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PITUITARY FUNCTIONAL IMAGE PATTERN ASSOCIATED WITH THE MECHANISM OF THE TSU-SAN-LI ELECTROACUPUNCTURE PRODUCED ANALGESIA IN RATS: A ^{14}C-2DG STUDY

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The multiple factors contributing to the output pain experience and behavior include the activity of the body's stress-regulation systems, including cytokines as well as the endocrine, autonomic, immune and opioid systems. In a previous report we found that 2 Hz electro- acupuncture-analgesia (EAA) activated glucose utilization of brain structures involved with: 1) multiple endogenous analgesia systems; 2) anti-inflammatory response and blood-pressure regulating properties (anterior and posterior pituitary); and 3) affective responses (limbic system) associated with pain and analgesia. The Pituitary gland is a critical organ of the neuroendocrine system and is essential for the maintenance of homeostasis, metabolism, reproduction, growth, and lactation. In order to know the role of the pituitary in the 2 Hz EAA, we evaluated the metabolic changes and patterns of the pituitary as related to the thermal tail-flick pain stimulation and the EAA. Dexamethasone was used to inhibit the ACTH release from the pituitary by a negative feedback mechanism. As a result, we demonstrated that 2 Hz EA at T-S-L acupoint induced analgesic effect which was verified by a 25 % elongation of the mean tail-flick latency (MTLF) and that dexamethasone blocked this EAA effect with only a 1 % MTLF elongation. Since ACTH and β-endorphin are co-released from the anterior pituitary lobe corticotrophs, the blockade of EAA by dexamethasone may indicate the involvement of β-endorphin. In addition, we found that in the "Pain+EA" group, the pituitary labeling pattern showed an unusual pattern with the greatest amount of labeling present in the intermediate lobe, followed by lesser but equal amounts in the posterior and anterior lobes, i.e., INP > POP = ANP, which was in contrast to the pattern in the control group of "POP > ANP > INP". In both the "Pain" and "Dexa+Pain+EA" groups, the pituitary labeling showed the same trend in LCGU values as that of the control group, i.e., POP > ANP > INP. This difference in labeling patterns of the posterior lobe appeared to be correlated with the EAA effect.

Key words: Pituitary, Pain, Tail-Flick Latency, Electroacupuncture Analgesia, LCGU.

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INTRODUCTION

"Pain has been a major concern of humankind since its beginning and the subject of ubiquitous efforts to understand and control it" cites Bonica[1,2]. Pain experience, including the sensation component that refers to the qualitative sensory experiences elicited by the stimulus and the reaction component that refers to the affective, emotional responses (such as suffering, anguish, and most importantly, **a private experience of hurting**), is subject to modulation by a variety of environmental factors, such as suggestion, stress, etc[3,4]. Although symptomatic pain has the important biologic function of indicating to the patient that something is wrong and often prompts him or her to seek medical counseling, certain types of acute pain and all forms of chronic pain are deleterious to the organism and also can disrupt the homeostatic regulation system of the brain. In 1999, Melzack proposed a new concept of the pain pathways termed the "neuromatrix theory of pain", which stated that "The brain possesses multiple inputs (neuromatrix) that act together to produce the pain feelings". Factors contributing to the output pain experience and behavior include the activity of the body's stress-regulation systems, which include cytokines as well as the endocrine, autonomic, immune and opioid systems[5]. The perception of pain can activate the stress response system called the "hypothalamic-pituitary-adrenal (HPA) axis" and stimulate the hypothalamic nuclei to release corticotrophin-releasing hormone (CRF), which in turn activate the pituitary to release adrenocorticotropic hormone (ACTH) and other substances such as β-endorphin[6,7]. The released ACTH in turn is transported via blood vessels to the adrenal cortex to release cortisol. Recently, the role of CRF as a new class of analgesics in chronic pain syndrome associated with the HPA-axis abnormalities has been recognized and is likely to attract further studies[7].

Acupuncture has been used as a medical modality of traditional Chinese medicine (TCM) for over 3000 years in China. Qi is a key concept in TCM and is postulated to flow through the body in precisely located pathways or channels called meridians. An imbalance of energy flow within these meridians will eventually cause illnesses. Acupuncture at particular points along meridians related to particular organ systems can restore the proper energy balance within the body and therefore restore good health[8]. In late 1997, The American National Institutes of Health Acupuncture Concensus Development Panel (NIHCDP) has offered a report of the evaluation of the efficacy of acupuncture for 14 medical conditions and concluded that acupuncture is effective for 2 conditions and may be useful for 12 others. In 2000, Mayer concluded in a review article that acupuncture was effective for the treatment of postoperative and chemotherapy-induced nausea and vomiting and that acupuncture may be useful for headache, low back pain, alcohol dependence, and paralysis resulting from stroke[8]. The findings of acupuncture studies prompted Han (2003) to propose a concept of "Neuropeptide mobility induced by a specific frequency of acupuncture" and states that specific neuropeptides can be mobilized by specific frequencies of acupuncture or electrical stimulation applied to certain body surface, such as acupoint[9]. In this review article, he suggested that endomorphins, enkephalins, and β-endorphin could possibly be released by low frequency (2 Hz) peripheral electroacupuncture (EA), which could be activated via the mu- and delta- opioid receptors. In contrast, dynorphin can only be released by high frequency

(100 Hz) peripheral EA, which could be activated via the kappa-opioid receptor[9]. In a previous report we found that the 2 Hz electroacupuncture analgesia (EAA) activated glucose utilization of the brain structures involved with: 1) endogenous analgesia systems; 2) anti-inflammatory response and blood-pressure regulating properties (anterior and posterior pituitary); and 3) affective responses (limbic system) associated with pain and analgesia[10].

The hypothalamo-hypophysial-adrenal axis has provided important clues for the studies of pain, stress, and analgesia. The hypothalamus forms the lowest part and the floor of the diencephalon and contains three regions: the anterior, the tuberal, and the posterior regions. The anterior region contains preoptic, supraoptic, and paraventricular nuclei. The tuberal region contains ventromedial, dorsomedial, and arcuate nuclei. The posterior region contains posterior and mamillary nuclei.

The hypothalamic centers influence all processes important for the maintenance of homeostasis and regulate the vital functions of sleep and waking rhythm, body temperature, water and thirst, hunger and satiety, electrolytic balance, cardiac function, stress, and sexual drive. Unmyelinated fiber bundles, which originate from the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus, project via the internal layer of the median eminence into the posterior lobe of the hypophysis (the neurohypophysis) and end on capillaries[11]. Electrical stimulation of these two nuclei cause increased secretion of the vasopressin and oxytocin. In addtion, unmyelinated fiber bundles, which originate from the ventromedial, dorsomedial, and arcuate nuclei of the tuberoinfundibular region, enter into the special vessels and capillary network of the adenohypophysis to control the release of the hormone of the adenohypophysis. The pituitary gland is a master organ of the neuroendocrine system and is essential for the maintenance of homeostasis, metabolism, reproduction, growth, and lactation. In addition, during mammalian development, the pituitary gland regulates organogenesis[12]. The pituitary consists of three lobes: 1) the anterior lobe, which is formed from an evagination of the primitive roof of the foregut known as Rathke's pouch and is an endocrine gland; 2) the posterior lobe, which is an evagination from the floor of the diencephalons and is part of the brain; and 3) the intermediate lobe. The anterior lobe of the pituitary gland contains six types of endocrine cells: they are the corticotropes, melanotropes, somatotropes, lactotropes, thyrotropes, and the gonadotropes. The synthesis and secretion of the trophic hormones from these distinct cell types is controlled by the neuropeptide releasing factors from the hypothalamus and by negative feedback loops from the peripheral organs.

The mammalian brain is a complex heterogeneous organ comprising many structural and functional components with differently and independently regulated levels of functional activity and energy metabolism. The majority of the glucose taken up by the brain is used for the maintenance of the membrane potentials and the electrical activities. The metabolic trafficking of an astrocyte-neuron lactate shuttle between astrocytes and neurons accounts for the ^{18}F- 2-deoxyglucose (DG)- PET as well as for the ^{14}C-2DG methods. Since neuronally-released glutamate triggers the cascade of events that leads to glucose uptake, the ^{18}FDG- PET as well as the ^{14}C-2DG signals will faithfully reflect activation of neuronal circuits[13]. Regional glucose utilization in the brain could thus be shown to be closely linked to

the functional activities[13,14]. The 2-DG method, which assesses rates of local cerebral glucose utilization (LCGU) can be used to determine the localization of functional action of a specific stimulation in the mammalian brain[14]. It is based on the use of [^{14}C]-2-DG in a tracer amount for the exchange as a glucose analogue between plasma and brain and its phosphorylation by hexokinase in the tissue. The [^{14}C]-2-DG is used because its product, [^{14}C]-2-DG-6-phosphate, is essentially trapped in the tissue and undergoes no further steps in the glycolytic pathway. This provides the basis for measurable parameters for the estimation of LCGU. This method gives evidence about the sites of stimulation in the brain, including not only areas directly affected, but also areas correlated with the stimulus extended functional effects.

In this study, we applied the 2-DG method to evaluate the metabolic changes of the structures of the hypothalamic-pituitary axis and patterns of the pituitary related to the thermal tail-flick pain stimulation and the EAA so that we can define the role of the pituitary in the 2 Hz EAA. Dexamethasone was used to inhibit ACTH and β-endorphin release from the pituitary in a negative feedback mechanism.

MATERIALS AND METHODS

A. Animals and Experimental design:

Adult male Sprague-Dawley rats weighting 250-350 gm were provided by the Animal Center of the Yang-Ming University. They were housed 3 per cage on a natural light-dark cycle with food-pellets and water available ad libitum. Six experimental groups were designed: 1) Control group (N = 4), which received non-noxious thermal (40 °C water) stimulation of the distal tail; 2) Pain group (N = 6), which received noxious thermal (56 °C) stimulation of the distal tail; 3) Pain + EA group (N = 6), which received both noxious thermal stimulation of the distal tail and EA stimulation of the proximal anterior tibial muscle (Tsu-San-Li acupoint); 4) Dexa+EA+Pain group (N = 6), which received dexamethasone IP pretreatment combined with both noxious thermal stimulation of the distal tail and EA stimulation of the proximal anterior tibial muscle (Tsu-San-Li acupoint); 5) EA group (N = 5), which received only EA stimulation at the Tsu-San-Li point; 6) EA-non-point group: which received only EA stimulation at the point outside the meridians. In the Dexa+EA+Pain group, we applied dexamethasone (0.6 mg/kg, i.m.), a potent cortisol analogue[15], to inhibit ACTH and beta-endorphin release from the pituitary via a negative feedback mechanism. Dexamethasone (9α-fluoro-16α-methylprednisolone, Sigma) was dissoloved in sesame oil and was injected intramuscularly in double doses [with the first dose (0.4 mg/kg) at 24 hours and the second dose (0.2 mg/kg) at 1 hour, respectively] prior to the 2DG injection.

B. Tail-flick Test

A modification of the D'Amour and Smith tail-flick test[16] was used as a behavioral test for determining: 1) that the animal detected the noxious thermal stimulation applied to the tail and 2) the efficacy of the EA produced analgesia.

Briefly, a servo-controlled water bath was used in which the water temperature was maintained at 56 °C. The tip of the rat's tail (a 3-cm segment was marked with ink) was immersed in the 56 °C water for each tail-flick test. Five tail-flick trials were conducted prior to EA treatment with a 30-sec interval between each trial to permit the tail to cool to prevent tissue damage.In this manner, a baseline tail-flick latency (TFL) was determined as the mean latency of the five trials. In order to avoid damage to the tail tissue in the Pain+EA group, the tail was removed from the 56 °C water by 7 seconds if a tail-flick response had not occurred by that time[10,16,17].

C. Electro-acupuncture (EA) and Behavioral Assessment for the Efficacy of Ea Produced Analgesia (EAA) and Good Responder Chosen:

Two sterilized stainless steel acupuncture needles (5 cm long, 0.2 mm diameter) were inserted into the anterior tibial muscle of the right hind leg of the rat. One needle was inserted into the proximal one-third of the anterior tibial muscle, a position equivalent to the Tsu-San-Li (ST-36) acupoint (cathode) and the other needle (anode) was inserted into the anterior tibial muscle at a point 1.5 cm distal to the first one. Anodal and cathodal leads from a battery operated Taiwanese acupuncture stimulator were connected to the two acupuncture needles. The Tsu-San-Li point was palpated according to acupuncture books[18,19]. This point has been used in clinical practice for the relief of tooth[20] as well as for relief of stomach pain[21]. In addition, we chose the Tsu-San-Li point because it is known to produce an increased tail-flick latency (analgesic effect) in response to noxious thermal stimulation of the tail[21]. EA was then started at 2 Hz and a stimulus strength, which produced twitching of the muscles of the right hind leg only, was utilized. The efficacy of the EA treatment was indicated as a "percentage of the elongated mean tail-flick latency after EA", that is to say, using the "mean tail flick latency (MTFL) during EA" minus the "baseline or the pre-EA MTFL" and then divided by the "baseline or the pre-EA MTFL". In order to pick up the real brain local glucose utilization change associated with EAA, good responders were selected based on the degrees of the effectiveness of EAA, i.e., an at least 25 % elongation of the mean-tail-flick response latency (MTFL). The good responders were assigned into the EA+Pain group or the EA group for the 2DG experiment.

D. Local Cerebral Glucose Utilization

The 2DG study was conducted according to previously published procedures[22]. Briefly, catheters were placed in one femoral artery and vein under 1 % halothane anesthesia. Rats were lightly restrained on wooden blocks. The animals were allowed at least 3 h to recover from the effect of anesthesia, then a dose of 100 uCi/kg of 2-[^{14}C]deoxyglucose (New England Nuclear; specific activity = 58.0 Ci/mmol) was injected through the venous catheter. Sixteen timed arterial blood samples were collected over the experimental period for the plasma [^{14}C] deoxyglucose counting and glucose analysis at 0.0, 0.25, 0.5, 0.75, 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 40 and 45 min. Forty-five minutes after the administration of the [^{14}C] deoxyglucose tracer, animals were sacrificed by an intravenous overdose of

sodium pentobarbital. Brains were rapidly removed, and frozen in isopentane (-50 °C). The brains were then coated with embedding medium and stored in a freezer at -70 °C and were sliced later on a coronal plane at -15 to -20 °C in a cryostat set for 20 μm sections. Every third section was placed on a glass slide cover, dried on a standard slide-warming tray at 65 °C and then placed against Kodak SB-5 X-ray films along with a set of [^{14}C] methylmethacrylate standards (Amersham, [^{14}C] Micro-Scales RPA 504L) previously calibrated for their equivalent ^{14}C concentration in 20 μm brain sections. Slides containing tissue sections from the experimental groups were randomized and placed in cassettes with calibrated Amersham standards [^{14}C] micro-scales. The Kodak SB-5 film was placed upon the brain sections in each prepared cassette and the cassette was closed. The resulting autoradiographs were analyzed using quantitative densitometry with a computerized-image processing system (MCID, BRS2). Tissue tracer concentrations were determined by densitometry of the autoradiograms with reference to the actual polymer activity value provided by the calibrated standards of Amersham, [^{14}C] micro-scales RPA 504L. Rates of local cerebral glucose utilization were then calculated from the local tissue [^{14}C]-concentrations, the time course of the plasma [^{14}C] deoxyglucose and glucose concentrations and the lumped constants derived using the operational equation of Sokoloff et al.[14]

E. Histological and Autoradiographic Procedures:

Perfusion, fixation and storage:

At the end of the 45 min. 2DG experiment, the animals were sacrificed with 1 mL of pentobarbital sodium (65 mg/mL; regardless of body weight) and immediately perfused intracardially with 3.3 % formalin, phosphate buffered to a pH of 7.4[23,24] for approximately one minute. Immediately following the perfusion, the brain and pituitary were removed and rapidly frozen by lowering into a Dewar flask filled with some liquid freon XXII (-50 °C). Brain sections were cut in 20 μm thickness in a coronal plane and every third section was picked up on a No.2 coverslip and placed, tissue side up, on a standard slide-warming tray at 65 °C for approximately 2 to 15 minutes for drying the section. Each coverslip with its attached dehydrated section was then glued (Duco cement; Dupont Co.) onto a 10 in. × 12 in. four-ply posterboard in serial order. The posterboard with the attached sections and a set of (C^{14})-methyl-methacrylate standards (New England Nuclear Corporation, Boston, Massachusetts) was placed in a standard 10 × 12 in. X-ray cassette in a darkroom under a safe light, and a sheet of Kodak SB-5 special blue-sensitive medical X-ray film was placed with its emulsion side (gray, non-shiny side) down, in direct contact with the dehydrated 2DG-labeled brain sections and the methyl-methacrylate standards. The cassette was then closed and stored in a cool, dry and dark place. The X-ray film was thus exposed to the sections for a period of 10 days. Following the appropriate exposure time, the X-ray films were developed and fixed according to the instructions supplied with the SB-5 film. Brain sections that produced the selected autoradiographic images were then stained with a cell body stain (thionin) suitable for 2DG sections[24].

F. Data analyses:

1. Glucose analysis:

Blood glucose values were determined using a Beckman glucose analyzer.

2. C^{14} liquid scintillation counting:

The precise amount of nCi of C^{14} per plasma sample was determined from scintillation counting of each of the 16 samples using a Tri-carb liquid scintillation counter (model 4530, Hewlett PACKARD, United Technologies). The [^{14}C] in local brain tissue is determined from optical densitometric readings of the autoradiographic image of the specific nucleus or brain structure using the methyl-methacrylate standards of known ^{14}C concentration.

3. LCGU analysis

The 2DG autoradiograms were analyzed using MCID Image Analysis System at the Instrument Center of the National Yang-Ming University. Rates of local cerebral glucose utilization were measured in 12 brain areas.

4. Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) and t-test for each structure.

RESULTS

In these studies, we demonstrated that the 2 Hz Tsu-San-Li EA effectively produce analgesia by the finding of the increasing of the average mean tail-flick latency (MTFL) from 2.86 ± 0.18 sec (before EA) to 3.57 ± 0.20 sec (30 min. after EA). Note the 25 % increase of the average MTFLs following the EA indicated that the effect of EA-produced analgesia (EAA). In addition, the dexamethasone treatment effectively reduced the EAA effect down to only a 1 % increasing of the MTFLs. In the following sections, we will describe the findings in different brain structures.

A. Changing of the Lcgu of the Telencephalic Sttructures Related to Pain and EA (Fig. 1)

Anerior Cingulate Gyrus (ACG): As compared with the Control group, a 18 % increase in the Pain group; 29 % increase in the Pain+EA group; and a 20 % increase in the Dexa+Pain+EA group were found.

Somatosensory Cortex I (SSI): As compared with the Control group, a 19 % increase in the Pain group; 32 % increase in the Pain+EA group; and a 18 % increase in the Dexa+Pain+EA group were found.

Medial Septum (MS): As compared with the Control group, a 22 % increase in the Pain group; 33 % increase in the Pain+EA group (significant, t-test, $p < 0.05$); and a 20 % increase in the Dexa+Pain+EA group were found.

Nucleus Accumbens (NAC): As compared with the Control group, a 10 % increase in the Pain group; 21 % increase in the Pain+EA group; and a 8 % increase in the Dexa+Pain+EA group were found.

The LCGU percentage changes of the telencephalic structures in each group as compared to the control group are indicated as below:

Telemcephalic structures \ Groups	Pain Group	Pain+EA Group	Dexa+Pain+EA Group
Anterior Cigulate Gyurs	18 %↑	29 %↑	20 %↑
Somatosensory I	19 %↑	32 %↑	18 %↑
Medial Septum	22 %↑	33 %↑*	20 %↑
Nucleus Accumbens	10 %↑	21 %↑	8 %↑

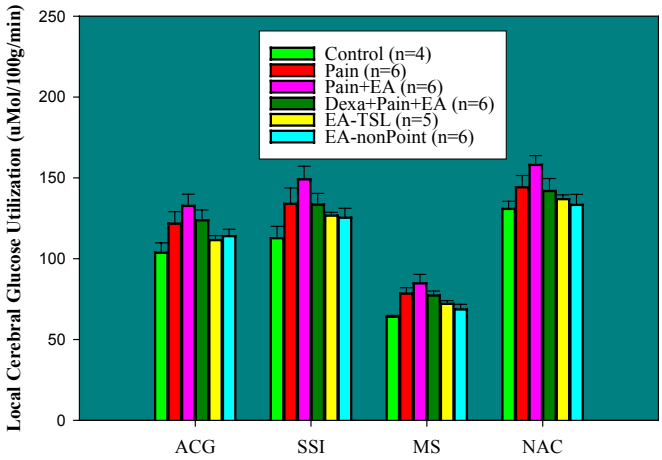

Fig. 1. Effects of thermal pain stimulation, followed by Tsu-San-Li electroacupuncture (combined with or without dexamethasone), on the LCGU changes in the telencephalic regions: anterior cingulate gyrus (ACG), somatosensory area I (SSI), medial septal (MS), nucleus accumbens (NAC). (* indicate that a significant difference exists as compared with the control group, t-test, $p < 0.05$).

B. Changing of the Lcgu of the Hypothalamic Structures Related to Pain and EA (Fig. 2)

Arcuate Nucleus (ARC): As compared with the Control group, a 5 % increase in the Pain group; 31 % increase in the Pain+EA group (significant, t-test, $p < 0.05$); and a 2 % increase in the Dexa+Pain+EA group were found.

Paraventricular Hypothalamic Nucleus (PVH): As compared with the Control group, a 27 % increase in the Pain group; 27 % increase in the Pain+EA group (significant, t-test, $p < 0.05$); and a 10 % increase in the Dexa+Pain+EA group were found.

Ventromedial Hypothalamic Nucleus (VMH): As compared with the Control group, a 11 % increase in the Pain group; 33 % increase in the Pain+EA group (significant, t-test, $p < 0.05$); and a 7 % increase in the Dexa+Pain+EA group were found.

The LCGU percentage changes of the hypothalamic structures in each group as compared to the control group are indicated as below:

Hypothalamic structures \ Groups	Pain Group	Pain+EA Group	Dexa+Pain+EA Group
Arcuate Nucleus	5 %↑	31 %↑*	2 %↑
Paraventricular Nucleus	27 %↑	27 %↑*	10 %↑
Ventromedial Nucleus	11 %↑	33 %↑*	7 %↑

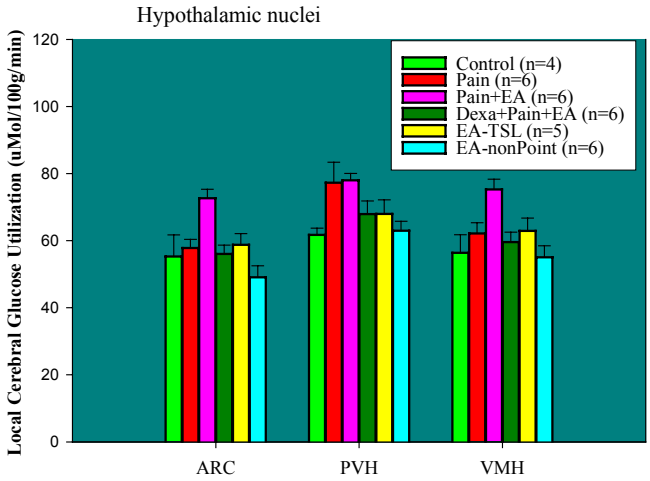

Fig. 2. Effects of thermal pain stimulation, followed by Tsu-San-Li electroacupuncture (combined with or without dexamethasone), on the LCGU changes in the hypothalamic nuclei, arcuate nucleus (ARC), paraventricular nucleus (PVH), ventromedial nucleus (VMH). (* indicate that a significant difference exists as compared with the control group, t-test, $p < 0.05$).

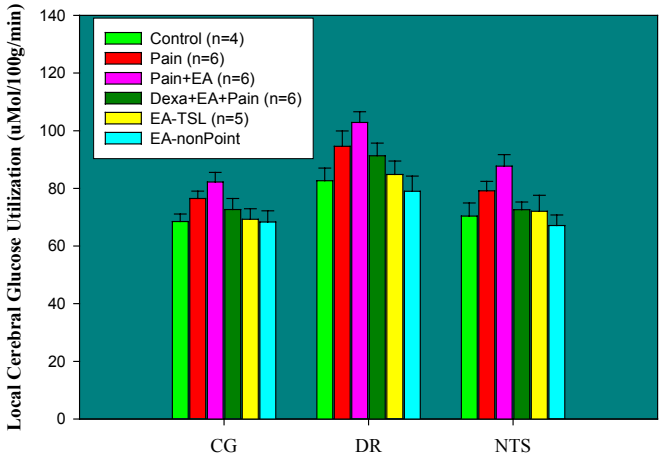

Fig. 3. Effects of thermal pain stimulation, followed by Tsu-San-Li electroacupuncture (combined with or without dexamethasone), on the LCGU changes in the central gray (CG), dorsal raphe (DR), and nucleus tractus solitaries (NTS) (* indicate that a significant difference exists as compared with the control group, t-test, $p < 0.05$).

C. Changing of the Lcgu of the Brain Stem Structures Related to Pain and EA (Fig. 3)

Central Gray (CG): As compared with the Control group, a 12 % increase in the Pain group; 21 % increase in the Pain+EA group; and a 7 % increase in the Dexa+Pain+EA group were found.

Dorsal Raphe (DR): As compared with the Control group, a 14 % increase in the Pain group; 24 % increase in the Pain+EA group (significant, t-test, $p < 0.05$); and a 10 % increase in the Dexa+Pain+EA group were found.

Nucleus Tractus Solitarius (NTS): As compared with the Control group, a 13 % increase in the Pain group; 25 % increase in the Pain+EA group (significant, t-test, $p < 0.05$); and a 4 % increase in the Dexa+Pain+EA group were found.

The LCGU percentage changes of the brainstem structures in each group as compared to the control group are indicated as below:

Brainstem structures \ Groups	Pain Group	Pain+EA Group	Dexa+Pain+EA Group
Central Gray	12 %↑	21 %↑	7 %↑
Dorsal Raphe	14 %↑	24 %↑*	10 %↑
Nucleus Tractus Solitarius	13 %↑	25 %↑*	4 %↑

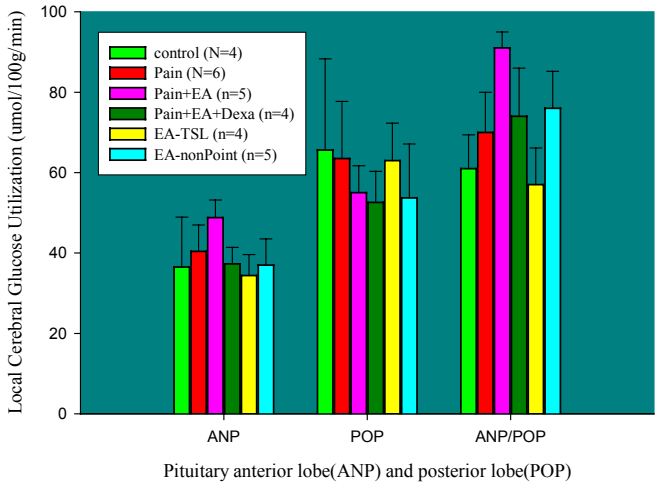

Fig. 4. Effects of thermal pain stimulation, followed by Tsu-San-Li electroacupuncture (combined with or without dexamethasone), on the LCGU changes in the pituitary of rat, ANP: anterior pituitary; POP: posterior pituitary.

D. Changing of the Lcgu of the Pituitary (Fig. 4)

Anterior Pituitary (ANP): As compared with the Control group, a 8 % increase in the Pain group; 32 % increase in the Pain+EA group; and a 1 % increase in the Dexa+Pain+EA group were found.

Posterior Pituitary (POP): As compared with the Control group, a 3 % decrease in the Pain group; 17 % decrease in the Pain+EA group; a 20 % decrease in the Dexa+Pain+EA group; and a 5 % decrease in the EA group were found.

Ratio of ANP/POP: As compared with the Control group, a 15 % increase in the Pain group; 49 % increase in the Pain+EA group; a 23 % increase in the Dexa+Pain+EA group; and a 7 % decrease in the EA group were found.

The LCGU percentage changes of the pituitary in each group as compared to the control group are indicated as below:

Pituitary lobe \ Groups	Pain Group	Pain+EA Group	Dexa+Pain+EA Group
anterior lobe	8 %↑	32 % ↑	1 %↑
posterior lobe	3 % ↓	17 % ↓	20 % ↓

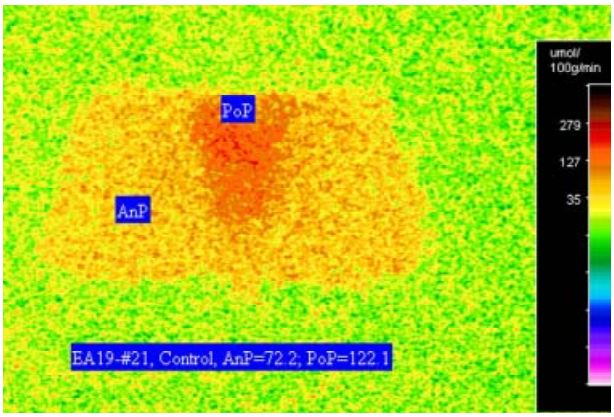

Fig. 5. A typical [C^{14}]-2DG labeling pattern of a pituitary of the Control group rat EA-19. This panel shows a computer-generated digitized image from a [C^{14}]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min. Note that the LCGU of the ANP is 72.2, while the POP is 122.1, and the trend of the LCGU is: POP > ANP >INP, where the ANP, POP and INP indicate the anterior lobe, posterior lobe and intermediate lobe of the pituitary, respectively. Although the ANP is an endocrine gland in itself, it still been labeled. However, it's functional reality may not follow the Sokoloff's equation.

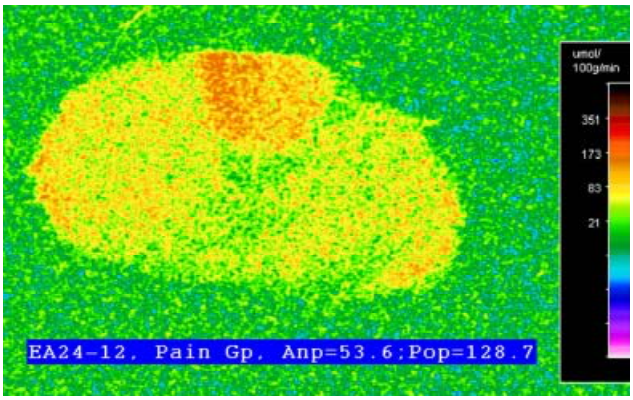

Fig. 6. A typical [C^{14}]-2DG labeling pattern of a pituitary of the Pain group rat EA-24. This panel shows a computer-generated digitized image from a [C^{14}]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min. Note that the LCGU of the ANP is 53.6, while the POP is 128.7, and the trend of the LCGU is: POP > ANP >INP.

E. Changing of the Labeling Pattern of Pituitary:

1. Control group (refer to Fig. 5):

The trend of the density of 2DG labeling of the Control group is "POP>ANP>INP (Intermediate lobe)". Fig. 6 shows this trend of labeling pattern in a 2DG autoradiogram of the pituitary gland from the rat (EA-19) of the control group.

2. Pain group:

In pain group rats, rat "EA-22" (Fig. 6) as a typical pattern, the pituitary labeling showed the same trend in

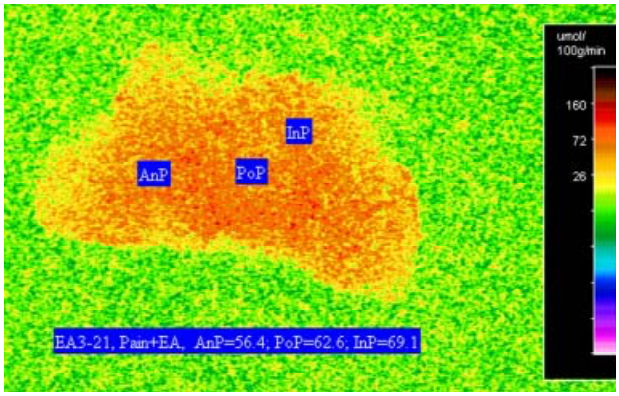

Fig. 7. A typical [C^{14}]-2DG labeling pattern of a pituitary of the Pain+EA group rat EA-3. This panel shows a computer-generated digitized image from a [C14]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min. Note that the LCGU of the ANP is 56.4, while the POP is 62.6, and the INP is 69.1, and the trend of the LCGU is: INP > POP = ANP. Furthermore, note that a ring of denser labeling on the outer border (the border facing the intermediate lobe of the pituitary) of the posterior lobe of the pituitary, while leaving the middle or central part of the posterior lobe lightly labeled at a density approximating that of the anterior lobe.

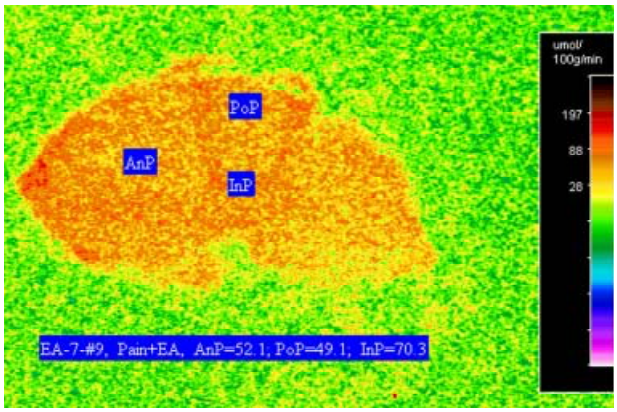

Fig. 8. A typical [C^{14}]-2DG labeling pattern of a pituitary of the pain+EA group rat EA-7. This panel shows a computer-generated digitized image from a [C^{14}]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min. Note that the LCGU of the ANP is 52.1, while the POP is 49.1, and the INP is 70.3, and the trend of the LCGU is: INP > POP = ANP.

LCGU values as that of the Control group, that is the posterior lobe had the densest labeling followed by the anterior lobe and lastly by the intermediate lobe (i.e., POP > ANP > INP).

3. Pain+EA group:

In this group, the pituitary labeling pattern showed an unusual pattern with the greatest amount of labeling present in the intermediate lobe, followed by lesser but equal amounts in the posterior and anterior lobes (i.e., INP > POP = ANP) (Figs. 7 and 8). However, in one of the animals (Fig. 7, EA-3), the posterior pituitary labeling pattern

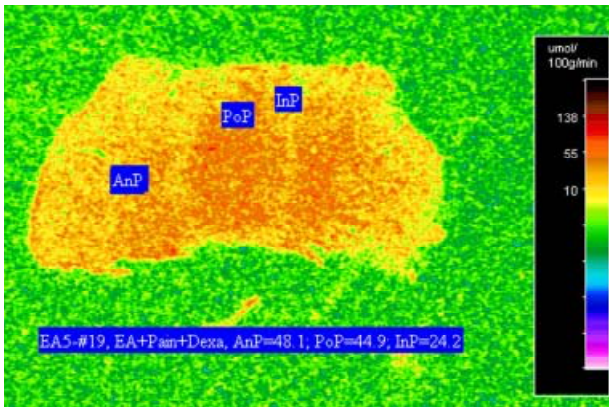

Fig. 9. A typical [C^{14}]-2DG labeling pattern of a pituitary of Dexa+pain+EA group rat EA-5. This panel shows a computer-generated digitized image from a [C^{14}]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min. Note that the LCGU of the ANP is 48.1, while the POP is 44.9, and the INP is 24.2, and the trend of the LCGU is: ANP = POP > INP. Furthermore, note that the anterior pituitary labeling pattern was quite heterogeneous in nature. A zone of denser labeling on the inner region (the region facing the intermediate lobe of the pituitary) exists in the anterior lobe of the pituitary.

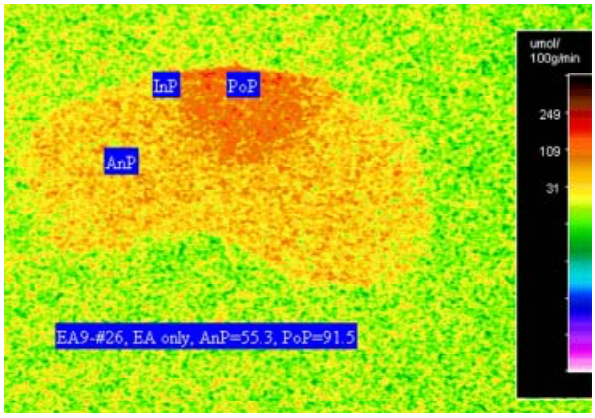

Fig. 10. A typical [C^{14}]-2DG labeling pattern of a pituitary of the EA group rat EA-9. This panel shows a computer-generated digitized image from a [C^{14}]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min.Note that the LCGU of the ANP is 55.3, while the POP is 91.5, and the trend of the LCGU is: POP > ANP = INP.

was quite heterogeneous.A ring of denser labeling on the outer border (the border facing the intermediate lobe of the pituitary) of the posterior lobe of the pituitary, while leaving the middle or central part of the posterior lobe lightly labeled at a density approximating that of the anterior lobe.

4. Dexa+Pain+EA group (Fig. 9):

In this group rats, the pituitary labeling showed the same trend in LCGU values as that of the pain group, that is the posterior lobe had the densest labeling followed by the anterior lobe and lastly by the intermediate lobe (i.e., ANP = POP > INP). The anterior pituitary labeling pattern was quite heterogeneous. A zone of denser labeling on the inner

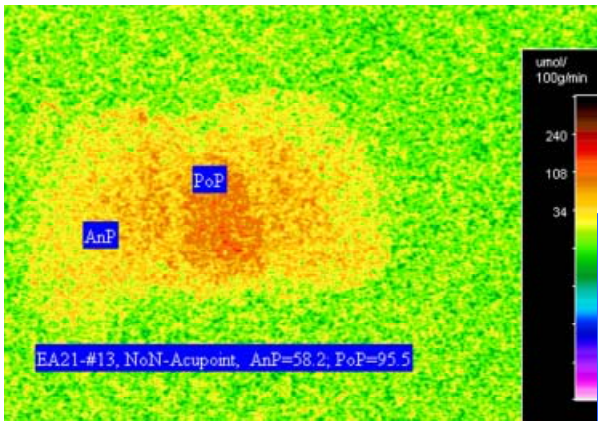

Fig. 11. A typical [C^{14}]-2DG labeling pattern of a pituitary of the EA-nonpoint group rat EA-21. This panel shows a computer-generated digitized image from a [C^{14}]-2DG autoradiogram. The color scale on the right side represents local cerebral glucose utilization (LCGU) in umole/100 gm of brain tissue/min. Note that the LCGU of the ANP is 58.2, while the POP is 95.5, and the trend of the LCGU is: POP > ANP > INP.

region (the region facing the intermediate lobe of the pituitary) existed in the anterior lobe of the pituitary.

5. EA group (Fig. 10):

In rats of this group, the general pattern of 2DG labeling in the pituitary was similar to that of the control group, that is, POP > ANP = INP.

6. EA-non-point group (Fig. 11):

In rats of this group, the general pattern of 2DG labeling in the pituitary was similar to that of the Control group, that is POP > ANP > INP. However, the anterior pituitary labeling pattern was quite heterogeneous. A zone of denser labeling on the inner border (the border facing the intermediate lobe of the pituitary) existed in the anterior lobe of the pituitary.

DISCUSSION

Our results showed that 2 Hz EA at the Tsu-San-Li point in rat produced elongation of the MTFL caused by the 56 °C water noxious stimulation, indicating the effect of the EA-produced analgesia (EAA). Furthermore, this EAA effect was blocked by dexamethasone, indicating the involvement of the ACTH and β-endorphin in these EAA effects.

In both the studies of the mouse and the rat brain, endomorphin and mu-opioid receptors have been proven to mediate the analgesic effect induced by 2 Hz, but not 100 Hz, EA stimulation[25,26]. The endogenous opioid system is also centrally important in mediating effects of the drugs of abuse and alcohol. It has been reported that 100-Hz EA or 100-Hz transcutaneous electrical nerve stimulation (TENS) is very effective in ameliorating the morphine withdrawal syndrome in rats and human. In addition, spinal dynorphin and kappa-opioid receptor play important roles in this effect[27,28]. In 2000, LaForge et al.[6] reported opioid receptor and peptide gene polymorphisms and indicated that

polymorphisms, including single nucleotide polymorphisms, have been identified in genes of the endogenous opioid receptors and peptides. The opioid receptors and peptides of the brain regions significant affected by the EAA, such as MS、ARC、PVH、VMH、DR、NTS, may deserve further studies.

Among the telencephalic structures studied (Fig. 1), the LCGU result in ACG (Brodmann area 24) showed that as compared to the control group, there is a little bit increase (29 % versus 18 %) in the ACG of the Pain+EA group than the Pain group, though not significant statistically. The ACG is involved in pain perception and contains nociceptive neurons[29,30], therefore the role of ACG in the EAA effect deserve further study. In the NAC, the result showed that as compared to the control group, a 21 % increase in LCGU of the Pain+EA group while only a 10 % increase in Pain group and an 8 % increase in Dexa+Pain+EA group. The NAC is a target structure of natural reward, abused drug, as well as antipsychotics[31,32]. In 1996, Orzi et al.[31], proved that morphine sulphate (0.2-0.4 mg/kg, IV) produced dose-dependent increases of glucose metabolism in the shell of the NAC without affecting functional activity in any other brain areas. The role of the NAC in the EAA effect may deserve further evaluate. Among the hypothalamus regions studied, the result in the LCGU of the PVH showed that as compared to the control group, a 27 % increase in both the Pain and the Pain+EA group (significant, t-test, $p < 0.05$); and a 10 % increase in the Dexa+Pain+EA group. The PVH is an important area involved in the functions of hypothalamic-pituitary-adrenal (HPA) axis, hypothalamic-pituitary-gonad (HPG) axis, and the stress response regulation. In 2002, Nestler et al.[33], proposed a conceptual model of depression stating that "Dysregulation of the HPA axis and hippocampus causes depression." and that "The corticotropin releasing factor (CRF)-containing parvocellular neurons of the PVN integrate information relevant to stress". The CRF is released by these neurons into the hypophyseal portal system and acts on the corticotrophs of the anterior pituitary to release ACTH. ACTH reaches the adrenal cortex via the bloodstream, where it stimulates the release of glucocorticoids. In addition to its many functions, glucocorticoids (including synthetic forms such as dexamethasone) depress CRF and ACTH synthesis and release. In this manner, glucocorticoids inhibit their own synthesis. At higher levels, glucocorticoids also impair, and may even damage, the hippocampus, which could initiate and maintain a hyper-cortisolemic state related to some cases of depression." In a sleep-deprived (maintained for 11-12d) study of the rat, Everson et al. reported that the average glucose utilization in the brain as a whole was unchanged in sleep-deprived rats, but regional decreases were found. The most marked decreases in local cerebral glucose metabolic rate (LCMRglc) were in the regions of the hypothalamus, thalamus, and limbic system (primarily in regions associated with mechanisms of thermoregulation, endocrine regulation, and sleep)[34]. The increase in the LCGU of the PVH of both the Pain+EA and Pain group may implicate the activation of the HPA axis.

In the posterior pituitary (POP), the results showed that as compared to the Control group, a 3 % decrease in the LCGU of Pain group; while a 17 % decrease in Pain+EA group; and a 20 % decrease in the Dexa+Pain+EA group. The neurohypophysis receives unmyelinated fiber bundles from the magnocellular neurons of the supraoptic and

paraventricular nuclei of the hypothalamus. These fibers project via the internal layer of the median eminence into the posterior lobe and end on capillaries[11]. Electrical stimulation of these two nuclei cause increased secretion of the vasopressin and oxytocin. Homozygous Brattleboro rats have a genetic inability to synthesize vasopressin and therefore manifest signs and symptoms of diabetes insipidus. Measurement of local cerebral glucose utilization in these rats has revealed increases specifically localized to the subfornical organ and pituitary neural lobe[35]. In rats, either given saline to drink or were deprived of water for 5 days, the rate of glucose utilization in the pituitary neural lobe was increased by 367 %[36]. Angiotensin II produces pressor and drinking responses and increases glucose utilization selectively in the subfornical organ and pituitary neural lobe but in no other brain structure studied[36]. The role of POP in the effect of EAA may deserve further study.

CONCLUSION

In summary, we conclude that: (1) as compared with the pain group, the EAA group showed increased LCGU in the anterior lobe, and decreased LCGU in the posterior lobe, as well as exhibiting different labeling pattern; (2) These findings appear to be correlated with the effect of the 2-Hz EAA, suggesting the involvement of the pituitary in the EAA, and warranting further research.

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大白鼠足三里電針止痛的中樞機轉：腦下垂體的 C^{14} 去氧葡萄糖代謝功能研究

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費城

痛覺是由許多不同層面的感覺傳入，經過認知徑路(包含身體的壓抑調節系統亦即內分泌、免疫、自主神經、和嗎啡系統）的調控，產生痛的感覺和行為反應（MelZack, 1999）。我們早先的研究結果（Huang, 1988）顯示大白鼠足三里低頻電針止痛的的中樞機轉是經由活化：1) 內生性止痛系統；2) 內生性嗎啡系統（endogenous opioid system），如下視丘室旁核、弓狀核、及腦下垂體前葉；3) 邊緣情緒系統，進而活化，引起止痛作用。腦下垂體是一個神經內分泌機制的調控關鍵器官，負責維持恆定、代謝能量、生殖、成長、泌乳等功能。為了解腦下垂體在低頻電針止痛中所扮演的角色，本研究藉由評估腦下垂體的葡萄糖代謝功能表現，並以 dexamethasone 阻斷腦下垂體前葉 corticotrophs 釋放 ACTH/β-endorphin，來探討此一問題。結果顯示 dexamethasone 有效阻斷低頻電針止痛，使閃尾反應的延長時間從打藥前的 25 %降低為 1 %。因此我們推斷 ACTH/β-endorphin 參與低頻電針止痛作用。再者，電針 + 痛刺激組的腦下垂體葡萄糖代謝功能表現順序為“中葉 > 後葉 = 前葉”與對照組的葡萄糖代謝功能表現順序“後葉 > 前葉 > 中葉”不同。電針 + 痛刺激組在後葉靠近中葉的葡萄糖代謝功能增加，且後葉葡萄糖代謝功能下降，推測與低頻電針止痛減低壓力作用有關。

關鍵詞：腦下垂體，痛，閃尾反射時間，電針止痛，葡萄糖代謝率。

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