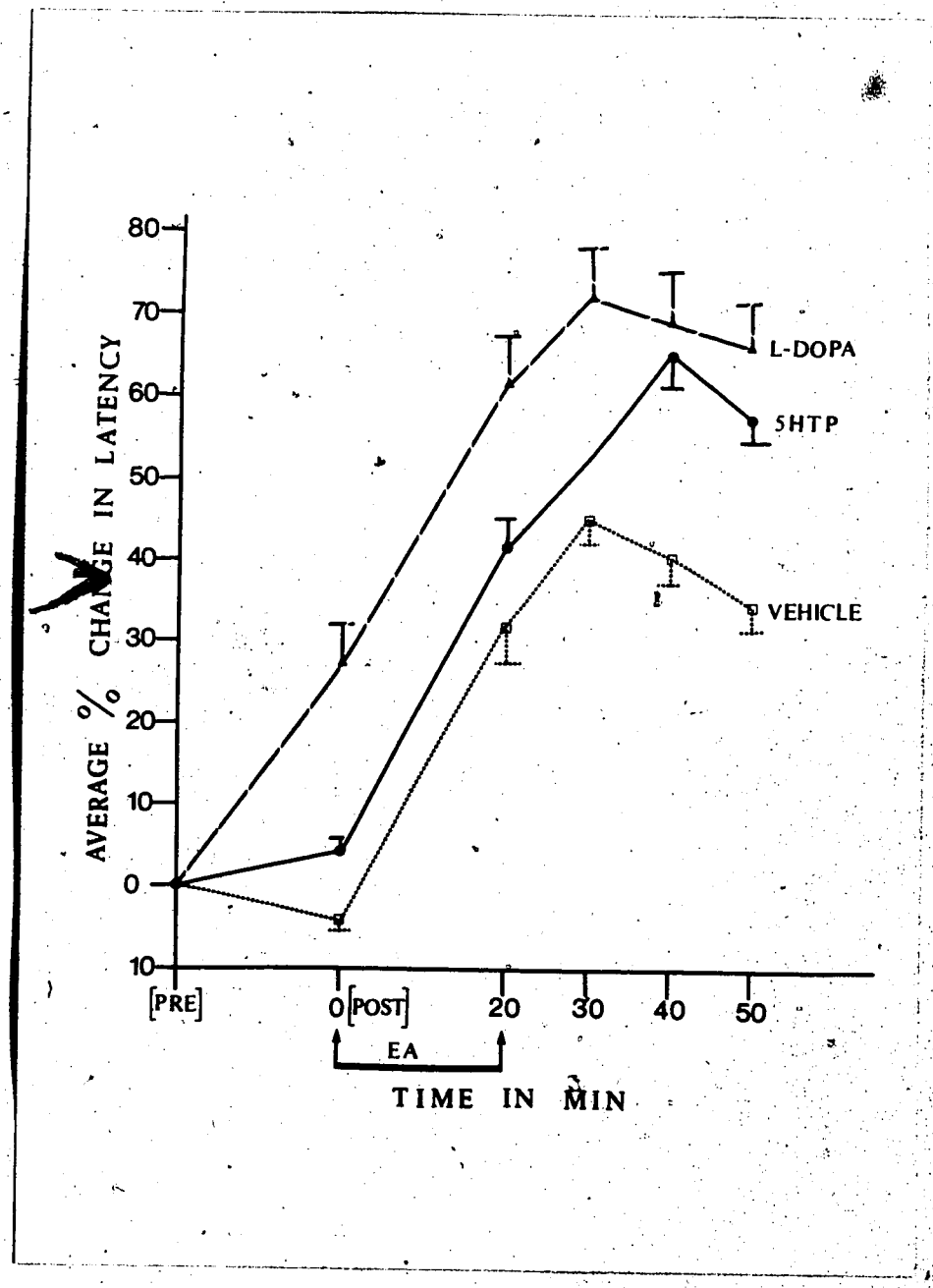
Effect of 5HTP or L-DOPA on baseline scores and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	3.95±0.08	3.78±0.09	5.32±0.12
Analgesia (%)		-4.05±1.01	39.4±3.41*
II. 5HTP:			
Latency (sec)	4.83±0.07	5.06±0.1	7.29±0.16
Analgesia (%)		4.09±0.66	53.18±3.0*
III. L-DOPA:			
Latency (sec)	4.57±0.1	5.62±0.18	7.04±0.14
Analgesia (%)		27.31±4.28*	67.54±6.28*

Table V and figure 5 shows the result of 5HTP and L-DOPA on EAA. EAA was increased by giving either of the cerebral monoamine precursors (Newman Keul's tests). 5HTP does not itself affect the baseline noxious responses (within group t-test). However, L-DOPA alone without EA produced significant analgesia (within group t-test) evidenced by a rise in post-drug baseline threshold to nociception. Hence the increase in EAA caused by L-DOPA is probably due to the analgesic effect of L-DOPA which occurs even before EA treatment is given (67.54 minus 27.31 leaves only 40.23% analgesia). In contrast 5HTP truly enhanced EAA acting in a synergistic manner with EA treatment.

Fig. 5

Effect of enhancing serotonin (by 5HTP) of or dopamine (by L-DOPA) on EAA. This figure is similar to fig I. Either L-DOPA or 5HTP increases EAA.



Probenecid

Probenecid was used to increase brain tryptophan levels (Kartizinel et al, 1976; Van Praag et al, 1973; Werdinius, 1967), the latter being a precursor for serotonin. Probenecid was dissolved in a few drops of NaOH solution and was adjusted to a pH of 7.4 by monopotassium phosphate buffer. Twelve mice were injected with probenecid (200 mg/kg), and twelve were injected with vehicle. EA was given 2 hours later. Table VI and Fig. 6 shows that probenecid produced no changes in baseline pain threshold (within group t-test) but enhanced EAA (between group t-test).

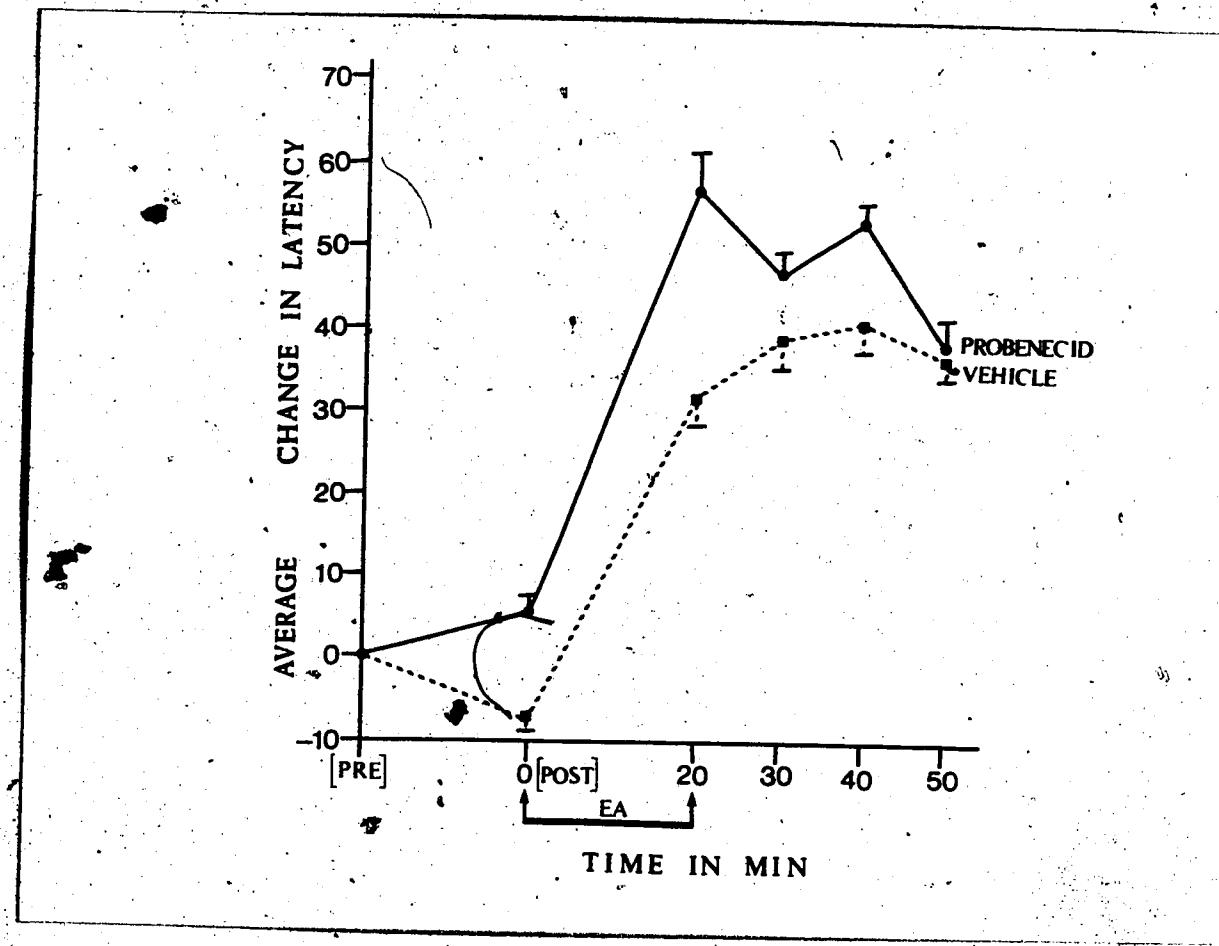
Table VI

Effect of probenecid on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	3.96±0.05	3.62±0.04	5.29±0.12
Analgesia (%)		-7.82±0.84	36.93±3.43*
II. Probenecid:			
Latency (sec)	4.1±0.07	4.31±0.08	6.13±0.11
Analgesia (%)		5.94±1.73	52.2±2.91*

Fig. 6

Effect of probenecid on EAA. Probenecid increases brain tryptophan enhancing EAA.



Cinanserin

Cinanserin was used as a serotonin receptor antagonist (Barasi and Roberts, 1975). Mice were injected with 20 mg/kg of cinanserin dissolved in 0.9% saline. EA was given 2 hours after injection. Experiments were paired with 0.9% saline controls. The results show that cinanserin completely inhibits EAA. Table VII and Fig. 7 demonstrate the effect. Even though cinanserin alone caused a small but insignificant 13% hyperalgesia (within group t-test), one cannot merely subtract this 13% from 45% and obtain a complete abolition of EAA; hence cinanserin must truly block EAA.

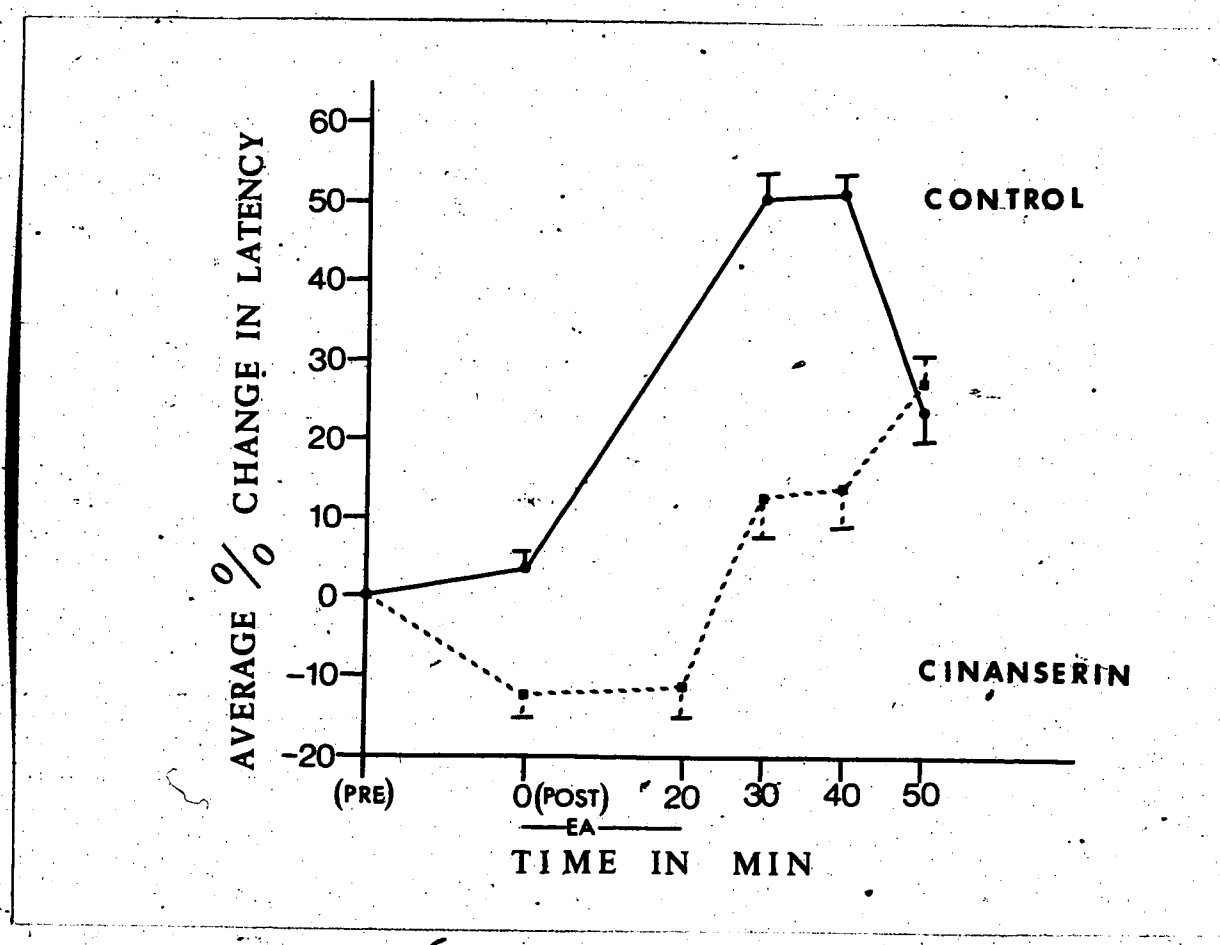
Table VII

Effect of cinanserin on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	4.00±0.66	4.16±0.10	5.74±0.15
Analgesia (%)		3.92±1.8	45.15±3
II. Cinanserin:			
Latency (sec)	4.02±0.1	3.37±0.13	4.05±0.16
Analgesia (%)		-12.65±3.15	4.8±5.29

Fig. 7

Effect of cinanserin on EAA. Cinanserin, a serotonin antagonist, inhibits EAA.



Apomorphine

Apomorphine is a selective dopamine receptor stimulator (Anden et al, 1967; Ernst, 1967). Group I was injected with 0.5 mg/kg apomorphine and group II with vehicle (0.9% saline). EA was applied 25 min after injection.

In contrast to SPA which is enhanced by apomorphine (Akil and Liebeskind, 1975), EAA was significantly reduced by apomorphine (between group t-test). Table VIII and Fig. 8 show the results. This was the major difference between EAA and SPA that we observed in the entire study.

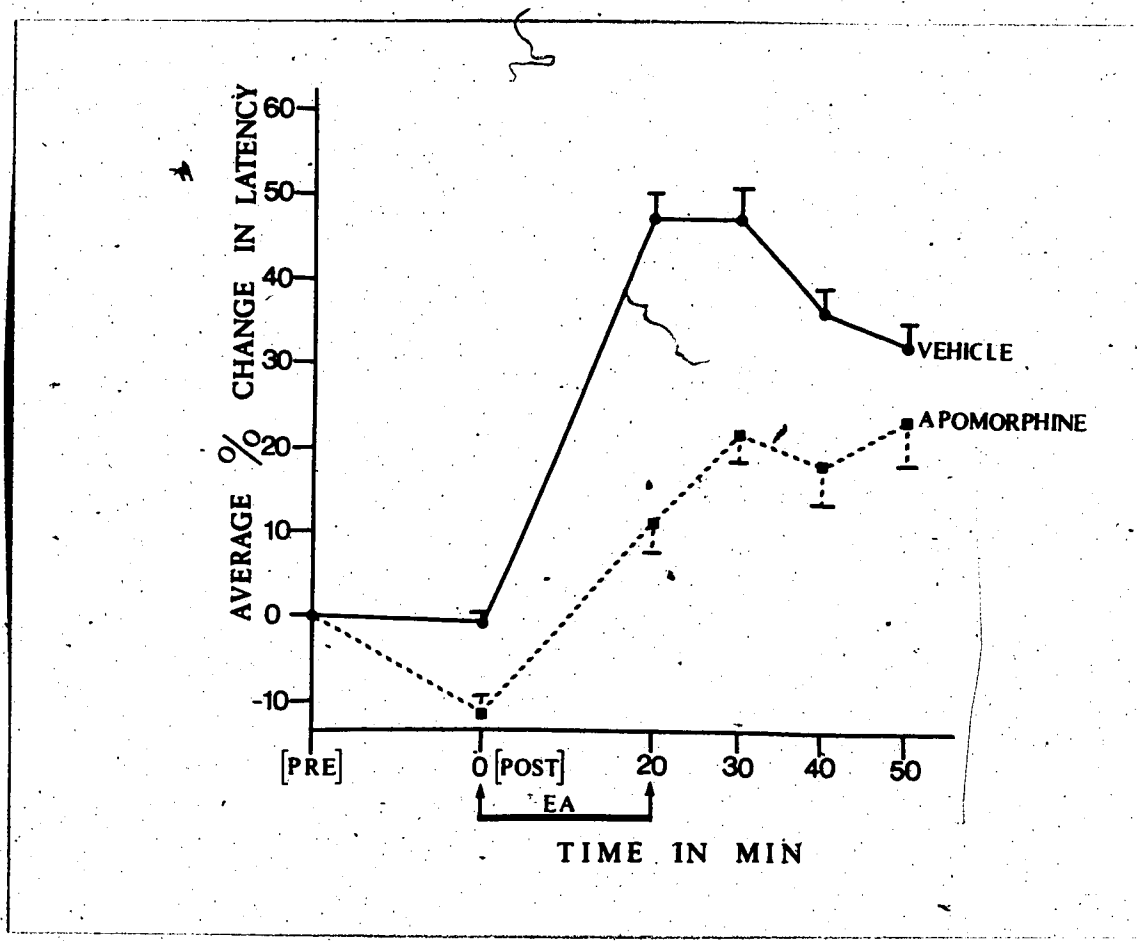
Table VIII

Effects of apomorphine on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	4.14±0.04	4.15±0.05	5.89±0.35
Analgesia (%)		-0.13±0.44	43.85±2.75*
II. Apomorphine:			
Latency (sec)	4.0±0.1	3.72±0.11	4.67±0.14
Analgesia (%)		-7.73±1.1	20.16±3.37*

Fig. 8

Effect of apomorphine on EAA. Apomorphine, a dopamine receptor stimulator, reduces EAA.



Haloperidol

Haloperidol is a dopamine receptor blocker (Cools et al., 1975). One group of mice was injected with 0.2 mg/kg haloperidol and one with the vehicle (5% dextrose). EA treatment began 90 min later.

Table IX and Fig. 9 shows that haloperidol does not change the baseline level of pain (within group t-test) but reduces EAA (between group t-test).

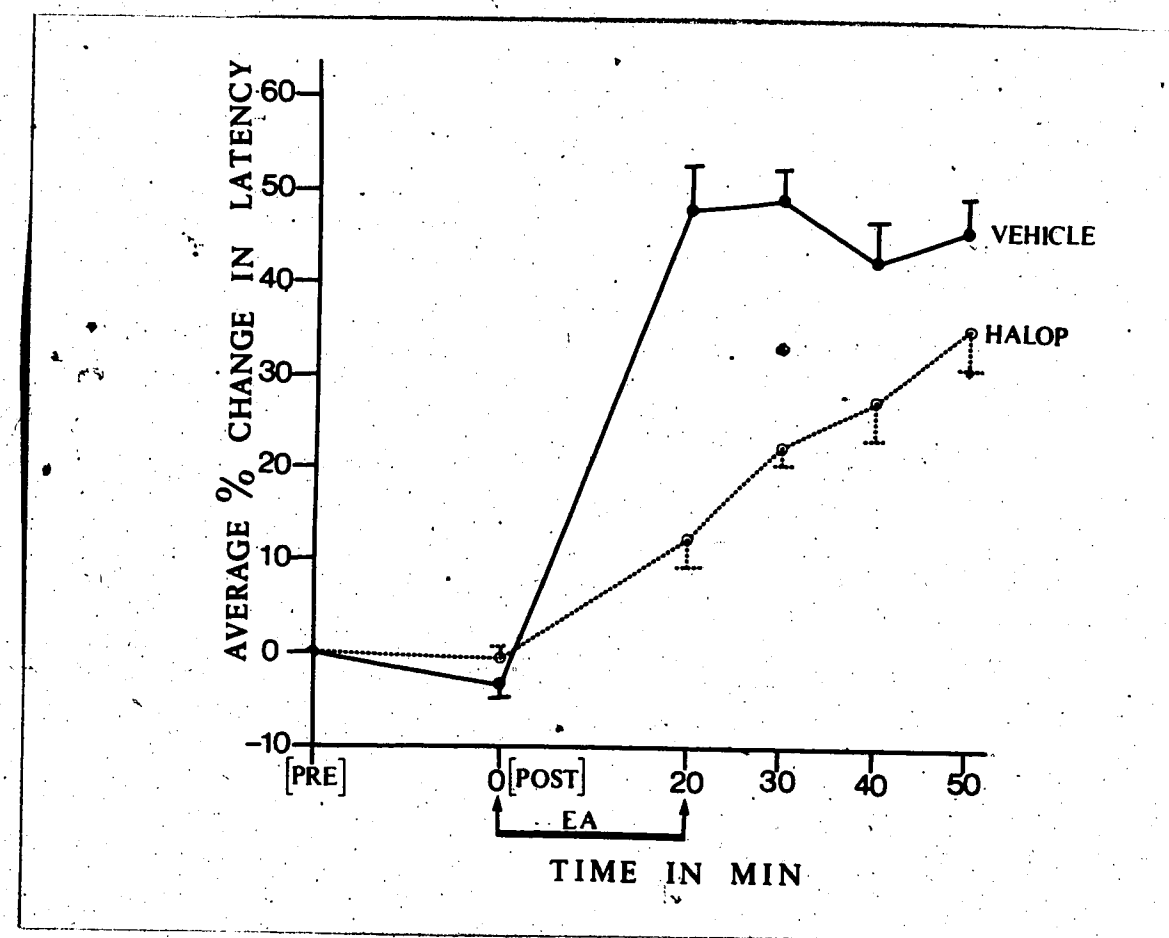
Table IX

Effects of haloperidol on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	4.12±0.07	3.97±0.08	5.82±0.14
Analgesia (%)		-3.27±0.92	46.6±4.15*
II. Haloperidol:			
Latency (sec)	4.76±0.08	4.75±0.1	5.65±0.39
Analgesia (%)		-0.87±0.46	20.9±2.63*

Fig. 9

Effect of haloperidol, a dopamine receptor blocker, on EAA. Haloperidol partially inhibits EAA.



Pimozide

Pimozide is a selective dopamine receptor blocker (Anden et al, 1970). One group of mice was injected with 0.5 mg/kg pimozide and one group with a matched volume of vehicle (a few drops of glacial acetic acid in 5% dextrose). These two groups of mice were given EA 3.5 hours later.

Table X

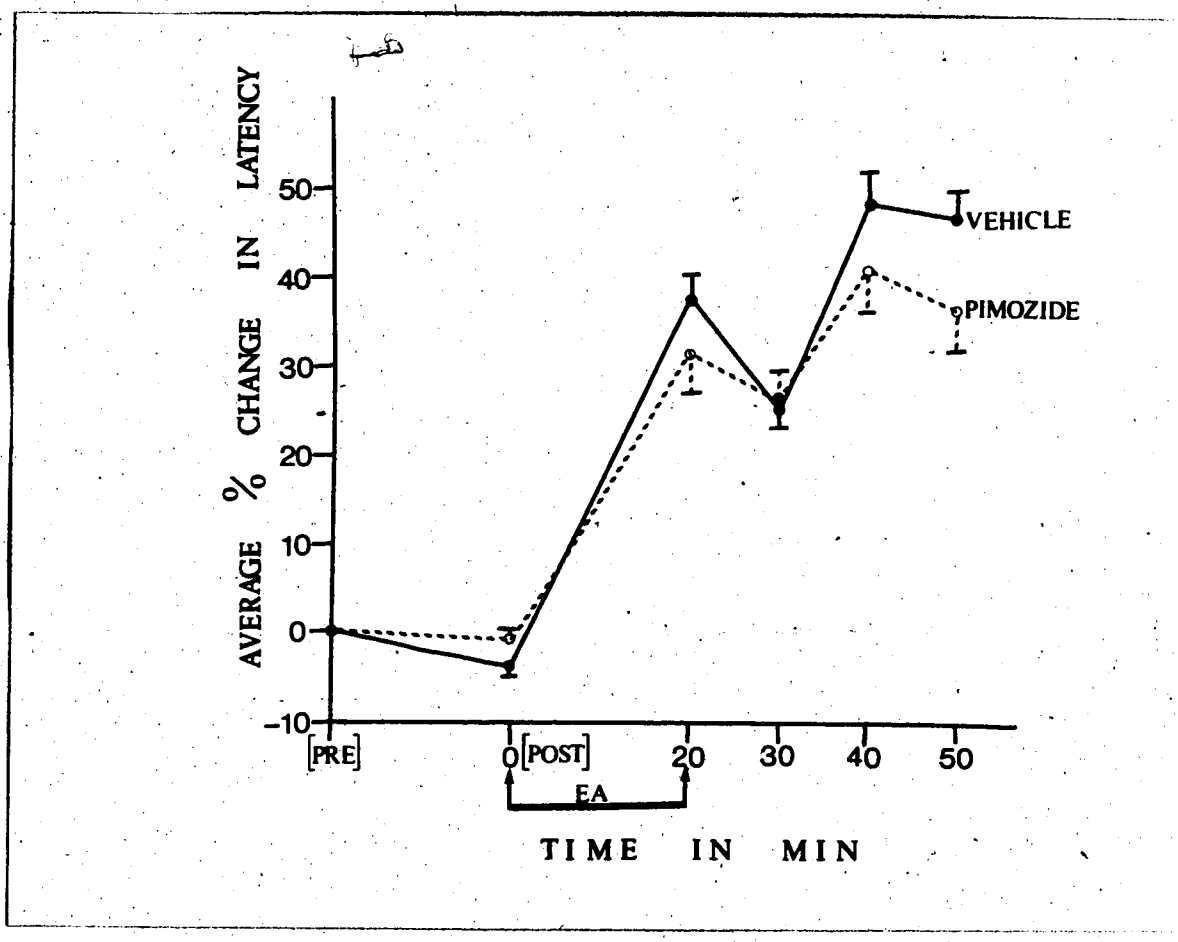
Effects of pimozide on baseline and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle:			
Latency (sec)	4.24±0.04	4.07±0.05	5.8±0.12
Analgesia (%)		-3.9±0.8	37.1±2.57*
II. Pimozide:			
Latency (sec)	4.28±0.1	4.27±0.11	5.39±0.12
Analgesia (%)		-o.76±0.29	33.06±3.93*

Table X shows that pimozide only slightly decreases the EAA which shows no significant different from the vehicle (control) value (between group t-test). Fig. 10 illustrates this result. In contrast, pimozide significantly reduced SPA (Akil and Liebeskind, 1975).

Fig. 10

Effect of pimozide, a selective dopamine receptor blocker, on EAA. Pimozide shows no obvious effect on EAA.



Yohimbine

It has been reported that yohimbine may block tonic descending inhibition descending from the lower brainstem (Koss and Bernthal, 1978) and antagonizes autoanalgesia (stress-induced analgesia) and morphine analgesia (Chance and Schechter, 1979). At low doses yohimbine is an alpha-adrenergic blocker and it is postulated to act preferentially at pre-synaptic sites (Bernthal et al, 1979).

To test the effect of yohimbine on EAA, mice (group I) were injected with yohimbine (5 mg/kg, I.P.). EA was applied 15 min later. These mice were paired with group II animals receiving similar treatments with vehicle (0.9% saline).

Table XI

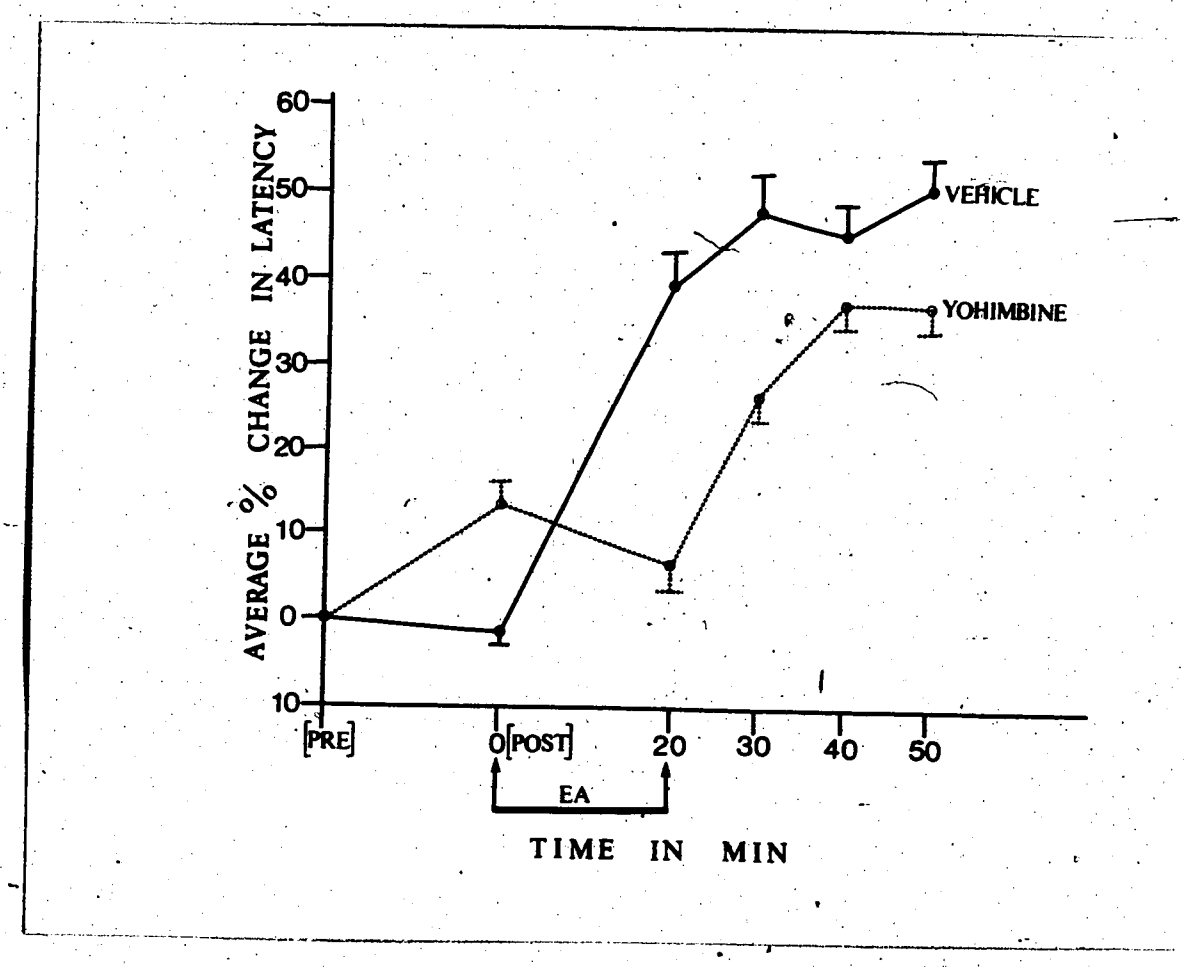
Effect of yohimbine on baseline scores and on EAA.

	Pre-drug	Post-drug	EAA
I. Vehicle			
Latency (sec)	4.21±0.06	4.15±0.07	5.9±0.12
Analgesia (%)		-1.53±0.57	44.38±3.9*
II. Yohimbine:			
Latency (sec)	4.51±0.07	5.03±0.09	5.5±0.12
Analgesia (%)		13.61±2.34	23.47±2.5*

Table XI and Figure 11 show the effect of yohimbine on EAA. EAA was slightly but significantly reduced (between group t-test) in yohimbine treated animals.

Fig. 11

Effect of yohimbine on EAA. Yohimbine partially inhibits EAA.



DISCUSSION

Table XII summarizes the major findings in this paper in relation to the putative effects of the various drugs, and compares the EAA results with the SPA results of Akil and Liebeskind (1975). As any given drug has known (and unknown) side effects, a battery of drugs giving consistent results is the best strategy to test for involvement of a transmitter. If an excitatory transmitter mediates a process, metabolic precursors should enhance, metabolic inhibitors should suppress, and receptor blockers should interfere with that process. Such consistent results in this paper were observed only for serotonin. In contrast, norepinephrine or dopamine manipulations did not produce convergent results. This could perhaps be explained by multiple effects of each of these transmitters producing conflicting effects in different parts of the brain. Similarly Akil and Liebeskind (1975) (see Table XII SPA column) also had inconsistent results with norepinephrine and dopamine drugs on SPA. By comparing the effects of the drugs on EAA and SPA, we observed many similarities. The only difference were that apomorphine blocked EAA but enhanced SPA and pimozide had no effect on EAA but reduced SPA (Akil and Liebeskind, 1975).

Table XII

Summary of monoaminergic drugs on EAA (200 Hz) and comparisons with SPA results.

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

S = Serotonin, D = Dopamine, N = Norepinephrine
R = Recovery of EAA or SPA after replacement drugs.
ND = Not done
* Only the data related to serotonin gave consistent EAA results.
\+ Results on SPA in rats from Akil and Liebeskind (1975)

Since serotonin manupulation gives a consistent result, the emphasis in the rest of the discussion will be on serotonin mechanisms in EAA. Although our present paper is the most extensive one on EAA with serotonin manipulations, there have been several previous papers implicating serotonin in EAA. In one study, McLennen et al (1977) showed that in 2 rabbits, PCPA (I.P.) blocked EAA, in two other rabbits, cyproheptadine (I.P.), a non-specific serotonin receptor blocker prevented EAA. There were more detailed drug studies from two groups in China on EAA in rats (Kin et al, 1979; Han et al, 1979). In one experiment (Kin et al, 1979) it was shown that cinanserin (I.P.) blocked EAA whereas 5HTP (I.P.) or L-tryptophan (I.P.) enhanced EAA. In another study (Han et al, 1979), it was found that 5HTP (I.P.) enhanced EAA, while L-tryptophan (I.P.) had no effect.

In addition to drug studies, there have been two other lines of evidence which strongly implicate the raphe serotonin system in EAA; raphe lesion experiments and biochemical studies on serotonin. In studies on cats three groups in China (Shen et al, 1975; Shen et al, 1978; Du et al, 1976) showed that cutting the dorsolateral fasciculus (DLF), which contains the descending axons from the raphe, abolishes EAA. This suggests that descending raphe serotonin projections are important (but these lesions cannot distinguish between serotonergic and other descending inhibitory systems). One group (Chiang et al 1979) also showed that lesions of the

raphe itself had the same effect. Using 5,6-dihydroxytrypamine they were able to lesion the raphe serotonin cells, which also suppressed EAA.

The biochemical studies are equally consistent. In one study (Yi et al, 1977), it was shown that radioactive serotonin was released into the cisterna magna of rabbits during acupuncture analgesia. In another study (Han et al, 1979) 5HIAA, a catabolite of serotonin was increased in brain extracts after EAA. In a third biochemical study, brain extracts after EAA showed a rise in both 5HT and 5HIAA (Kin et al, 1979).

Thus three lines of evidence, pharmacological, surgical and biochemical all converge on the conclusion that the raphe-DLF-serotonin system mediates EAA. The relationship of the EAA-endorphin system and this descending effect is still unclear. In a previous paper (Cheng and Pomeranz, 1979) we showed that different stimulus frequencies applied to the acupuncture needles produced different results. EAA at 4 HZ was blocked by naloxone but not by PCPA while EAA at 200 Hz was blocked by PCPA but not by naloxone. Hence in the present paper all the studies were done at 200 Hz. The enkephalin input to the raphe nucleus may be in series or in parallel with the raphe-DLF serotonin system. The results to date in the pain literature are confusing on this point (Basbaum and Fields, 1978). Hence it is unclear at the moment, whether the endorphin and serotonin systems are in

series or in parallel in the EAA phenomenon, although the differences between the 4 Hz and 200 Hz results may suggest a parallel rather than a serially organized system. In the spinal cord, the enkephalin input to the dorsal horn may be parallel with the DLF-descending inhibitory neurons since intrathecal application of naloxone does not block the analgesia induced by microinjecting morphine into the PAG (Yaksh and Tyce, 1979). Although more work is needed to resolve this latter question, there can be no doubt that serotonin mediates at least some of the EAA phenomena.

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CHAPTER 7: CORRELATION OF GENETIC DIFFERENCE IN ENDORPHIN SYSTEMS WITH ANALGESIC EFFECTS OF D-AMINO ACIDS IN MICE

SUMMARY

To test the idea that D-amino acids may produce analgesia via the endorphin system, an experiment was undertaken to examine the effect of D-leucine and D-phenylalanine on mice with congenitally abnormal endorphin systems. In three strains of mice D-amino acid analgesia ranks in the order of ob/ob>B6AF1/J>CXBK. This correlates with the endorphin abnormalities in these mice: ob/ob high in pituitary beta-endorphin and CXBK low in opiate receptors.

INTRODUCTION

Recent preliminary reports suggest that systemic treatment with D-amino acids (D-AA) can cause analgesia (anti- nociceptive response) in man and mice (Ehrenpreis et al, 1978). D-leucine and D-phenylalanine were postulated to cause pain relief through the endorphinergic system, since the analgesia they produced was naloxone- reversible. To further test the D-amino acid-endorphinergic hypothesis we compared D-AA effects in 3 strains of mice. One strain (CXBK) is low in opiate receptors (Baran et al, 1975) and exhibits poor electroacupuncture (Peets and Pomeranz, 1978)

and morphine (Baran et al, 1975) analgesia; another strain (ob/ob) was recently found to have abnormally high levels of pituitary endorphins (Grevert and Goldstein, 1977). A third strain B6AF1/J, was used as control as it exhibits "normal" electroacupuncture analgesia which is naloxone-reversible (Pomeranz and Chiu, 1976); all 3 strains are related as they are descended from C57BL. If the hypothesis is true, we predict that the D-AAs should produce the following rank order in analgesia: ob/ob > B6AF1/J > CXBK based on differences in the endorphin systems. The present results bore out this prediction. Moreover, the D-AA analgesia was naloxone-reversible. The combined genetic and naloxone evidence provides a strong proof of the D-AA-endorphin hypothesis.

METHODS

To study anti-nociceptive response a hot-plate method was used to obtain a measure of pain threshold. This was done by placing a mouse on a hot-plate, 55 C(+ 0.5 C) and measuring the time elapsed until the onset of hindpaw-licking. The following protocol was used: (1) two pretreatment values were obtained for each mouse at 30 min apart; (2) D-phenylalanine (125 mg/kg) and D-leucine (125 mg/kg) were given in a single i.p. injection; and (3) pain threshold was measured at 60, 120 and 180 min after the injection. The last 3 measurements were averaged to give a

post-drug value for later statistical analysis. Fifteen mice from each strain were used in the above D-AA experiment. In order to test for naloxone-reversibility, another 15 mice from each strain were tested with naloxone plus D-AAs using doses similar to those previously reported (Ehrenpreis et al, 1978), namely 20 mg/kg naloxone, 125 mg/kg D-leucine, 125 mg/kg D-phenylalanine (all i.p.). The injections of the D-AAs with or without naloxone were done in a "blind" manner; mice were randomly assigned to either group. All the experiments were done at the same time of day (from 14.00 to 18.00 h).

RESULTS

The results with D-AAs are shown in Figs. 1 and 2 and in Table I (1 and 4). There was a significant analgesia in B6AF1/J and ob/ob mice but not in CXBK (see Table I 1(i) - D-AA vs pre-D-AA). There was a significant difference in analgesia between B6AF1/J and ob/ob mice (Table I 4). Thus the prediction, based on endorphinergic disturbances, proved correct: the analgesia ranking was ob/ob > B6AF1/J > CXBK, and the differences were all significant by Newman Keul's test (Table I 4). This result supports the hypothesis that the D-AAs act through the endorphin system. Moreover it was confirmed that the D-AA effects were naloxone-reversible in B6AF1/J and ob/ob (Figs. 1 and 2, Table I 2).

Fig. 1

A histogram shows the effect of the D-AA on pain thresholds in 3 strains of mice and its reversal by naloxone. Pain threshold was measured by recording the time elapsed (latency) before beginning hindpaw-licking after mice were placed on a hot-plate at 55 C + 0.5 C. The changes in latencies (comparing before and after DAA treatments) were measured at 60, 120 and 180 min. after D-AA and were averaged to give one value for each mouse. Thus the ordinate represents the average change in the latency (+S.E.) of all the mice under the same treatment. Abscissa represents the strain of mice used, i.e. CXBK, B6AF1/J and ob/ob. Solid bars indicate D-AA treatment. Open bars indicate D-AA plus naloxone treatment. Each bar is the average value of 15 mice. Stars (** and *) indicate significantly different D-AA analgesia in the order of (**) ob/ob>(*)B6AF1/J>CXBK. Naloxone reverses these analgesic effects in ob/ob and B6AF1/J mice.

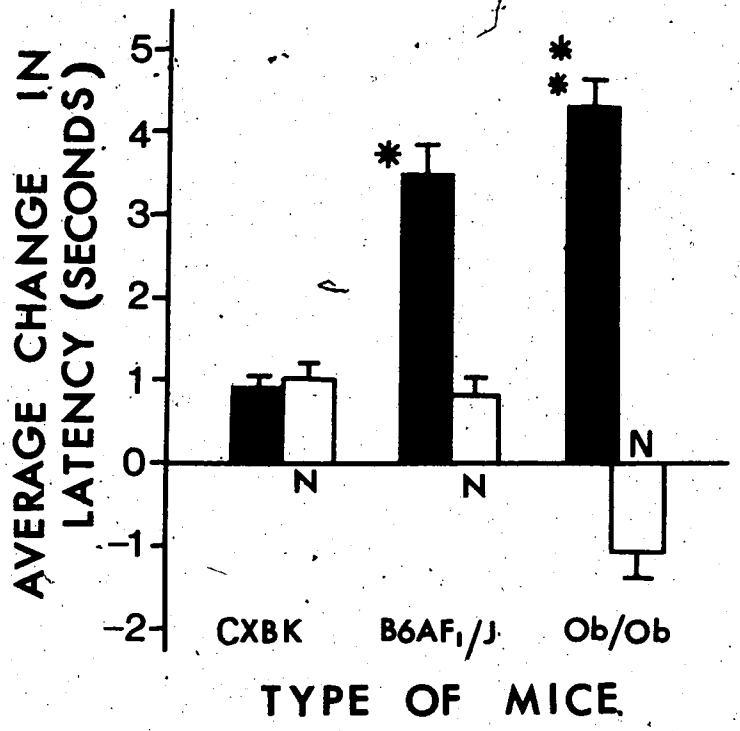

Fig. 2

A graph shows the effect of D-AA (D-leucine and D-phenylalanine) on the pain thresholds on 3 strains of mice. The ordinate represents the change in the latency of hindpaw-licking of the mice after the drug treatment as compared to the control values. The abscissa is the time after the injection of the drugs. The upper 3 lines show the D-AA effect on B6AF1/J, ob/ob and CXBK mice, while lower 3 lines show the effect of naloxone on D-AA treated animals. Each point is the mean for 15 mice. Bars show S.E. Arrow indicated the time of injection after control pain thresholds were taken.

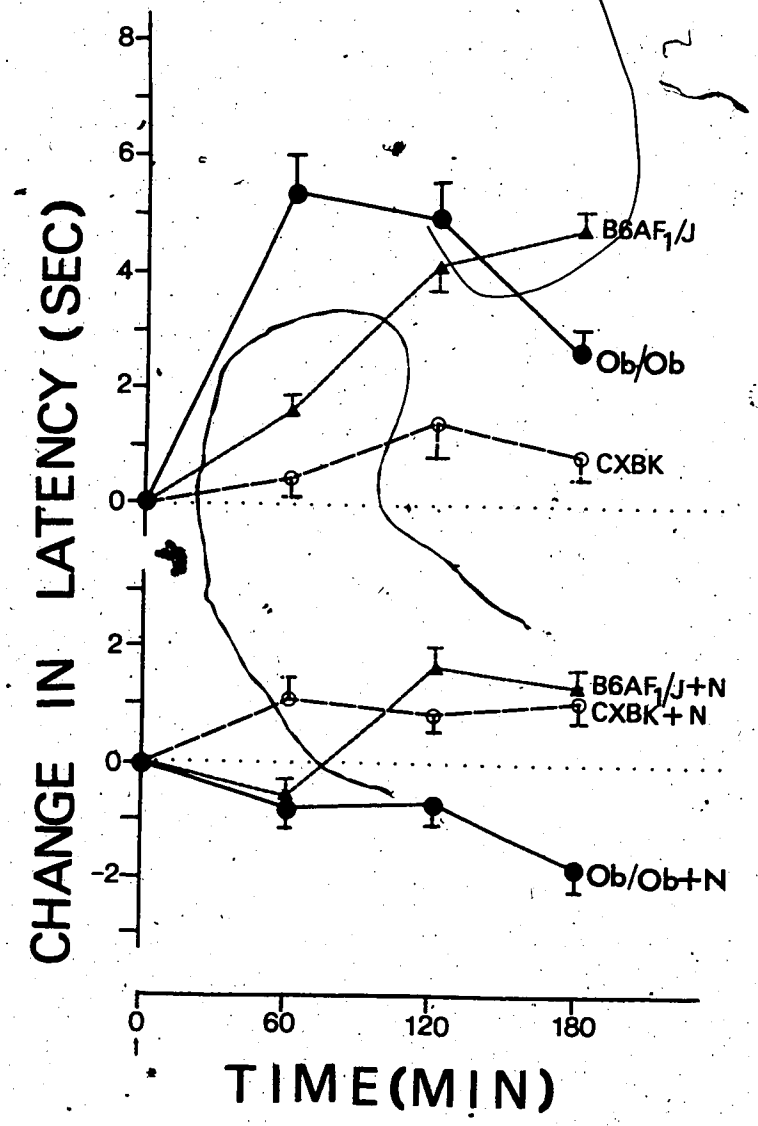

TABLE 1

Statistical analysis to compare D-amino acids and the naloxone-reversible effects on the 3 strains of mice

1:(i): Student's t-test was used to test the D-AA effect in 3 strains of mice; (ii) Student's t-test was used to analyze D-AA plus naloxone effect in 3 strains of mice; 2: a between group analysis by using Student's t-test to show the difference between the D-AA effect in 1(i) and the D-AA plus naloxone effect in 1(ii). In sections 3, 4 and 5 we did between-strain comparisons using the two-step procedure: first an ANOVA, second the Newman Keul's test. 3: baseline-line (pre-D-AA) pain thresholds in the 3 strains of mice compared; 4: D-AA effect on the 3 strains of mice compared; 5: D-AA plus naloxone results on the 3 strains of mice compared. D-AA, D-amino acids; pre-D-AA, before D-AA treatment.

1 Within animal comparisons			
	B6AF1/J	CXBK	ob/ob
(i) t-test (D-AA) vs (Pre-D-AA)	p<0.05*	p>0.1	p<0.005*
(ii) t-test (D-AA+Naloxone) vs (Pre-D-AA)	p>0.4	p>0.2	p>0.2
2 Between group comparisons (within strain comparisons)			
t-test (D-AA) vs (D-AA + Naloxone)	p<0.005*	p>0.2	p<0.001*
Between strain comparisons			
3 AVONA of pre-D-AA	p<0.01* for all 3 strains		
Newman Keul's of pre-D-AA	CXBK vs B6AF1/J		p>0.05
Newman Keul's of pre-D-AA	CXBK vs ob/ob		p<0.01*
Newman Keul's of pre-D-AA	B6AF1/J vs ob/ob		p<0.01*
4. ANOVA of D-AA analgesia	p<0.005* for all 3 strains		
Newman Keul's of D-AA	CXBK vs B6AF1/J		p<0.01*
Newman Keul's of D-AA	CXBK vs ob/ob		p<0.001*
Newman Keul's of D-AA	B6AF1/J vs ob/ob		p<0.05*
5 ANOVA of D-AA + Naloxone	p>0.1 for all 3 strains		
Newman Keul's of D-AA+Naloxone	Cannot be done as ANOVA not significant		

* Statistically significant result.

DISCUSSION

It should be noted that the average (+S.E.) baseline pretreatment control values are 12.11 + 0.11 sec for 30 ob/ob mice, 8.73 +0.13 sec for 30 B6AF1/J mice and 8.78 + 0.11 sec for 30 CXBK mice. Thus it was found that the ob/ob mice had a significantly higher pretreatment pain threshold level than the B6AF1/J and CXBK strains, but there was no difference between B6AF1/J and CXBK (Table I 3). This result is consistent with a higher baseline level of pituitary endorphins in ob/obs. The lack of difference in the other two strains suggests that the acute pain of paw-lick may not release endorphins in CXBK or B6AF1/J. A similar conclusion was reached by Grevert and Goldstein (1977) who noted that naloxone did not cause hyperalgesia (pain enhancement) as measured by paw-licking in Swiss Webster mice. They only noted hyperalgesia if they measured latency to jump, which involves a more prolonged pain. Frederickson et al (1977), using the Cox strain, also observed a much greater naloxone hyperalgesia with jumping than with paw-licking behaviour. Finally, note that the D-AAs had a faster analgesic effect on ob/ob mice than B6AF1/J mice (Fig. 2). This may be due to a higher baseline level of pituitary endorphins in ob/obs, which is more readily available for release. Despite this higher baseline level, ob/ob still gave the highest D-AA analgesia.

As stated in the introduction, we predicted that D-AAs should produce different effects in the 3 strains of mice: ob/ob > B6AF1/J > CXBK. This was based on a higher level of pituitary B-endorphins in ob/ob (5 times the normal level) (Margules et al, 1978) and a low level of opiate receptors in the brains of CXBK mice (about 2/3 of the normal level) (Baran et al, 1975). The present results support this prediction. Moreover we have biochemical evidence that there are no significant differences in brain levels of endorphins in these 3 strains as measured by radioreceptor binding assay and radioimmunoassay (Roy et al, 1979). This suggests that the failure of D-AA effects in CXBK mice is entirely caused by the deficiency in opiate receptors. Although the receptor binding is only decreased by 50% in this strain (Baran et al, 1975), a complete absence of D-AA analgesia is observed. Similarly, electroacupuncture analgesia was also absent in this strain (Peets and Pomeranz, 1978). It is still not possible to explain these extensive consequences of a small deficit of receptors; since receptors in the total brain were measured (Baran et al, 1975) perhaps the deficit was more marked in critical areas such as periaqueductal gray. Our biochemical evidence in ob/ob mice, showing the presence of normal levels of endorphins in the brain confirms the work of others (Margules et al, 1978) localizing the abnormality to the pituitary gland. The question arises whether or not these elevated levels of pituitary B-endorphins can cross the blood-brain barrier to

cause analgesia in the brain. These lines of evidence suggest that the barrier in mice is not as pronounced as in the rat. Firstly, systemically injected B-endorphin causes analgesia in mice (Tseng et al, 1976) but not in rats. Secondly, in mice pituitary endorphins are implicated in electroacupuncture analgesia (Cheng et al, 1979; Pomeranz et al, 1977). Thirdly, in the present paper it was observed that the ob/ob mice had a higher baseline pain threshold than the other two strains, a result which is consistent with elevated pituitary endorphins causing analgesia. Moreover, there may exist a reverse flow from pituitary to brain in the portal system which could bypass the blood-brain barrier completely (Bergland and Page, 1979). In conclusion, D-AAs may be the first potent non-addictive analgesics for humans (Ehrenphreis et al, 1978); hence it is essential to know their mechanism of action. The combined genetic and naloxone evidence implicates the endorphin system in D-AA analgesia.

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CHAPTER 8: A COMBINED TREATMENT WITH D-AMINO ACIDS AND ELECTROACUPUNCTURE PRODUCES A GREATER ANALGESIA THAN EITHER TREATMENT ALONE; NALOXONE REVERSES THESE EFFECTS

SUMMARY

The D-amino acids (DAA), D-phenylalanine and D-leucine, produce naloxone reversible analgesia; electroacupuncture (EA) also produces analgesia which is blocked by naloxone. Combining the two treatments produces an additive effect with a larger analgesia than that produced by either treatment given alone; this combined effect is also blocked by naloxone. Moreover only 62% of the mice show EA analgesia and 53% show D-amino acid (DAA) analgesia; 80% of the animals show marked analgesia with both EA plus DAA treatment. Perhaps the combination of EA with DAA will provide a potent method for the treatment of clinical pain.

INTRODUCTION

Analgesia induced by endorphins is relatively short lasting, owing to rapid degradation by carboxypeptidase A, leucine aminopeptidase (Hughes, 1975) and other enzymes. Ehrenpreis et al (1978) have shown that the D-amino acids (DAA) which block carboxypeptidase A and leucine aminopeptidase (D-phenylalanine and D-leucine respectively) induce

analgesia which is naloxone reversible in man and mice. In a recent study, we demonstrated that ob/ob mice, which have high levels of pituitary beta-endorphin (Margules et al, 1978), showed marked DAA induced analgesia (Cheng and Pomeranz, 1979); in contrast CXBK mice which were genetically deficient in opiate receptors (Baran et al, 1975) showed poor analgesia with DAA treatment (Cheng and Pomeranz, 1979). All the above studies suggest that DAA produce analgesia via the endorphin system possibly by protecting endorphins from enzymatic degradation. The present study was undertaken because recent expriments indicated that electroacupuncture (EA) may release endorphins (Sjolund et al, 1977). If these released endorphins are protected by DAA, a higher analgesia may be observed. Thus this paper is designed to study the analgesia produced by a combination of EA plus DAA.

METHODS

The methods used in the present study were similar to those in the previous work (Pomeranz and Chiu, 1976). Briefly, a behavioral pain latency measurement was used in female mice (10 - 12 weeks old) of strain B6AF1/J from Jackson Laboratories; the latency to squeak was determined in response to noxious heat stimuli (this is done by shining a hot lamp at the nose of the mice). The latency was measured from an audiogram of the squeak. Three control tests were

given, 3 minutes apart, to the restained mice. The mean of 3 tests gives zero time control values for each mouse. Only those mice giving 3 reproducible responses in the control period, with latencies between 3 to 5 seconds, were used for subsequent treatments. Then different doses for two D-amino acids (D-phenylalanine plus D-leucine at 150 mg/kg, 300 mg/kg or 600 mg/kg of each drug in combined injection of two drugs) or saline (for control) were injected I. P. in a blind manner; there were 4 groups of mice and 15 mice per group. Pain (squeak) latencies were again measured at one hour after the injection before EA was given. EA was then administered to the mice by inserting fine needles (34G. stainless steel) in the first dorsal interosseus muscle on each forepaw (this point is called Hoku or L.I.4 in the acupuncture literature, and is extrapolated from veterinarian atlases for small animals, Shanghai Institute, 1973). Electrical stimulation was applied for twenty minutes by means of square pulses from a Grass SD9 stimulator at 4 Hz, 0.1 msec. duration and with a voltage from 8 to 12 volts (voltage was adjusted to be above threshold for muscle contraction but below threshold for pain vocalization). Squeak latencies were measured again just before the EA needles were removed (i.e. after 20 minutes of EA treatment); next the squeak latencies were measured at 30 and 40 minutes (i.e. 10 and 20 min. after removing the needles). Then levo-naloxone (10mg/kg I.P.) was administered to the mice at 40 min. Squeak latencies were measured at 50 and 55 minutes

(i.e. 10 and 15 minutes after naloxone injection). In another 4 groups of mice (15 per group), similar doses of drugs were given to the animal except that there was no EA treatment. Mice were randomly assigned to each group. Statistics were done with Student's t-test, two-tailed.

In another experimental design naloxone was given at the onset of EA (instead of 40 minutes after onset of EA). In four groups of mice (15 per group), similar pain threshold tests were done on the mice treated with DAA (350 mg/kg), DAA plus EA, DAA plus naloxone (10 mg/kg) or DAA plus EA plus naloxone (10 mg/kg). The DAA were injected at 60 minutes before EA administration.

RESULTS

Figure 1 and 2 show the results; figure 2 was derived from figure 1 by plotting the 40 minute values; this was selected because it is the peak time for EA effects as shown in a previous study (Pomeranz and Chiu, 1976), and is also the time for maximum DAA analgesia (Cheng and Pomeranz, 1979; Ehrenpreis et al, 1978). The solid line in figure 2 shows that the DAA and EA produce additive effects at doses above 300 mg/kg. Both the 300 and 600 mg/kg values are significantly higher than the 0 mg/kg values ($p<0.05$), indicating that DAA and EA produce additive effects. Moreover, the EA plus DAA results, the solid line in figure 2, were always

significantly above the effects of DAA alone, the dotted line in figure 2, ($p<0.01$ for each dose). It is noteworthy that DAA alone (dotted line, figure 2) causes significant analgesia only at above 300 mg/kg which is the same dose required to show additive effects with EA analgesia ($p<0.01$). The analgesia caused by EA plus saline ($p<0.01$), or EA plus DAA, or DAA alone, all were reversed by naloxone given at 40 minutes ($p<0.01$, figure 1, A - D). Previous studies showed that EA analgesia (Pomeranz and Chiu, 1976) and DAA analgesia (Cheng and Pomeranz, 1979; Ehrenpreis et al, 1978) last for 1 - 2 hours in the absence of naloxone (i.e. there is no spontaneous recovery after 40 minutes). A control study indicated that the restraint used in this experiment induced insignificant pain threshold changes through the two hour period (figure 1A dotted line). Figure 3 shows that naloxone given at zero time completely abolishes both DAA analgesia or the DAA plus EA analgesia. Figure 4 shows that 28 out of 45 mice (62%) have marked EA analgesia (>16%) and 24 out of 45 (53%) mice show marked DAA analgesia, while 34 out of 45 mice (80%) shows marked analgesia by the combined treatment of EA and DAA. This shows that EA plus DAA treatment can induce analgesia in some animals that show poor response to either EA or DAA treatment alone.

Fig. 1

The effect of D-amino acids (DAA)(D-leucine plus D-phenylalanine) plus electroacupuncture (EA) analgesia. Ordinate shows percentage change in latency to squeak as compared to pretreatment control value (before the -60 min.). Positive values denote analgesia. Abscissa shows the time of measurement. Injections were given 1 h before acupuncture began (i.e. -60 min.). Pre drug tests were done just before the -60 min. period and post drug tests at 0 min. Electroacupuncture was administered from 0 to 20 min. Each point is the mean of observations on 15 mice. A: solid line shows EA + saline (0.9%); dashed line shows saline alone. B: solid line shows EA + DAA (150 mg/kg I.P.); dashed line shows DAA (150 mg/kg I.P.) alone. C: solid line shows EA + DAA (300 mg/kg I.P.); dashed line shows DAA (300 mg/kg I.P.) alone. D: solid line shows EA + DAA (600 mg/kg I.P.); dashed line shows DAA (600 mg/kg I.P.) alone. Bars show standard errors. Arrows indicate time of EA (0-20 min.) and naloxone injection (at 40 min.).

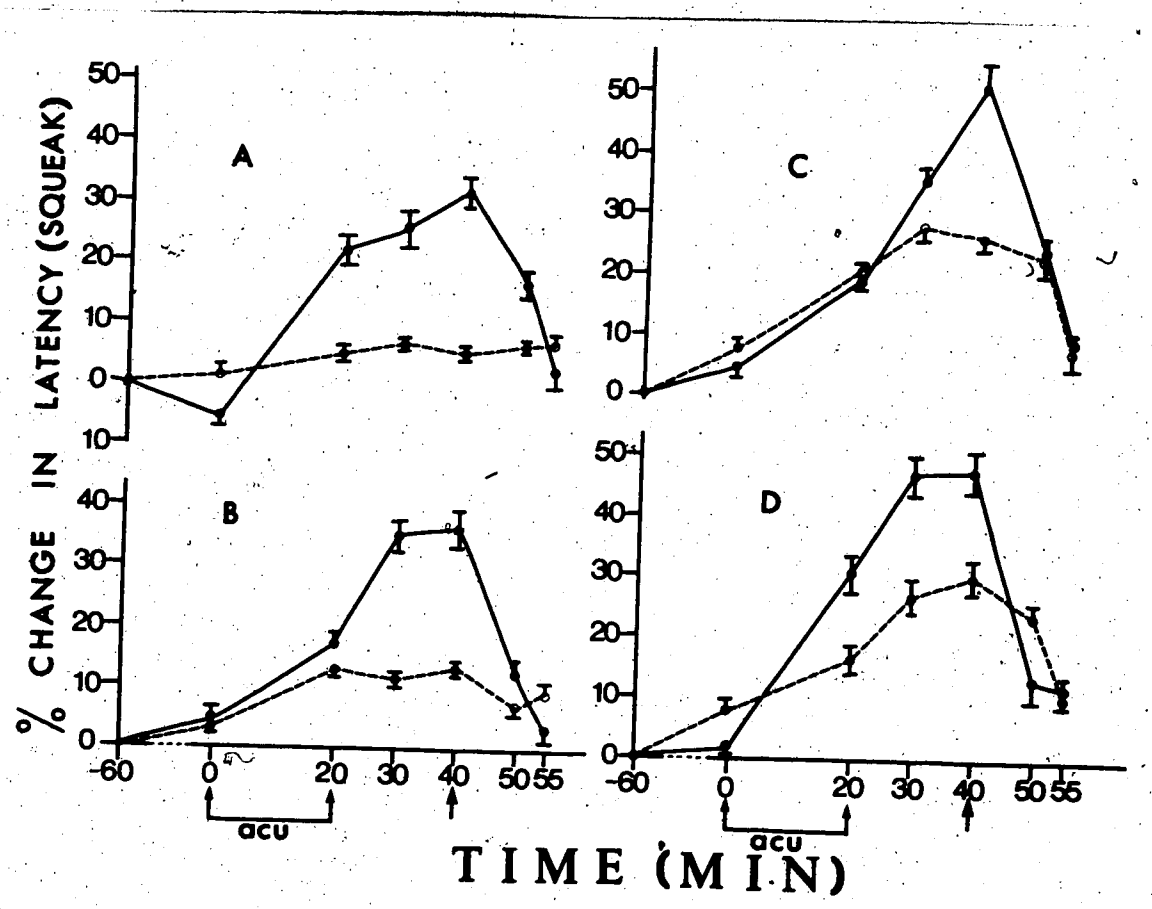


Fig. 2

Dose-response curves of EA + DAA and DAA (D-leucine + D-phenylalanine) in mg/kg. Ordinate shows % of analgesia at 40 min. (i.e. % change of average squeak latency at 20 min. after EA). Each point is the average of observations on 15 mice. Bars show standard error. Upper curve shows EA + DAA. Lower curve shows DAA alone. At point 0 mg/kg of DAA, saline (0.9%) injection was used. + shows significant EA analgesia ($p<0.01$, t-test two tailed). ++ indicante EA plus DAA analgesia which is significantly higher than EA plus saline.

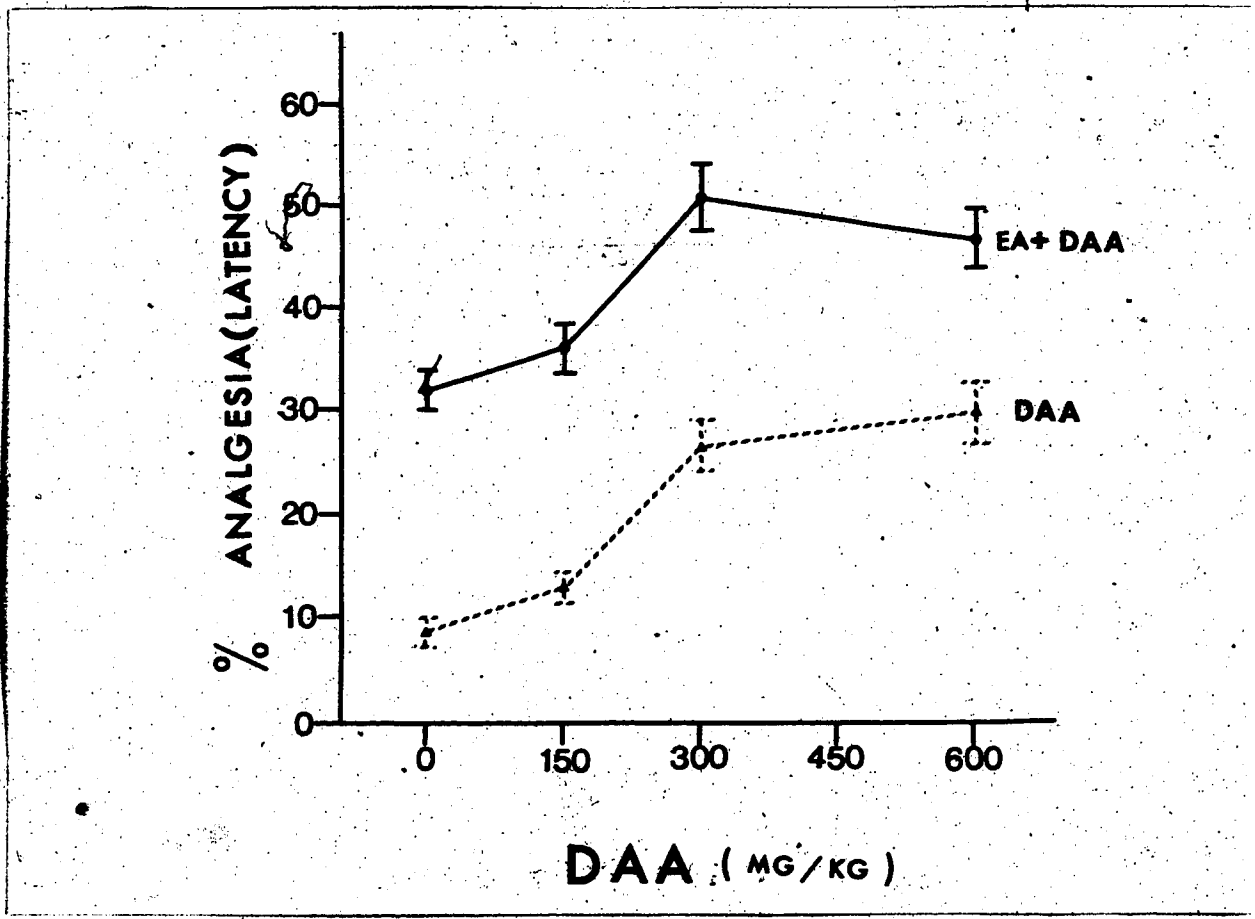


Fig. 3

The effect of naloxone given at zero time (10 mg/kg) on EA and EA plus DAA treatment in mice. Ordinate shows the average % change in latency to squeak as compared to pre-treatment control value (before the -60 min.). Positive values denote analgesia. Abscissa shows the time of measurment. D-Phenylalanine (350 mg/kg) and D-leucine (350 mg/kg) were injected at -60 min. Each curve represents the average of observations on 15 mice. I: mice treated with DAA + EA + saline. II: mice treated with DAA + saline. III: mice treated with DAA + naloxone. IV: mice treated with DAA + EA + naloxone. The injections of naloxone or saline were done in a blind manner after the 0 time measurements. The 40 min. values were taken for statistical analysis. Naloxone significantly reverses the DAA and DAA + EA effect ($p<0.01$, t-test two-tailed). Arrows indicate the time of injections and bars represent standard error.

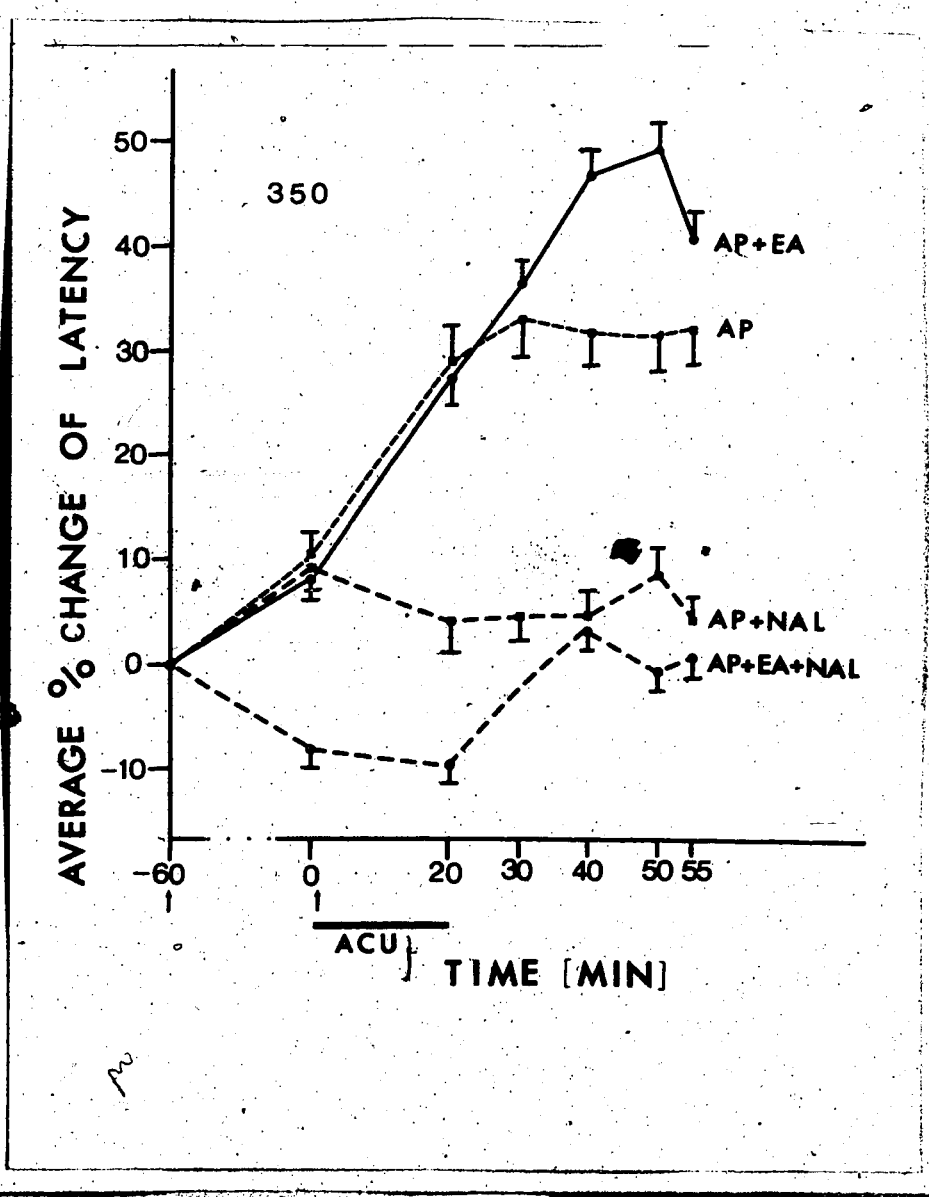
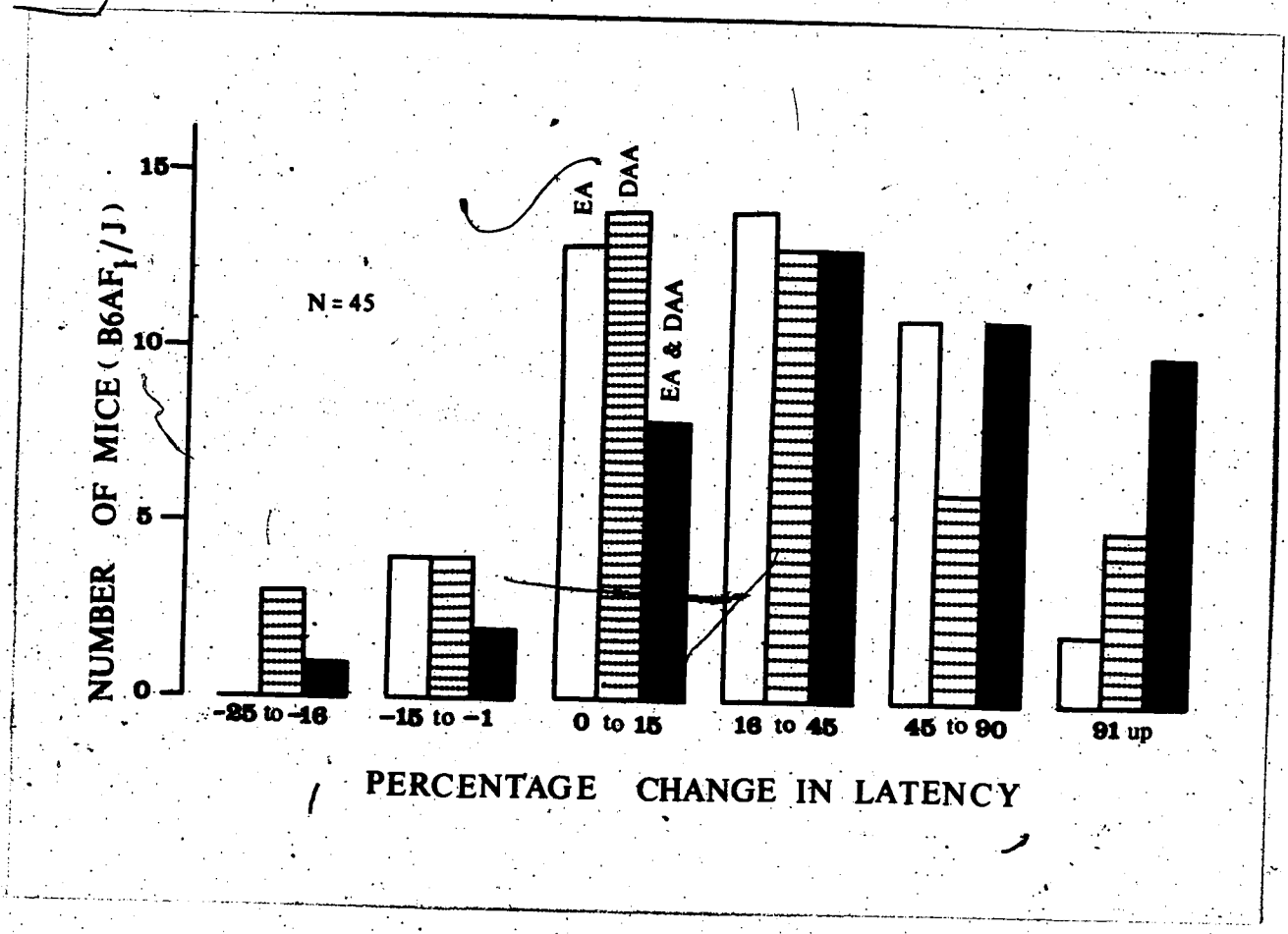


Fig. 4

A histogram shows the effect of EA, DAA (300 mg/kg) and DAA (300 mg/kg) plus EA on groups of 45 B6AF1/J mice. Ordinate indicates the number of mice. Abscissa shows the percentage change in squeak (analgesia) at 40 min. after the initiation of EA (EA was applied for 20 min.; refer to Fig. 3). Open bars: mice treated with EA. Striped bars: mice treated with DAA. Solid bars: mice treated with DAA plus EA. The combined EA and DAA effect is significantly higher th[illegible] the effect of EA or DAA alone ($p<0.01$, ANOVA and Ne[illegible]n-Keuls tests).



DISCUSSION

The present studies show that the analgesia produces by EA and DAA are additive. This effect is blocked by naloxone, supporting the possibility of the role of endorphins. Presumably, the EA releases endorphin, the DAA prevent its destruction and the two together give an additive effect. This combined treatment could be clinically useful to improve the results with EA.

Only about 50% to 60% of the mice show marked EA or DAA analgesia. Other reports indicate that EA (Gunn et al, 1977) or DAA (Ehrenpreis et al, 1978) work on a similar percentage of humans. However, more animals (80%) show marked analgesia by the combined EA and DAA treatments. This combination of EA with DAA may be a useful clinical pain treatment for many patients in whom EA alone is ineffective. It had been shown that chronic D-phenylalanine and/or D-leucine treatment cause no addiction in humans or mice (Ehrenpreis et al, 1978); the dose required to produce DAA analgesia becomes smaller and smaller after repeated injections; also after chronic treatment there was no sign of abstinence withdrawal.

Recently, it was demonstrated that D-phenylalanine entered the mouse brain in about 30 to 45 min by systemic injection (Okafor et al, 1980). This time course corresponded to the slow onset of D-AA analgesia.

Furthermore, brain enkephalinases were also found to be inhibited by D-phenylalanine and D-leucine in guinea pig ileum bioassay (Greenberg et al, 1980). More research on the mechanisms of these D-amino acid effects may lead to the discovery of a potent non-addictive method for treatment of pain.

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CHAPTER 9: TWO ELECTROACUPUNCTURE TREATMENTS THREE HOURS APART CAN INDUCE A HIGHER ANALGESIA THAN ONE TREATMENT ALONE

SUMMARY

Electroacupuncture analgesia (EAA) is enhanced after a second EA treatment three hours later and 100% (15/15) of the mice showed significant EAA. Only 53% (8/15) of the mice showed significant EAA when they were treated with sham and normal EA treatments at three hours apart. Additionally, it was demonstrated that EA produced significant analgesia while 'sham' EA had no effect on mice.

INTRODUCTION

Clinically, it has been observed that the acupuncture effect accumulates after repeated treatments. In many chronic pain therapies, substantial pain-relief is observed only after several treatments (Chen and Hwang, 1977; Leung, 1979). However, this increased electroacupuncture effect has never been properly studied nor reported in animal experimentation. Thus the present experiment is undertaken to find out whether electroacupuncture analgesia can be enhanced in the second treatment at three hours later as compared to the results of one EA treatment.

METHODS

The technique of electroacupuncture is similar to that described in previous papers (Pomeranz and Chiu, 1976; Cheng and Pomeranz, 1979b; Cheng and Pomeranz, 1980a). Briefly, two groups of mice (15 per group) were put into paper receptacles. Noxious stimulation was applied by shining a hot lamp onto the noses of the mice at 9 cm apart. Pain (squeak) latencies were taken three times, then one group of mice was treated with EA and the other groups of mice were treated with sham EA for 20 minutes. For sham EA, needles were placed subcutaneously at the back and electrical current was adjusted to below muscle vibration (voltage ranged from 1-4 volts, 4 Hz and 0.1 ms duration). For true EA (needles inserted into the first interosseus muscles) the voltage was adjusted to produce muscle vibration (voltage ranged from 8-12 volts, 4Hz and 0.1 ms duration.) Then the animals were removed from the paper holders and put back into the cage. Three hours later, both groups of mice were again put into the paper holders, pain latencies were again measured and true EA was applied to both groups. Pain thresholds were then measured at 20 minutes (before needles were removed) and again at 30, 40 and 50 minutes (i.e. 210, 220 and 230 minutes - see fig 1).

RESULTS

Figure 1 shows that two EA treatments produce a higher analgesia ($p<0.001$, t-test two tailed). On average mice that were treated with two true EA treatments had double the amount of analgesia as compared to those mice that had been treated with one sham and one true EA. A histogram (Fig. 2) indicated that only 8/15 (53%) of the mice showed significant EAA (>15%) 20 minutes after the true EA treatment (i.e. at 220 min. in Fig. 1), when animals were treated with both sham and true EA at 3 hr apart. In two true EA treatments, 100% of the mice showed significant EAA (>15%) at 20 min after the second treatment (i.e. 220 min. in Fig 1). Furthermore, figure 3 showed that the true EA produced a significant analgesia ($p<0.001$, t-test, two tailed) in a group of 15 mice, while sham EA had no effect ($p>0.05$, t-test, two tailed).

Fig. 1

This figure showed the effect of two EA treatment (at 3 hours apart) on mice. Ordinate indicated the average % change in squeak latency, positive denoted analgesia. Abscissa showed the time of measurement. Solid line, solid squares represented the average change of 15 mice that were treated with two EA at 180 min. apart. Dashed line, open square represented a group of 15 mice that were treated firstly with sham EA and then EA. Mice that had two real EA treatments gave a higher analgesic response.

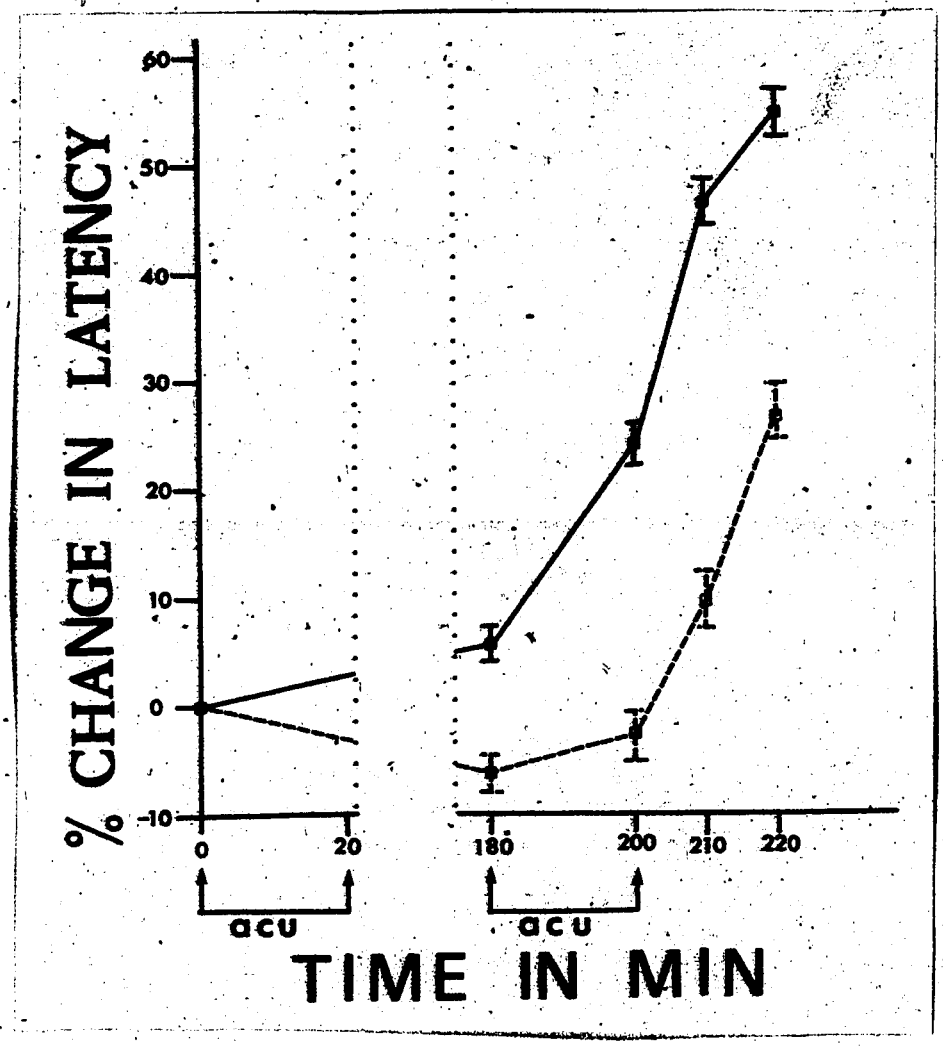


Fig. 2

A histogram indicated that the differences of EAA in two groups mice that were treated with two true EA (solid bars) or sham EA plus true EA (open bars). Ordinate showed the number of mice. Abscissa showed the % of analgesia at 220 min. (refer to Fig. 1).

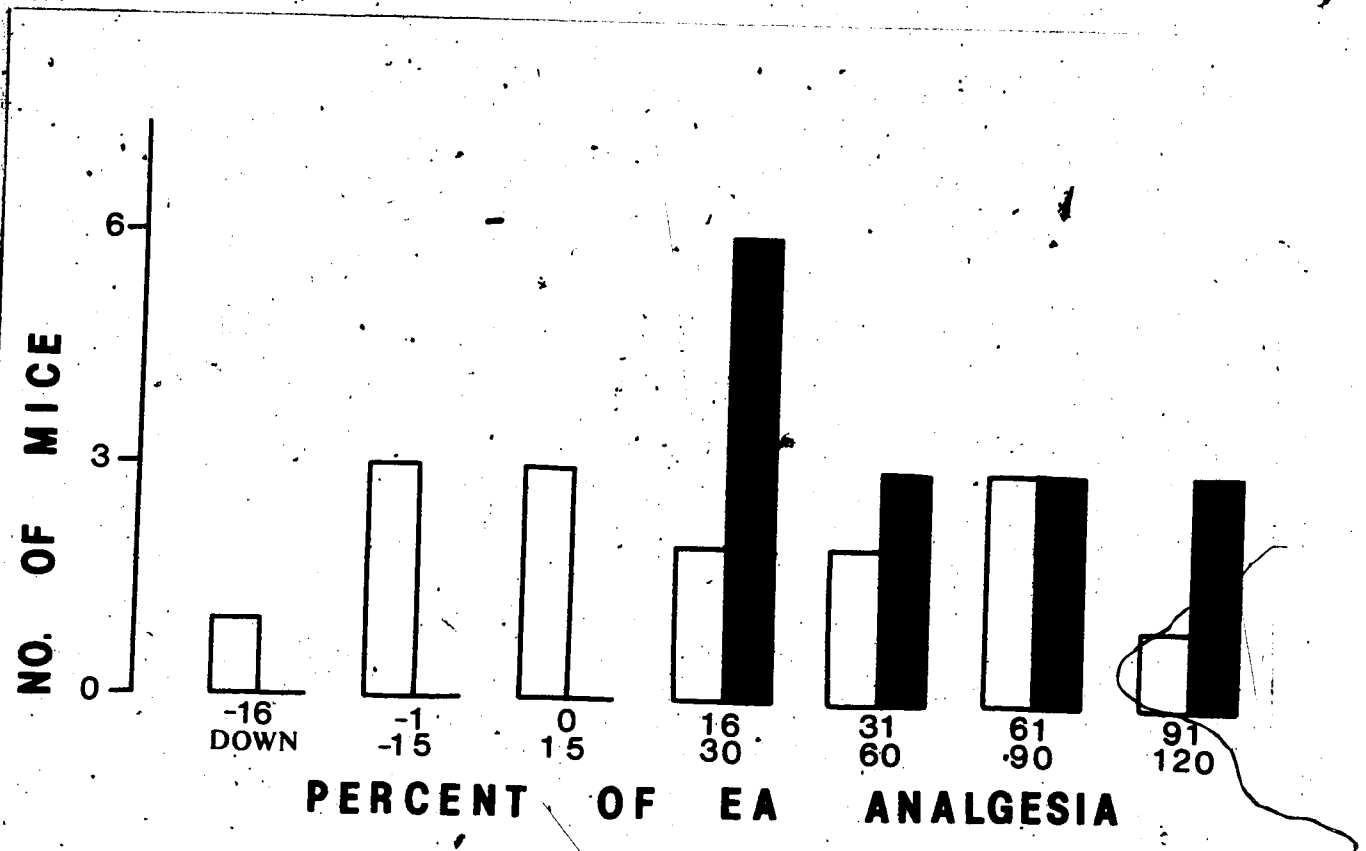
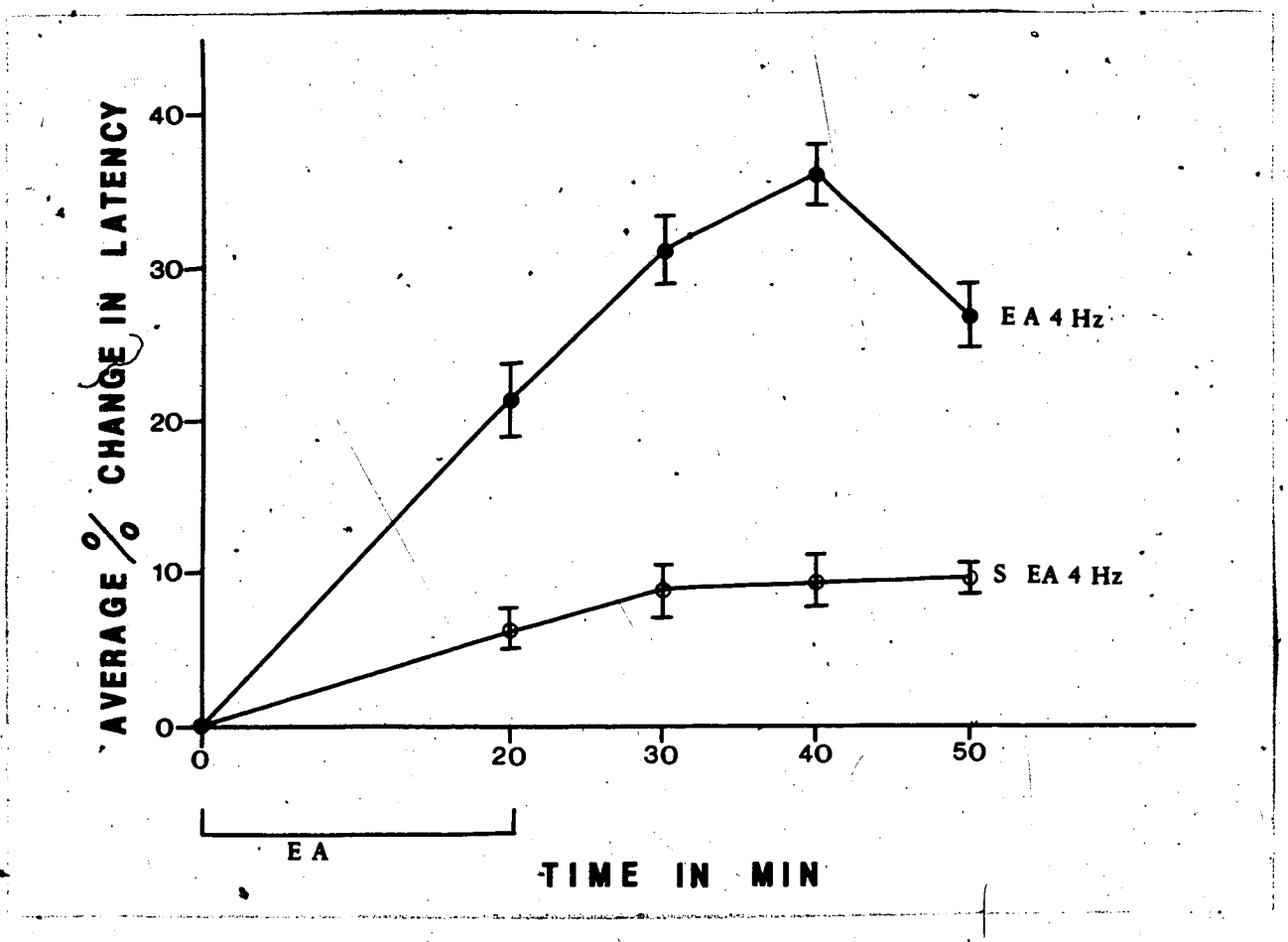


Fig. 3

This figure is similar to Fig. 1 except that the mice were treated once either with true EA (solid circles) or sham EA (open circles).



DISCUSSION

The enhanced EAA in mice after the second treatment corresponds to the clinical observations which indicated that EA effect is cumulative. Several lines of evidence have indicated that acupuncture analgesia is mediated by endorphins (Mayer et al, 1977; Pomeranz and Chiu, 1976; Sjolund et al, 1977; Cheng et al, 1979f; Pomeranz and Cheng, 1979) and serotonin (Han et al, 1979; Cheng and Pomeranz, 1979b). Recently, it was demonstrated that low frequency (4 Hz) EA may be mediated by endorphins while high frequency (200 Hz) EA may be partly mediated by serotonin (Cheng and Pomeranz, 1979b). Thus the cumulative effect of EA is most probably due to increase of endorphin levels.

In a previous paper, it was also demonstrated that EAA showed no cross-tolerance with morphine (Cheng et al, 1980g). Repeated EA treatments are unlikely to cause addiction. Instead, a higher analgesia is observed after repeated EA treatments. Furthermore, normally about 50 to 70% of the patients (Chen and Hwang, 1977; Leung, 1979; Gunn et al., 1977) and mice (Cheng and Pomeranz, 1980e) demonstrated significant EAA after single EA treatment. The present results indicate all the mice show significant EAA after the second EA treatment three hours later. Since acupuncture has variable effects on human subjects and only about 60% of the population show EAA, this may be due to the

differences in endorphin systems as proposed by Peets and Pomeranz (1980). However, if EA releases the endorphins which are prevented from enzymatic degradation by D-phenylalanine and D-leucine, more profound analgesia is observed in a higher percentage (80%) of mice (Cheng and Pomeranz, 1980e). Recent biochemical evidence shows that D-phenylalanine and D-leucine inhibit the action of enkephalinases in guinea pig ileum assay (Greenberg et al., 1980). Thus the increase in endorphin levels is essential for endogenous pain-relief. Perhaps two EA treatments at three hours may be another technique enhancing endogenous endorphins and may set an example for a time course for EA treatments in clinical practice.

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CHAPTER 10: ELECTROACUPUNCTURE TREATMENT OF MORPHINE DEPENDENT MICE REDUCES SIGNS OF WITHDRAWAL, WITHOUT SHOWING CROSS TOLERANCE

SUMMARY

Morphine pellets (75 mg morphine base per pellet) were implanted subcutaneously in mice (B6AF1/J) and were surgically removed after 3 or 8 days. During morphine abstinence (7 h after pellet removal), the mice were treated with electroacupuncture (EA). The results indicate that EA analgesia shows no cross-tolerance to morphine. Additionally, EA reduced withdrawal behaviour (jumping) in 50% of the mice during morphine abstinence.

INTRODUCTION

Clinical reports suggest that electroacupuncture (EA) may be effective in the therapy against opiate dependence in human addicts (Wen, 1977). Moreover, recent evidence suggests that EA analgesia may be mediated by endorphins, morphine-like peptides in the brain (Cheng et al, 1979; Mayer et al, 1977; Pomeranz and Cheng, 1979; Pomeranz and Chiu, 1976; Sjolund et al, 1977). Perhaps the therapy of addicts is somehow related to the release of endorphins, as met-enkephalin levels in CSF are elevated during the treat-

ment of addicts (Ho et al, 1978). This hypothesis raises an important question. Does morphine tolerance in addicts cause them also to be tolerant to the endorphins released by EA (i.e., is there cross tolerance between morphine and EA-stimulated endorphins)? Recently it has been shown that stimulus produced analgesia (SPA) from midbrain implanted electrodes, which also releases endorphins (Hosebuchi and Li, 1978), shows cross tolerance with morphine (Mayer et al, 1975). However reports show that another form of analgesia, stress-produced-analgesia, which also involves endorphins (Madden et al, 1977), does not exhibit cross tolerance to morphine in rats (Akil et al, 1978; Bodnar et al, 1978), but does show cross tolerance in mice (Chesher and Chen, 1977). We therefore designed the following experiment with mice to determine if EA showed cross tolerance with morphine. Additionally, we investigated the effectiveness of EA in treating morphine withdrawal.

MATERIALS AND METHODS

Examining the relationship between EA and addiction produced methodological difficulties. The foregoing clinical EA studies have not ruled out placebo effects; moreover they are unreliable because of the problem of objectively assessing withdrawal in human patients (Wen, 1977 and Blum et al, 1978). Additionally, the EA studies in rats (Ng et al, 1975) and mice (Ho et al, 1978) may be difficult to

interpret because they employed naloxone (a morphine antagonist) to precipitate withdrawal in morphine dependent animals. We felt that the naloxone would also block the endorphins presumably released by EA (Sjolund et al, 1977). To circumvent these problems, we used an animal model which did not employ naloxone. For this purpose we employed the acute abstinence method described by Way (Way et al, 1969; Brase et al, 1977). It consisted of implanting a subcutaneous slow-release pellet (containing 75 mg morphine base) for 3 days, followed by surgical removal of the pellet. This produces withdrawal signs (especially jumping) 7 h after pellet removal. Preliminary results on 12 mice showed that animals treated with morphine pellets showed marked tolerance to morphine analgesia.

We used B6AF1/J female mice, 10-16 weeks of age, obtained from Jackson Laboratories. They were housed in our facilities for at least one week prior to the study, with a 12 h light-cycle 7AM to 7PM. All tests were made between 4PM and 7PM.

For true EA, needles were placed in the first dorsal interosseus muscle on each forepaw (this point is called Hoku or L.I.4 in the acupuncture literature, and it is extrapolated from Veterinarian atlases for small mammals). Electrical pulses were applied to the needles with a frequency of 4 per sec, 0.1 msec duration, 8-12 volts (which is below pain levels), for 30 min (Pomeranz and Chiu, 1976).

For sham EA, needles were placed subcutaneously over the back, in non-acupuncture sites, and the same electrical pulses were applied. In a previous study, this sham EA treatment did not produce analgesia, unlike true EA at Hoku (Pomeranz and Chiu, 1976). Placement of sham EA needles in Hoku (without electrical current) is not a suitable control as clinical reports suggest that the mere placement of needles in true acupuncture points could cause effects even in the absence of electrical stimulation. Mice were randomly assigned to 7 groups; 12 mice per group. Table 1 summaries the protocols of groups I-VII.

Groups I-III were employed in the study of cross tolerance between EA and morphine using a method of measuring EA analgesia previously reported (Pomeranz and Chiu, 1976). Each mouse was placed in a cardboard restrainer and was tested for pain threshold before and after EA. Threshold was determined by the latency to squeak (vocalization) after turning on a hot lamp directed at the nose.

Groups IV-VI were used to study the effectiveness of EA in treating withdrawal. Our quantitative method, extensively studied by Way (Way et al, 1969 and Brase et al, 1977), consisted of measuring jumping behaviour 7 h after pellet removal. Six mice at a time were placed on a platform, 50 cm high, to determine whether or not they jumped within 15 min. Only the mice that jumped were given a subsequent EA treatment.

After this paper was completed, a preliminary report from China (Han et al, 1979) indicated that cross tolerance to EA in rats develops after 8 days (but not after 3 days) of daily injections of morphine. Thus in group VII morphine pellets were removed on day 8 and tested for EA cross tolerance 7 h later. For statistical analysis to compare the data obtained from group I, II, III and VII we used ANOVA and Newman-Keul's tests. The F test for proportions was used to compare the difference of groups IV, V and VI. For within group studies (before and after EA comparisons) we used the paired t-test, two tailed.

RESULTS

Table II and Fig. I summarize the cross tolerance results. In groups I-III we showed that EA analgesia (EAA) did not show cross tolerance to morphine after 3 days (Fig. 1). Group I (M3+EA), addicted to morphine, still showed a strong EA analgesia with an average pain threshold of 59 + 4% higher than pre EA levels ($p<0.001$). To test whether this analgesia was due to EA, and not caused by an artifact (such as stress), group II (M3+S) received sham EA, and showed an insignificant change in pain threshold ($p>0.05$) (no higher than pre EA control levels). Group II (M3+S) was significantly different from group I (M3+EA) (Newman Keul's test, $p<0.01$). Next we compared the EA effects in addicted mice, group I (M3+EA), with those in normal mice, group III (EA) and found that the analgesia was enhanced in the

addicted animals (Newman Keul's test, $p<0.01$). Moreover, instead of demonstrating cross tolerance, group I showed the complete opposite effect (i.e. EA was enhanced). These results seem to suggest that EA analgesia does not show cross tolerance with morphine. In group VII (M8+EA), EAA was similar to group I (M3+EA) ($p>0.1$, Newman Keul's test). Hence the results, after 3 days and 8 days of morphine treatment, showed the same lack of cross tolerance to EA contrary to the results of Han et al (1979) (after 8 days of morphine addiction in rats).

Table II also summarizes the effects of EA on withdrawal behaviour. Group IV-VI were used to test the effectiveness of EA in the treatment of jumping behaviour (withdrawal sign). Group IV (addicted mice, treated with EA) showed significantly less jumping (only 6 out of 12 mice jumped), compared with the control group (11 out of 12 mice jumped; $p<0.005$, F-test). Group VI, an additional control group, showed that normal, non-addicted mice do not jump (zero out of 12 miceejumped).

FIG. 1

Electroacupuncture effects on morphine-addicted mice seven hours after morphine-pellet removal. Ordinate shows average percentage change in latency to squeak; positive values denote analgesia. Abscissa shows the time of measurements. Each curve represents the average values from a group of 12 mice. "M3+EA" indicates the EA effect on mice that have been implanted with morphine pellets for 3 days. "M8+EA" shows the EA effect on mice that have been implanted with morphine pellets for 8 days. "EA" indicates the EA effect on non-addicated mice. "M3+S" shows the effect of sham EA on mice that have been implanted with morphine-pellets for 3 days. Bars show standard error. Arrows indicate time of EA treatment.

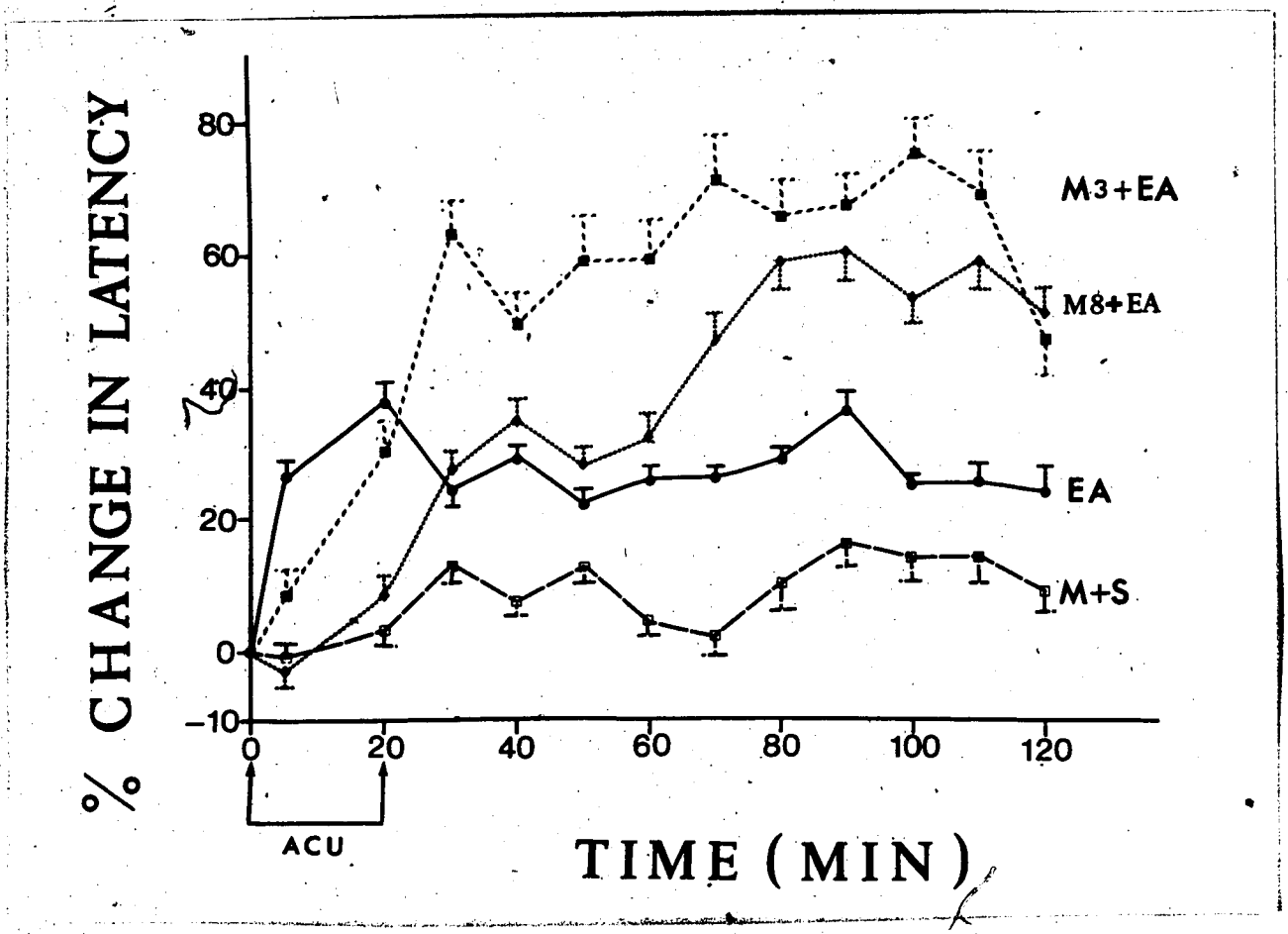

TABLE 1 (METHODS)

			After pellet removal	
	Pellet	Pre-response	Treatment	Post-response
I (M3+EA)	M. Pellet 3 days	Squeak	EA	Squeak
II (M3+S)	M. Pellet 3 days	Squeak	Sham EA	Squeak
III (EA)	None	Squeak	EA	Squeak
IV	M. Pellet 3 days	Jumping	EA	Jumping
V	M. Pellet 3 days	Jumping	Restraint	Jumping
VI	None	Jumping	Restraint	Jumping
VII (M8+EA)	M.Pellet 8 days	Squeak	EA	Squeak

M. = Morphine EA = Electroacupuncture

S = Sham EA M3 = Morphine pellet(3 days)

M8 = Morphine pellet(8 days)

TABLE II (RESULTS)

Group n=12	Pre-response	Post-response	% Change (pre vs Post)	Statistics
I	3.8±0.3*	5.9±0.3**	+59±4%	P<0.001 t-test
II	4.8±0.2	5.3±0.4	+13±2%	P>0.05 t-test
III	4.6±0.2	5.9±0.15	+25±1.5%	P<0.001 t-test
	Anova, P<0.01 Newman Keul's test for III and I, P<0.01; for II and I, P<0.01			
IV	12 jumped	6 jumped	-50% (6/12)	P<0.005, F-test for proportion
V	12 jumped	11jumped	-8% (11/12)	
VI	0 jumped	------	---------	----------
VII	4.5±0.2	5.8±0.3	+35±3%	P<0.01 t-test
	ANOVA, P<0.01; Newman Keul's test for III & VII P>0.1			

* In Groups I, II, III and VII these values were averages of 3 control values per mouse; then the average of 12 mice was obtained. (+S.E.)

**In groups I,II,III and VII these values were averaged from 20 min - 40 min for each mouse; then the average of 12 mice was obtained. (S.E.)

DISCUSSION

Two clear results emerge from this paper. First that EA in mice does not show cross tolerance with morphine; secondly that EA produces beneficial effects on opiate withdrawal.

The significance of the lack of cross tolerance is not clear since the literature on cross tolerance is full of conflicting evidence. In rats SPA and EA both show cross tolerance (Mayer and Hayes, 1975; Han et al, 1979), but stress analgesia does not (Bodnar et al, 1978). In mice stress analgesia shows cross tolerance (Brase et al, 1977) but now we find that EA does not produce cross tolerance in the mouse. It is not clear whether species differences or subtle differences in methodology between investigators contributes to these differences. Until the mechanisms of addiction are better understood, this conflict of results cannot be easily resolved.

Our experiment on sham EA (group II) was designed to rule out the effects of stress in the EA phenomenon. Moreover the EA experiment is designed to minimize stress; voltages are used which are well below the threshold for A-delta pain fibers (Pomeranz and Paley, 1979); the mice do not squeak during the EA treatment suggesting that EA is not a stressful procedure.

The significance of the beneficial effects of EA in suppressing the signs of withdrawal is evident. It is consistant with clinical results which show that EA helps the addict through the difficult stages of opiate withdrawal. Moreover, our results agree with those in rats by Ng et al (1975) and in mice by Ho et al (1978). even though we used an entirely different method of producing withdrawal (they used naloxone, we used acute abstinence). Thus the presence or absence of naloxone during withdrawal does not interfere with the effects of EA in reducing withdrawal signs. If EA operates by releasing endorphins, then both paradigms would benefit from the acupuncture treatment. In the acute abstinence experiment, the released endorphins would compensate for the drop of morphine levels. In the naloxone precipitated withdrawal paradigm, the released endorphins would compete with naloxone for the opiate receptors and overcome the blockade which causes withdrawal effects.

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GENERAL DISCUSSION

I. Lines of evidence supporting the EAA-endorphin hypothesis:

Presently, there are numerous papers that have been published on the topic of acupuncture analgesia in the East and West. More importantly, this multidisciplinary research gives a consistent picture suggesting that EAA may be mainly mediated by endorphin or serotonin. Collectively, many lines of evidence indicate that EAA may be mediated by endorphins:-

(i) EAA is reversed by systemic naloxone in humans (Mayer et al, 1977; Chapman and Beneditti, 1977) and animals (Pomeranz and Chiu, 1976; Pomeranz and Cheng, 1979; Cheng and Pomeranz, 1980a; Zhang et al, 1979; Han et al, 1979; Takeshige et al, 1978). Microinjections of naloxone (10 ul) into the PAG, caudate, nucleus accumbens or hypothalamus decreases acupuncture analgesia in rats and rabbits, while other areas which do not contain endorphins show no naloxone effects (Zhang et al, 1979; Zou et al, 1979). This suggests that certain brain regions controlling the endogenous pain-relieving systems can be triggered by acupuncture.

(ii) EAA is mediated by stereospecific opiate receptors;, dextronaloxone, an inactive isomer, has no effect on EAA while Type I opiate antagonists, levo-naloxone, naltrexone, cyclazacine and diphrenorphine all block EAA (Cheng and

Pomeranz, 1980a) in mice. EAA is also blocked by naloxazone (Zhang, 1980), a high affinity opiate receptor blocker which also blocks morphine analgesia (Pasternak et al, 1980).

(iii) Peets and Pomeranz (1978) demonstrated that mice (CXBK) genetically deficient in opiate receptors showed poor electroacupuncture analgesia. They speculated that there are genetic variations in both humans and animals and that about 30 to 40% of them would respond poorly to acupuncture due to a poor endorphin system. This idea was further confirmed by Takeshige et al (1978) who observed that those rats (40%) demonstrating no acupuncture analgesia had a deficiency in total brain endorphins measured by receptor binding assay.

(iv) A more direct verification of the acupuncture-endorphin hypothesis is to measure the endorphin levels in brain tissues, CSF and the spinal cord before and after acupuncture. Sjolund, Terenius and Erickson (1977) reported that transcutaneous electroacupuncture (TEA) on the lower lumbar spine (segmental TEA) in humans elevated lumbar CSF endorphin (fraction I) concentrations, while TEA to HoKu (non-segmental TEA) did not. This 'fraction I' endorphin extract was found to cross-react with dynorphin antibodies (Terenius - personal communication). Since dynorphin is found in the dorsal root ganglia (Goldstein et al, 1979), it is hypothesized that acupuncture can induce a local segmental release of endorphins; possibly dynorphin which may also

presynaptically inhibit the release of substance P. However, H. Akil has shown that injecting dynorphin into the PAG or ventricular CSF produces no analgesic effects in rats (personal communication). Thus it is unclear whether dynorphin is an analgesic substance.

K. Tsou and his colleagues (1979) also found that EA caused an increase of endorphin (fraction I) levels in the cisternal CSF in rabbits. Additionally, they found that intraventricular injections of bacitracin (retarding the enzymatic degradation of enkephalins) plus acupuncture treatment increased met-enkephalin concentrations by 3 times, while either treatment alone has no significant effect (Tsou et al, 1979). Another study by Zhang and colleagues (1979) demonstrated that fraction I endorphin release was elevated in the PAG after acupuncture treatment. This experiment was done on awake rabbits by using push-pull cannulae. However acupuncture had no significant effect on the fraction I levels in the caudate and nucleus accumbens. Moreover, Candace Pert and L.K.Y. Ng also showed that endorphin levels (fraction I) were increased in the ventricular CSF but decreased in the hypothalamus after ear-acupuncture in rats (in press). All these results suggest that acupuncture releases endorphins from brain into CSF.

(v) It was shown that hypophysectomy abolishes EAA (Cheng, M.Sc. thesis, 1977) and also that dexamethasone, which inhibits the release of ACTH and beta-endorphin, and

2% saline treatment, which depletes pituitary endorphin all reduce EAA (Cheng et al, 1979f). These findings suggest that pituitary endorphin is at least partially involved in mediating EAA. Recently, it was reported that hypophysectomy inhibits morphine analgesia (Katz, 1980) and naloxone hyperalgesia (Grevert et al, 1978). This indicates that the pituitary is a corequisite for opiate analgesia. Finally an indirect experiment indicated that EA may release beta-endorphin and ACTH, which were released together into the blood (Guillemin et al, 1977), since cortisol levels were elevated after EAA in horses (Cheng et al, 1980h) and rabbits (Zhang, 1980).

(vi) .It was demonstrated that D-phenylalanine and D-leucine produced naloxone-reversible analgesia in humans and mice (Ehrenpreis et al., 1978; Cheng and Pomeranz, 1979d; Cheng and Pomeranz, 1980e). It was postulated that these D-amino acids protected endorphins from enzymatic degradation. Recent biochemical evidence supports this hypothesis; D-phenylalanine and D-leucine can cross the blood-brain barrier (Okafor et al., 1980) and inhibit enkephalinases in guinea pig ileum assay (Greenberg et al., 1980). Combining the D-amino acids and EA treatments produced a higher analgesia (and in larger numbers of mice) as compared to either treatment alone (Cheng and Pomeranz, 1980e). Presumbably EA releases endorphins which are protected by the D-amino acids from enzymatic degradation: these combined effects produce a profound analgesia.

II. Lines of evidence suggesting EAA-serotonin mechanisms:

There are also many lines of evidence indicating that EAA is also mediated by serotonin:

(1) The descending inhibition by the raphe-serotonin system was first demonstrated by the Shen and co-workers in 1975. They found that lesioning of the dorsolateral fasciculus (DLF) completely abolished acupuncture analgesia in rabbits (Shen et al, 1975; Shen et al, 1978). In 1976, Du and Chao (1976) showed that lesioning the raphe magnus inhibited acupuncture analgesia. This result was repeated by McLennan et al (1977) who showed that both electrical and chemical lesions of the raphe nucleus reduced EAA in rabbits and rats. It was also found that the DLF system mediates morphine analgesia and SPA (Basbaum and Fields, 1978). Intracerebral injection of 5,6-dihydroxytrytamine in raphe nucleus inhibited EAA (Chiang et al, 1979). Similarly, microinjection of naloxone into the PAG partially reversed EAA (Zhang et al, 1979). 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-EAA effect may by-pass the brainstem enkephalin system. Recent results demonstrated that while low frequency (4Hz) EAA might be mediated by endorphins high frequency (200Hz) EAA might be mediated by serotonin (and not endorphins):- the former was reversed by naloxone and the later was partially reduced only by P-CPA, a serotonin

synthesis inhibitor (Cheng and Pomeranz, 1979b).

(2) EAA was also abolished or reduced by many different chemicals that either depleted or antagonized the effect of brain serotonin, while drugs that enhanced the serotonin level in the CNS increased EAA (Han et al, 1979; Kin et al, 1979; Cheng and Pomeranz, 1980c).

(3) Another important piece of evidence for the EAA-serotonin hypothesis indicated that serotonin and its catabolite 5HIAA were elevated in the CSF (Yi et al, 1977), raphe nucleus and locus coerulus (Tung et al; Han et al, 1979), spinal cord (Chan, 1978; Han et al, 1979), and the whole brain (Han et al; 1979; Kin et al, 1979) during EAA. The above elevation of serotonin and its metabolites was positively correlated to the effect of EAA. Han and coworkers (1979) further demonstrated that the serotonin content and its turnover rate increased in the telencephalon, diencephalon, brain stem and spinal cord after one hour 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 5HT synthesis in the lower brain stem and spinal cord but a marked increase of 5HT turnover in diencephalon and telencephalon. Thus they concluded that the forebrain might also play an important role in EAA.

The above lines of evidence seem to converge suggesting that EA releases endorphin and/or serotonin for analgesia. Additionally, other hormones or neurotransmitters may directly or indirectly be involved (Cheng and Pomeranz, 1980c). It appears that EA works through a multi-factorial system. These may be triggered simultaneously or independently which may be achieved by altering the frequencies of electrical currents.

III. Other considerations:

Iontophoretically applied 5HT or noradrenaline inhibits the response of dorsal horn cells evoked by nociceptive stimulation (Randic and Yu, 1976; Headly et al, 1978), while intrathecal application of 5HT or noradrenaline in the spinal cord also produces profound analgesia in rats, rabbits and cats (Yaksh and Wilson, 1978). Furthermore, it was demonstrated (Yaksh 1979) that application of serotonin or noradrenaline in the spinal cord abolished analgesia from morphine injected in the PAG. All these results implicate descending systems, which may modify spinal sensory processing by means of serotonin or noradrenaline. Han and colleagues (1979) found that naloxone only partly blocked EAA in rats. When the rats were injected with P-CPA which blocked the synthesis of serotonin, EAA was also only partly reduced. However, when they combined the naloxone and P-CPA treatments in rats, EAA was completely abolished. It should

be noted that Han et al (1979) used noxious electrical current of 5 mA to induce EAA which was 50 times greater than the threshold for A-beta fiber stimulation (threshold = 0.1mA; Pomeranz and Paley, 1979). Zhang et al (1979) found that "moderate strength EAA" (7.5 - 8 mA) was readily reversed by naloxone in rabbits, while "superstrength EAA" (12.5 - 15 mA) was not. This would suggest that such high current stimulation may evoke a stress-induced analgesia which may be mediated by non-endorphin systems; possibly serotonin and/or noradrenaline are involved. In contrast, non-noxious electrical currents of low frquency EA (4 Hz, 0.2-0.3 mA at 0.1 ms duration) and high frequency (200 Hz, 0.1-0.2 mA at 0.1 ms duration) were able to trigger the endorphin and non-endorphin systems independently (Cheng and Pomeranz, 1979b).

The results of the various studies of the mechanism of EAA are summarized in Tables A and B. Table A shows results of 4 Hz and 200 Hz EAA while table B shows the results of only 200 Hz stimulation.

Table A

Treatment	Action	EAA(4Hz)	EAA(200Hz)
1 Hypophysectomy*	Deplete pituitary endorphin	↓	
2 Dexamethasone	Inhibit beta-endorphin release	ND (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 hr apart	Accumculative effect	↑	ND
8 Morphine addicted mice during withdrawl	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.
* from my M.Sc. thesis ND = not done

Table B

Summary of monoaminergic drugs on EAA at 200 Hz.

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

S = Serotonin, D = Dopamine, N = Norepinephrine

R = Recovery of EAA after replacement drugs.

* only the data related to serotonin gave consistent EAA results.

IV. AN INTRICATE SYSTEM IS PROPOSED FOR EAA

By combining the results in this thesis with those in the literature I propose an intricate system for the mechanisms of EAA (see figure B). Namely, EA at 4 Hz may stimulate the sensory receptor in the deep muscle causing the midbrain PAG to release enkephalins which activates the dorsal raphe nucleus or nucleus raphe magnus and nucleus reticularis magnocellularis (Rmc) to send down descending inhibitions along the DLF to the spinal cord. The raphe-DLF system may be mediated by the neurotransmitter, serotonin (while the Rmc-DLF systems may be mediated by noradrenaline). In parallel, EA (4 Hz) may stimulate the beta-endorphins neurons in the hypothalamus. These hypothalamic neurons project to different areas of the brain (e.g. PAG, periventricular nucleus of thalamus, accumbens, amygdala) and may release beta-endorphin for pain-relief. Additionally, the hypothalamus may also produce a releasing factor to stimulate the release of pituitary endorphins and ACTH. The pituitary endorphins may be released into the blood circulation, or may backflow into the CSF (the endorphins in the circulation have to pass through the blood-brain barrier to bind to the opiate receptors for analgesia). If the acupuncture points and the painful areas are in the same segmental levels, EA (4 Hz) may also directly stimulate the endorphins in the spinal cord. The spinal cord endorphins may presynaptically inhibit the release of the neurotramsitters small primary afferent fibres and

Figure A

At low frequency (4Hz), 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(4Hz) 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 (200Hz) EA appears to stimulate directly the RN and Rmc descending inhibitory systems, bypassing the endorphin system.

(Details of these systems are described in the text.)

thus block the pain message (nociception). Furthermore, high frequency (200 Hz) EAA may activate sensory nerves and directly stimulate the DLF-serotonin descending inhibitory systems, by-passing the PAG-endorphin systems.

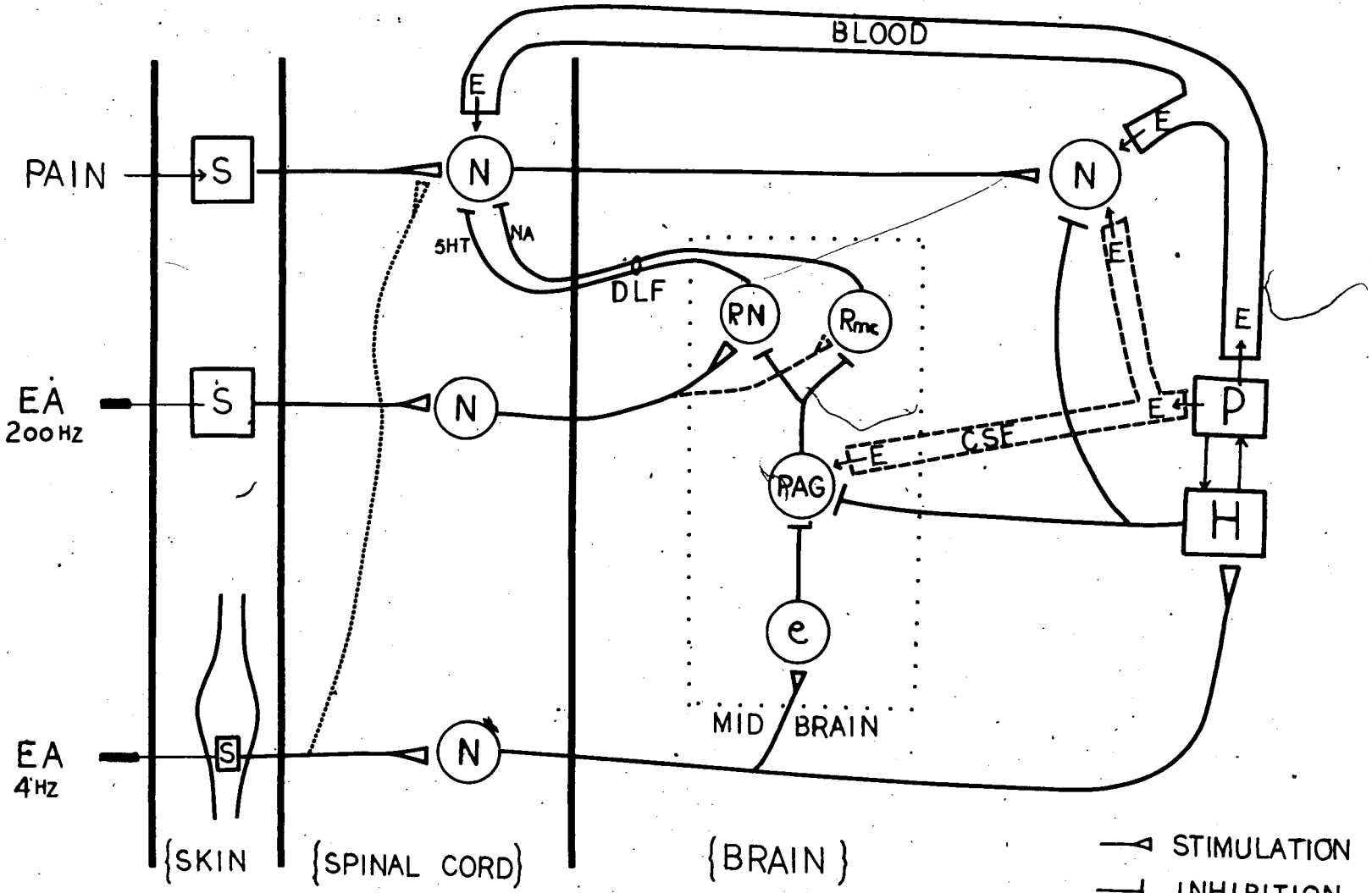
In summary, low frequency (4 Hz) EA may stimulate the release of endorphins in the spinal cord, midbrain, hypothalamus and pituitary for pain-relief, while high frequency (200 Hz) may directly stimulate the DLF-inhibitory systems, thus avoiding the endorphin links. The details and evidences for this intricate system of EAA are discussed according to the sites of EA actions as follows:

V. Possible anatomical sites of EAA mechanisms:

Collectively, the acupuncture-endorphin hypothesis may be divided into three levels of analgesia:

(A) Local : Such analgesia is probably localized over a small area due to a segmental release of endorphins in the spinal cord according to the area of stimulation. Several lines of evidence have suggested that substance P is the neurotransmitter at the terminals of small primary afferent (A-delta and C) fibres responsible for pain transmission (Leeman, 1979). Immunohistochemical analysis has revealed that enkephalin and substance P neurons have an intimate spatial relationship in the spinal cord and brain stem (Hokfelt et al, 1977). Depleting substance P by capsaicin produces analgesia and enhances morphine analgesia

Fig A



EA : ELECTROACUPUNCTURE
S : SENSORY RECEPTOR
N : INTERNEURON
e : ENKEPHALINERGIC NEURON
E : ENDORPHIN (β-ENDORPHIN or DYNORPHIN)
NA : NORADRENALINE

H : HYPOTHALAMUS
P : PITUITARY
PAG : PERIAQUEDUCTAL GRAY
RN : RAPHE NUCLEUS
RMC : RETICULAR MEGNOCELLAR NUCLEUS
DLF : DORSOLATERAL FUNICULUS

(Yaksh, 1979). Moreover, it has been demonstrated (Mudge et al, 1979; Jessell and Iversen, 1977) that endorphins are able to suppress the release of substance P. Consequently, the release of endorphins may presynaptically inhibit noxious input (Duggan et al, 1976). If substance P is the neurotransmitter that plays a role in the perception of pain, the closely associated enkephalinergic neurons will inhibit noxious input rapidly, since it takes about 1 msec. for the released endorphin to cross the synapse and cause an affect.

Clinically, it is often observed that EA produces greater analgesia if the painful area and the acupuncture points are at the same specific segment(Chapman et al 1976). Sjolund and colleagues (1977) demonstrated that transcutaneous electroacupuncture (TEA) treatment upon the lumber area elevates endorphin levels in human lumbar CSF but TEA at a distal site (Hoku - First Interosseus muscle on the hand) does not change the endorphin level in the human lumbar CSF. Terenius and his colleagues (personal communication) have found that low frequency transcutaneous electroacupuncture increases endorphin levels in human lumbar CSF but that high frequency EA has no effect on CSF endorphins suggesting that low frequency EA is a better technique for releasing spinal endorphins for segmental pain-relief. Application of opiates or endorphin on the spinal cord reduces the noxious output of lamina V neurons (Duggan et al, 1976) or produces a behaviorally defined analgesia in rabbits, rats, cats and pimates (Yaksh and Rudy, 1976; Yaksh and Rudy, 1977; Wong,

1977; Yaksh, 1978). Systemic injection of naloxone also antagonized the EA suppression of the noxious output of lamina V in cats (Cheng, M.Sc. thesis, 1977; Pomeranz and Cheng, 1979). Recently, Peets and Pomeranz demonstrated that microinjection of naloxone in the spinal cord abolished transcutaneous nerve stimulation analgesia in rats (personal communication).

(B) Regional : Regional analgesia may be due to the release of enkephalins in the midbrain. These enkephalins stimulate the raphe nucleus promoting descending inhibition to noxious input via the dorsolateral fasciculus to spinal regions. Mayer et al (1971) and Liesbeskind et al (1973) advocate the existence of regional mapping in the midbrain and that stimulation of certain midbrain areas will cause analgesia in only part of the body. Acupuncture may exert a similar regional-analgesic effect. For example, low frequency (4 Hz) EA may activate the periaqueductal gray (PAG) to release enkephalins (Pomeranz and Cheng, 1979; Cheng and Pomeranz, 1979b) which can act on the brainstem area (Watson et al, 1977). It has been demonstrated that the nucleus raphe magnus releases serotonin while the nucleus reticularis magnocellulis releases noradrenaline through the DLF (Basbaum and Fields, 1978). It should be noted in relation to EAA that the micro-injection of naloxone into PAG partly reversed EAA (Zhang et al, 1979), while lesioning of the DLF (Shen, 1975) and raphe nucleus (Shen,1976; McLennan et al,

1977) abolished EAA. Recent evidence suggests that microinjection of morphine into the PAG evokes the release of serotonin from the spinal cord (Yaksh and Tyce, 1979). Consequently, the inhibition of the spinal sensory system by the PAG opiate complex associated with antinociception appears to be mediated by the joint excitation of serotonin and noradrenaline terminals in the spinal cord via the DLF (Yaksh, 1978). In the PAG, the release of enkephalin may bind to opiate receptors on the neurons that inhibit the nucleus reticularis magnocellularis (NRM) or nucleus raphe magnus (NRM) (Basbaum and Fields, 1978). This enkephalin-opiate receptor binding inhibits the neuronal firing, promoting descending inhibitory systems.

At high frequency stimulation, EA probably stimulates the raphe neurons also producing a descending inhibition to the spinal cord through the dorsolateral fasciculus and this seems to be mediated by serotonin. Since high frequency EAA is not blocked by naloxone and is only partially reversed by depleting serotonin (Cheng and Pomeranz, 1979b), other descending systems such as the nucleus reticularis magnocellularis may also be stimulated. From other studies on analgesia, it is most probable that noradrenaline is involved in this system (Yaksh, 1979; Basbaum and Fields, 1978). Thus high frequency EA may directly stimulate descending inhibitory neurons by-passing the endorphin-link mapping in the PAG.

(C) General : EA was shown to exert a segmental and non-segmental effect. Chapman et al (1975) observed that non-segmental EA (Hoku on the hand) produced a 40% analgesia on tooth-pain, while segmental EA (cheek area) produced 185% analgesia. However, it was demonstrated by Chiang et al (1973) that the non-segmental EA stimulation on Hoku caused an increase in pain threshold over the entire body without significant differences among different body areas. This non-segmental EAA is quite different from the local segmental pain relief and may be mainly due to brain and/ or pituitary endorphin release. Therefore, in a parallel system, especially at low frequency electrical current, EA may stimulate the hypothalamus and pituitary to release beta-endorphin and ACTH (Cheng et al, 1979f), which may serve both pain-relieving and therapeutic functions. Pituitary endorphins can either be released into the blood stream or directly into the CSF. Evidence for pituitary-brain transport has been demonstrated by Mezey and colleagues (1978). The backflow of pituitary hormones to the hypothalamus may be partly vascular via the hypothalamic hypophysial portal system and to other brain areas via the CSF. Hypothalamic beta-endorphin neurons in the arcuate nucleus are also shown to project to the PAG, locus coerulus, reticular formation, medial amygdala, accumbens and the periventricular nucleus of thalamus (Barchas et al, 1978). It is still not clear,however, whether EA has a direct effect on stimulation of these long projecting beta-endorphin neurons of the

hypothalamus. If indeed, EA has a strong effect in releasing hypothalamic and pituitary endorphins, the analgesic effect will probably cover the whole body and be of long duration, since these high molecular weight endorphins can reach a wide variety of brain regions by passing through the blood-brain barrier or via the CSF in eliciting general analgesia. Recently, a novel endorphin, dynorphin, was found in the posterior pituitary gland (Goldstein et al, 1979) while beta endorphin was found in the anterior pituitary (Goldstein 1976). Further research is required to find out whether dynorphin is involved in EA or stress-induced analgesia.

GENERAL SUMMARY

This thesis is composed of ten chapters showing different approaches to investigate the mechanisms of EAA:-

(1) Chapter 1: Two different noxious responses were measured and compared in the same mice by shining a hot lamp on the heel . Two kinds of noxious responses were obtained: measuring the latencies for leg withdrawal and vocalization (squeak). In 58 mice, the average leg-withdrawal latency was 2.47±0.02 sec. and average squeak latency was 3.64±0.02 sec. These two responses showed a high correlation coefficient. Thus the vocalization method was used to study the pain threshold during electroacupuncture analgesia.

(2) Chapter 2: Dextro-naloxone, a recently synthesized stereoisomer with much less opiate receptor affinity than levo-naloxone, produces no reversal of electroacupuncture analgesia (EAA) in mice. Since levo-naloxone completely reverses EAA, this suggests that stereospecific opiate receptors are involved. Moreover it has been reported that there are two classes of opiate receptors: Type I and Type II. Type I opiate receptors may be responsible for opiate analgesia. In this experiment I show that antagonists of Type I receptors, levo-naloxone, naltrexone, cyclazocine and diprenorphine, all block electroacupuncture analgesia at low doses. All together, these results strongly support the hypothesis that electroacupuncture analgesia is

mediated by opiate receptors. Possibly Type I receptors are the major component of this system.

(3) Chapter 3: Dexamethasone, a cortisol analogue which inhibits ACTH and endorphin release in a negative feedback system, partially reduces EAA in mice. In addition, mice fed 2% saline for 3 days (this reduces pituitary endorphin levels) had a complete loss of EAA. These two experiments support the previous finding that hypophysectomy abolishes EAA. Altogether, these results implicate pituitary endorphins in EAA.

(4) Chapter 4: It was hypothesized that electroacupuncture releases beta-endorphin and ACTH from the pituitary. Since ACTH induces the release of cortisol from the adrenal glands, blood cortisol level should be enhanced by electroacupuncture. The present result shows that the blood cortisol levels of awake horses are significantly increased after 30 min. of electroacupuncture treatment while the sham acupuncture treatment (control) shows an insignificant effect.

(5) Chapter 5: This experiment shows different levels of EAA induced by three different frequencies of stimulation (i.e. 0.2, 4 and 200 Hz); highest analgesia is induced at 200 Hz and lowest at 0.2 Hz. Naloxone (1 mg/kg) completely reverses the EAA effects at low frequency stimulation (4 Hz) but produces no inhibition at high frequency stimulation (200 Hz). Conversely, parachlorophenylalanine (320 mg/kg),

a drug which suppresses serotonin levels partially blocks the high frequency (200 Hz) analgesia but produces no effect on the low-frequency (4 Hz) EAA. This suggests that EAA induced by low frequency stimulation may be mediated by endorphins while high frequency stimulation is not endorphinergic but may be partly due to serotonin. The serotonin mechanism is further explored in the next chapter.

(6) Chapter 6:

This experiment was carried out to study the effects of systemic injections of monoamine depletors, enhancers and receptor blockers on EAA in mice. The following results emerged:

(i) EAA is reduced by using non-specific depletors of monoamines (tetrabenazine, TBZ) or specific depletors (para-chlorophenylalanine, P-CPA for serotonin, alpha-methyl-paratyrosine, AMPT for catecholamines). Conversely, depletion of noradrenaline by disulfiram enhances EAA.

(ii) Replacement of depleted cerebral monoamines after TBZ by their precursors (5-hydroxytrytophan, 5HTP or L-DOPA) restores EAA.

(iii) EAA is enhanced by potentiating serotonin and dopamine by probenecid. It is also enhanced by the administration of precursors of L-DOPA(for dopamine) and of 5HTP (for serotonin). The specific dopamine receptor agonist, apomorphine reduces EAA.

(iv) EAA is also reduced by dopamine receptor blockade such as haloperidol, or specific blockade of noradrenaline (by yohimbine) or serotonin (by cinanserin). However, blockade of dopamine by pimozide has no significant effect on EAA.

These results were similar to SPA (stimulation-produced-analgesia by implanting electrodes in the brain) for most of the drugs tested. Consistent results were obtained only with manipulations of serotonin, this indicated that EAA (at 200 Hz) is mediated by serotonin. Since previous studies show that raphe or DLF (dorsolateral fasciculus) lesions abolished EAA, it is postulated that the descending axons from the raphe nucleus inhibit spinal cord nociception during EAA by releasing serotonin. Recent biochemical studies on EAA also support this postulate.

(7) Chapter 7: It has been hypothesized that D-phenylalanine and D-leucine produce analgesia by protecting endorphins from enzymatic degradation. Thus the idea that D-amino acids may produce analgesia via the endorphin system is tested by examining the effect of D-leucine and D-phenylalanine on mice with congenitally abnormal endorphin systems. In three strains of mice D-amino acid analgesia ranks in the order of ob/ob>B6AF1/J>CXBK. This correlates with the endorphin abnormalities in these mice: Obesity mice (ob/ob) are high in pituitary beta-endorphin and CXBK low in opiate receptors. This result, along with the nalox-

one reversibility of the analgesia, supports the involvement of endorphins in DAA analgesia.

(8) Chapter 8: The D-amino acids (DAA), D-phenylalanine and D-leucine, produce naloxone reversible analgesia; electroacupuncture (EA) also produces analgesia which is blocked by naloxone. Combining the two treatments produces an additive effect with a larger analgesia than that produced by either treatment given alone; this combined effect is also blocked by naloxone. Moreover only 62% of the mice show EA analgesia and 53% show DAA analgesia; 80% of the animals show marked analgesia with both EA plus DAA treatment. Perhaps the combination of EA with DAA will provide a potent method for the treatment of clinical pain.

(9) Chapter 9: EAA is enhanced by two treatments given three hours apart. In addition, 15/15(100%) of the mice showed significant EAA after the second EA treatment, while only 8/15 (53%) of the mice showed significant EAA when they were treated with sham and normal EA treatments at three hours apart.

(10) Chapter 10: Mice were addicted to opiate using morphine pellets (75 mg morphine base per pellet) implanted subcutaneously; the pellets were then surgically removed after 3 or 8 days. During morphine abstinence (7 h after pellet removal), the mice were treated with electroacupuncture. The results indicate that EA analgesia shows no cross-tolerance to morphine. Additionally, EA reduced

withdrawal behaviour (jumping) in 50% of the mice during morphine abstinence.

In conclusion, an intricate system is proposed for the mechanisms of electroacupuncture analgesia (refer to "GENERAL DISCUSSION").

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APPENDIX (A):

TABLE 1 - Comparing three control squeak latencies at three minutes apart before EA.

	Time (min)	3'	6'	9'
N(1)	latency (sec)	4.25±0.05	4.11±0.06	4.51±0.05
=15	% change		-2.85±1.02	7.18±1.00
N(2)	latency (sec)	4.33±0.03	4.61±0.04	4.53±0.04
=15	% change		7.34±1.09	5.23±1.04
N(3)	latency (sec)	4.51±0.04	4.60±0.04	4.56±0.04
=15	% change		3.13±1.10	2.22±0.9
N(4)	Latency (sec)	4.03±0.04	4.06±0.04	4.06±0.04
=15	% change		1.48±1.05	1.96±1.17
N(5)	latency (sec)	4.20±0.05	4.09±0.05	4.32±0.05
=15	% change		-2.17±0.92	4.10±1.06
N(6)	latency (sec)	4.34±0.02	4.27±0.03	4.46±0.03
=55	% change		-0.06±0.79	4.22±0.62
N(7)	latency (sec)	4.26±0.01	4.40±0.01	4.41±0.01
=55	% change		4.71±0.32	4.91±0.03

N(8)	latency (sec)	4.30±0.006	4.33±0.007	4.44±0.007
=109	% change		2.30±0.20	4.56±0.16

Note: ANOVA among 3, 6, and 9 minutes; $p>>0.05$
t-test two tailed; $p>>0.05$ which indicate that there no significant difference among these control latencies.

APPENDX (B):

LIST OF ABBREVIATIONS

ACTH	=	corticotropin
ALT	=	anterolateral tract
AMPT	=	alpha-methyl-para-tyrosine
CSF	=	cerebrospinal fluid
DAA	=	D-amino acids (D-phenylalanine and D-leucine)
DLF	=	dorsolateral fasciculus
EA	=	electroacupuncture
EAA	=	electroacupuncture analgesia
EEG	=	electroencephalogram
FSH	=	follicle stimulating hormone
5-HTP	=	5-hydroxytryptophan
Hz	=	Herz
L-DOPA	=	L-3,4-dihydroxyphenylalanine
LH	=	luteinizing hormone
LPH	=	lipotropin
MLC	=	morphine-like compound
MSH	=	melanocyte stimulating hormone
NRM	=	nucleus raphe magnus
PAD	=	primary afferent depolarization
PAG	=	periaqueductal gray
PAH	=	primary afferent hyperpolarization
PCPA	=	parachlorophenylalanine
RIA	=	radioimmunoassay
RN	=	raphae nucleus

RRA = radio-receptor assay

SPA = stimulation-produced analgesia

TBZ = tetrabenazine

Acupuncture points:

B13 : 6 cm from the popliteal fossa

B18 : in the middle of the gluteal sulcus

B47 : 4 cm beside the lower end of the spinous process of the first lumbar vertebra.

B65 : On the natural line of the hair, right above the medial end of the eyebrow.

G29 : middle of the depression above the suprasternal notch.

GB26 : on the same level as the umbilicus, right on the mid-axillary line.

GB30 : on the postero-superior side of the greater trochanter.

Ki 1 : one-third the distance from the center to the front of the planta, in the depression which is present when the foot is raised.

Ki 2 : in the depression on the inferior border of the tuberosity of the navicular bone.

LI 4 : first interosseus muscle.

PC 4 : three cm lateral to the nipple, over the 4th intercostal space.

(C) GENERAL HISTORY OF ACUPUNCTURE IN CHINESE MEDICINE

The ancient art of treating diseases and relieving pain by acupuncture is an integral part of Chinese medicine. Although there is no longer a record of its initial discovery, some famous legends concerning acupuncture have persisited for about 5,000 years. According to one story, acupuncture was first discovered by a group of warriors after being wounded by an arrow they sometimes noted a sensation and "miraculously" recovered from ailments by which they had been palqued for many years (The Academy of Traditional Chinese Medicine, 1975). Another story tells of a wise man who accidently struck his lower leg against a sharp stone and relieved the pain in certain parts of his body. Accordingly, the first needles were made of sharp pieces of stones called "pien", and this work eventually came to refer to the curring of diseases by pricking with a stone (Shuo Wen Ji Zi Analytical Dictionary of Characterers, compiled during Han Dynasty 206 BC - A.D. 200). Later Needles were made from bamboo, copper, iron, gold, silver and finally stainless steel. The steel needles frequently used at present vary from 1 to 20 cm in length and from 26 to 32 gauge in thickness (0.45 mm to 0.26 mm in dia.).

The earliest record of a successful cure by means of acupuncture is described in a book called Shik Chi (Historical Records) written 2,000 years ago. The book states that

PienChueh, a famous physician of Warring States Period (475-221 BC) used acupuncture to revive a dying patient already in coma. However, the classical method of acupuncture is first described in Ling Shu, a special chapter in Wang Di Nei Ching (The Yellow Emperors's Book of Internal Medicine) in which treatment of sickness, meridians and points, alleviation of pains in the head, ear, tooth, back, stomach, abdomen and the joints ar discussed in detail and embellished with Chinese medical theories.

In the Tsin Dynasty (A.D. 265-420) acupuncture was in popular usage and a more comprehensible and systematic text-book named the "Chen Chiu Chia Yi Ching (An Introduction to Acupuncture and Moxibustion) was published. This book listed 649 acupuncture points, maped out 349 basic points on the human body and clearly reviewed the theory of acupuncture and the needling techniques.

In the Tang Dynasty (618-907) acupuncture was taught in the Imperial Medical College organized by the Chinese Government, and the method of needling spread to Korea, Japan and India.

In the Ming Dynasty (1364-1644), extensive records of practical knowledge were accumulated and summarized in a book known as the "Chen Chiu Ta Cheng" (compendium of acupuncture and moxibustion) which is still in common use today.

During the Ching Dynasty (1644-1911) and Nationalist Chinese rule (1911-1949), the practice of acupuncture was suppressed, and authorities banned the art. China was at this time being exposed to the vast body of Western science and medicine. Yet the practice of acupuncture and other traditional healing arts still persisted among the common people. Often knowledge was kept secret within families and passed along from father to son. After the founding of the People's Republic of China, an attempt was made to combine the ancient traditional healing arts with the relatively newly acquired Western technology.

There were many new developements during this last time period, such as the use of electronic instruments and acupuncture anesthesia starting in 1958. Since then, more than 400,000 major and minor surgical operations (Small T.J., 1974) have been performed on both adults and children by means of acupuncture anesthesia.

Acupuncture was first introduced in Europe by a German, Dr. E. Kampfer in 1683. But the significant evaluation of this subject was done by George Soulie' de Morant who wrote: L' Acupuncture en Chine et la Reflextherapie Moderne' and Les Arguilles et les Moxas en Chine in 1863. Acupuncture was gradually accepted by some countries in Europe; notably France, Germany and Austria. Only very recently (after President Richard Nixon visited China in 1970), was acupuncture or specifically North America. A surge of

interest and scientific investigations in the ancient oriental art began to emerge.

(D) TRADITIONAL THEORY OF ACUPUNCTURE

The classical theories of Chinese medicine are integrated with the ancient philosophy called is composed of a cosmic field of force in which two basic elements, Yin and Ying, are perpetual complements in continuous change. Yang, the positive, corresponds to such things as sun, day, heat, light, dryness, male and life. Ying, the negative, corresponds to the moon, night cold, darkness, water, female, death and many others. Yin and Yang are said to be dynamically opposed, yet are harmonizing energies in the universe. Accordingly, these two elements are considered to comprise the balance of man's 'life energy' called "chi", and the human body is treated as a small universe system. The chi circulates continuously throughout the body along invisible pathways—known as meridians, each of which originates in one of the principal internal organs and then surfaces to run along the outside of the body, sometimes as close as a millimeter or two beneath the skin. The meridians are able to transmit signals of internal illness in any major organ to the outside, and can also transmit stimuli back to internal organs from the body surface.

In all, there are seventy-one meridians in the human body and they are classified into channels (Ching) and collaterals (Luo'). Channels are the main pathways running lengthwise and are made up of twelve main channels and Eigh

Extra-Channels, while the collaterals are divisws into major collaterals and the sub-collaterals which connect one cannel to another. Hence the entire 'Chingluo' system is distributed over the whole body and connects the viscera with the four extremities, skin and the sense organs, making the body an organic whole.

The twelve Channels are (An outline of Chinese Acupuncture, 1975):-

1. The Lung Channel of Hand-Taiyin (Lu).
2. The large Intestine Channel of Hand-Yangming (L.I.).
3. The stomach Channel of Foot-Yangming (St.).
4. The Spleen Channel of Foot Taiying (Sp.).
5. The Heart Channel of Hand-Shaoyin (H.).
6. The small intestine Channel of Hand-Taiyang (S.I.).
7. The Urinary Bladder Channel of Foot-Taiyang (U.B.).
8. The Kidney Channel of Foot-Shaoyin (ki).
9. The Pericardium Channel of Hand Jueyin (P.).
10. The Triple Warmer of Hand-shaoyang (T.W.).
11. The Gall Bladder Channel of Foot-Shaoyang (G.B.).
12. The Liver Channel of Foot-Jueyin (Liv.).

There are also two important meridians running along the midline of the body:-

(1) The Governing vessel (Go) runs along the back of the body midline.

(2) The conception vessel (Co) runs along the front of the body midline.

Acupuncture points lie along all these meridians and can be used to affect the internal organs of the body for treating illnesses. Traditional theory states that sickness arises when there is too much Yang (over tonification) or too much Yin (over sedation). Therefor the two forces are not in balance and the chi does not circulate smoothly throughout the meridians. Acupuncture will either tonify or sedate the body to restore the smooth flow of this 'life energy', hence balancing the Yang and Yin forces and eliminating the illness.

It should be borne in mind that this is not a 'scientific theory' but rather a methodology based on an ancient philosophical system. However, it reveals the profound difference between the systems of thoughts in the East and the West.

LES MECANISMES DE L'ANALGESIE DE L'ELECTROACUPONCTURE DANS LEUR RELATION AVEC LES ENDORPHINES ET LES MONOAMINES; UN SYSTEME COMPLIQUE EST SUGGERE

Abstrait d'une thèse soumise en conformité avec les règlements pour le diplôme de Docteur en Philosophie à l'Université de Toronto

RICHARD SHING SOU CHENG, 1980

Il a été suggéré (Cheng, thèse de M. Sc., 1977) que l'analgésie de l'électroacuponcture (AEA) peut être transmise par des endorphines, les peptides endogènes qui ressemblent à la morphine. Selon cette hypothèse, l'électroacuponcture (EA) peut stimuler la substance grise du périaqueduc (GPA du cerveau moyen) à décharger des enképhalines qui activeront le système inhibitif descendant du raphe-FDL (faisceau dorsolatéral) pour bloquer les signaux de douleur (nociception) au niveau du cordon médulaire. Parallèlement, l'EA peut stimuler la glande pituitaire à décharger des endorphines pour produire l'analgésie.

Afin d'explorer cette hypothèse plus profondement, plusieurs études ont été faites sur les mécanismes de l'AEA dans leur relation avec les endorphines et les monoamines (serotonine, dopamine et norepinephrine). Les expériences d'EA furent faites sur des souris B6AF1/J qui furent placées dans des réceptacles en papier. Les réactions nocives à la chaleur rayonnante furent mesurées par les intervalles entre couinements. L'EA fut appliquée en insérant des aiguilles d'acier

inoxidable au point d'acuponcture, HoKu (les prémiers muscles dorsaux interosseux). Sans savoir laquelle était utilisée, les drogues furent injectées dans le péritoine. Les résultats suggèrent que l'AEA est transmise par des récepteurs stéréospécifiques opiacés; la composante principale étant peut-être les récepteurs opiacés de type I (ceux-ci sont situés principalement dans les régions analgésiques du système nerveux central et sont peut-être responsables de l'analgésie opiacée). L'évidence démontre aussi que l'AEA peut induire des béta-endorphines et de l'ACTH qui sont habituellement déchargées ensemble. La dexamethasone, un dérivé de la cortisone, a supprimé l'AEA, probablement par une réaction inhibitive négative de la décharge pituitaire. Une solution saline à 2%, qui diminue les endorphines pituitaires, réduit aussi l'AEA. L'ACTH déchargée par l'EA est cause de niveaux élevés de cortisol et une étude démontre que l'EA augmente les niveaux de cortisol du sang chez les chevaux. Plusieurs expériences (y compris une pour cette thèse) suggèrent que les amino-acides-D (AAD), la phenylalanine-D et la leucine-D produisent l'analgésie en protégeant les endorphines de la dégradation enzymatique. Les traitements combinés avec l'EA et les AAD ont produit une analgésie supérieure chez les souris que l'un des deux traitements seul. Ceci suggère que l'EA peut décharger des endorphines qui sont protégées par les AAD et ainsi l'EA et les AAD ensemble produisent une analgésie supérieure. Cette étude montre aussi que l'AEA agit chex les souris toleérant la morphine et que l'EA réduit les symptômes d'abstinence chez la souris morphinomane. Uneautre expérience démontra que l'AEA à basse fréquence (4 Hz) peut être transmise par les endorphines tandis que l'AEA à haute fréquence (200 Hz) peut être transmise par la serotonine. D'autres expériences de l'EA montrent que

les drogues qui modifient les niveaux de dopamine et de norepinephrine ne donnent pas de résultats cohérents tandis que celles qui modifient les niveaux de serotonine sont très régulières.

Ajoutant les résultats de cette thèse et ceux d'autre ouvrages, je suggère un système compliqué des mécanismes de l'AEA. L'EA à 4 Hz stimule peut-être les nerfs sensoriels afférents à plusieurs régions du cerveau. Dans la GPA du cerveau moyen, elle décharge des enképhalines qui activent les noyaux du raphe à envoyer des inhibitions descendantes le long du FDL au cordon médulaire afin de bloquer les signaux de douleur qui arrivent. Le système inhibitoire descendant du FDL est peut-être transmis en partie par le neurotransmetteur, la serotonine. Parallèlement, l'EA (4 Hz) peut stimuler les neurons de la béta-endorphine dans l'hypothalamus et la glande pituitaire. Les endorphines pituitaires peuvent être déchargées dans la circulation sanguine, ou peuvent recouler directement dans le FSC (les endorphines dans la circulation doivent passer par la barrière sang-cerveau pour être liées aux récepteurs opiacés du cerveau pour l'analgésie). Si les points d'acuponcture et les régions douloureuses sont dans les mêmes niveaux segmentaires, l'EA (4 Hz) peut aussi stimuler directement la décharge des endorphines dans le cordon médulaire. L'AEA à haute fréquence (200 Hz) peut activer les nerfs sensoriels qui stimulent directement les systèmes inhibitoires de serotonine descendant du FDL, évitant ainsi les systèmes d'endorphines de la GPA.

Pour résumer, l'EA à basse fréquence (4 Hz) peut stimuler la décharge des endorphines dans le cordon médulaire, le cerveau moyen, l'hypothalamus et la glande pituitaire pour apaiser la douleur, tandis

que l'EA à haute fréquence (200 Hz) peut stimuler directement les systèmes inhibitoires de serotonine du FDL, évitant ainsi les liens d'endorphine.