99-14-170

2 7 3
1

Influence of Electro-acupuncture on Pain Threshold in Horses and its Mode of Action

Huisheng Xie, DVM, Ph.D.; Edgar A. Ott, Ph.D.; Daniel Harkins, DVM, Ph.D.; Thomas Tobin, DVM, Ph.D.; Patrick T. Colahan, DVM

Objective— To determine the effectiveness of electro-acupuncture in experimental pain in the horse, and to determine how acupuncture relieves pain.

Design— A parallel arm design with 2 factors

Animals — Twenty-two Thoroughbred horses without any clinical symptoms.

Procedure

Twenty-two Thoroughbred horses were used to evaluate the effectiveness of electro-acupuncture (EA) on pain perception. The six treatments were assigned at random: 4 acupuncture groups, 1 positive control group and 1 negative control group. Focused radiant light/heat was used as a noxious stimulus and was directed onto the pastern to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) is defined as the time (seconds) between lamp illumination and the withdrawal of the hoof. The HWRL was measured at -15, -5, 0, 15, 30, 60 ,90, 120, and 180 minutes. Zero was the time immediately following treatment termination. Plasma concentrations of β-endorphin, ACTH and cortisol were also measured at the same time intervals.

Results

The second acupuncture treatment (using 4 local points with frequency of 80-120 Hz) increased ($P<0.01$) the HWRL and plasma β-endorphin concentration. The third acupuncture treatment (using 4 local points with frequency of 20 Hz) did not induce the HWRL in left limb (where local acupuncture stimulation took place) ($P>0.05$), but increased the HWRL by 37% in right limb (control limb) ($P<0.05$). This may indicate that EA using low frequency of 20 Hz) induced a stronger analgesia in other body areas than the local areas which was close to the stimulated acupoints. The fourth acupuncture treatment (2 local point with frequency of 80-120 Hz) increased ($P<0.01$) the HWRL but did not change the plasma β-endorphin concentration. The first acupuncture treatment (2 distal points and 2 local points with 80-120 Hz) saline treatment did not alter the pain threshold or the plasma β-endorphin concentrations. None of the acupuncture treatments altered the ACTH concentrations.

Conclusions and Clinical Relevance

Acupuncture treatments varied in their effect on pain threshold in horses. Acupuncture stimulation using local acupuncture points with high frequency (80-120 Hz) is more effective than the use of distal points with low frequency (20 Hz). The acupoints close to the painful areas need to be stimulated with high frequency EA while the acupoints far from the painful areas may be stimulated with low frequency EA. The release of β-endorphin may be one of the pathways in which electro-acupuncture relieves the experimental pain.

Acupuncture is best known for its effect on pain relief in both horses and people.[1,2] Acupuncture may be defined as the stimulation of a specific point on the body, known as an acupuncture point, or acupoint, with a specific method, resulting in a therapeutic or hemostatic effect. The origin of this therapy can be traced to China as early as 2,200 to 3,000 B.C.[3] Historically, veterinary practitioners have used conventional needles, bleeding needles, heat, moxa, cauterization and massage to stimulate acupoints.[4] Modern technology, such as laser, electricity, injectable agents and microwaves, can also be used to stimulate acupoints.[4] Electro-acupuncture (EA) is a method in which an electric current is passed to the animal through a needle inserted an acupoint. EA is generally considered to have stronger effects than other types of acupuncture methods.[5] EA has been reported to be effective for treatment of various musculoskeletal pain-producing conditions including cervical, thoracolumbar and lumbosacral hyperpathia,[1,6] chronic lameness [7], degenerative joint diseases [8], back pain [9], colic [10]. EA stimulation has also been found to produce an analgesic effect, generally called electro-acupuncture analgesia (EAA).[11] However, few controlled studies have been conducted on how acupuncture relieves pain in the horse. The objective of this study was to determine the effectiveness of electro-acupuncture in experimental pain in the horse, and to determine how acupuncture relieves pain.

Materials and Methods

Subjects and Groups

Twenty-two healthy Thoroughbred horses aged 2 to 13 years were used for this study. Seventeen of these subjects were mares and 5 were geldings. They all were chosen from the Horse Farm at the University of Kentucky. Ten of them were assigned to the study only one time, and 12 of them were assigned twice. For those horses used twice, the washout period was 1 week. The selected horses were required to be free of any pain relief drugs for at least 2 week to avoid any confounding influences from these drugs. They were randomly assigned to one of 6 treatment groups including 4 acupuncture treatment groups, a positive and a negative control group:

Acu-I: Electrical stimulation at acupoints Bai-hui (GV-20a), Duan-Xue (GV-6), left Qiang-feng (SI-9) and left San-yang-lo (SYL) with a frequency of 80-120 HZ.

Acu-II: Electrical stimulation at acupoints left SI-9, left SYL, left Qian-chan-wan (QCW) and left Qian-jiu (QJ) with a frequency of 80-120 Hz.

Acu-III: Electrical stimulation at acupoints left SI-9, left SYL, left QCW and left QJ (same as Acu-II) with a frequency of 20 Hertz.

Acu-IV: Electrical stimulation at acupoints either left SI-9 and left SYL, or left QCW and left QJ, with a frequency of 80-120 Hertz.

Positive control group (PCG): Two milliliters of 0.5 % bupivacaine HCL (10mg/2ml, made in Abbott Lab, North Chicago, IL) was subcutaneously injected into the area of the lateral palmar nerve where it passes lateral (abaxial) to the lateral sesamoid bone.

Negative control group (NCG): Two milliliters of saline was subcutaneously injected into the same area as described for the PCG group.

For each group, the test was conducted in the barn in the Horse Farm of University of Kentucky. Two hours before the animals were to be tested, they were kept in the quiet stall. They were provided free access to water, but no feed was provided.

One hour before the test, the left and right front pastern were clipped. The clipped areas were blackened with stamp ink (Sanford, Beillwood, IL). A catheter was placed in the left jugular vein, filled with heparinized saline and taped to the neck.

Pain Threshold Measurement: Hoof Withdrawal Reflex Latency

This measurement is based on Harkins' method.[12] The hand-held KHT radiant heat lamp was used to elicit the focused radiant light (diameter: 0.4 cm), which was used as a noxious stimulus and was directed onto the pastern to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) is defined as the time (seconds) between lamp illumination and withdrawal of the hoof. The unfocused light beam (nonpainful light) was also used on the pastern in order to avoid the visual influence from the light. The time could be adjusted by varying the intensity of the heat output of the lamp. The duration of light exposure was limited to 20 seconds for acupuncture and saline treatment groups, and limited to 10 seconds for bupivacaine treatment group to prevent damage to the skin.

The HWRL was measured at -15, -5, 0, 15, 30, 60, 90 and 180 minutes for each subject in all 6 groups. The 0 minute time period was the time immediately after completion of any treatment. For 4 acupuncture treatment groups, the HWRL was also measured at "during" period which is 15 minutes after the beginning of the 30-minute acupuncture stimulation. There was no "during" period in 2 control groups. The HWRL was measured on both front pasterns at each time period. The HWRL at -15' and -5' time period were considered as baseline: HWRL baseline = (HWRL at -15' + HWRL at -5') ÷ 2.

In order to limit the individual variance, the data were transformed to the percent of the baseline:[12,27] % change in HWRL = (HWRL ÷ baseline) × 100.

Acupuncture Procedure

The following acupuncture procedure was based on experience and other research results.[6,9,13] A filiform needle (0.30 mm X 75 mm, Suzhou Medical Instrument Factory, Jiangsu, China) was inserted into each acupoint. An electro-acupuncture instrument (Model WQ6F, Donghua Electronic Instrument Factory, Beijing, China) was used to supply electric current. Electrodes were attached to needles that were inserted into the acupoints. Two acupoints were hooked together with electrical wire by GV-20a with GV-6, SI-9 with SYL, and QCW with QJ. The wires were connected to the EA instrument. The electric current was carried through the needle and acupuncture points to the body. Electrical stimulation was conducted for 30 minutes in all 4 acupuncture treatment groups. Different acupuncture groups varied by acupoints and electrical frequency. The location of each acupoint and its manipulating method are described in table 1.

Blood Chemistry Tests

Blood samples were collected at -15, -5, 0, 15, 30, 60, 90 and 180 minutes for each subject in all 6 groups. The "0" time period was the time immediately after the end of any treatment. There were two sessions each day: morning and afternoon sessions. The morning session (n=17) started at 9:00 am, and the afternoon session (n=17) started at 12:00 pm. Blood samples (20 ml) were collected from jugular vein after clearing the catheter of heparin. Blood was collected into a venipuncture tube containing EDTA as the anticoagulant. The sample tube was immediately inserted in ice, and blood was centrifuged within 30 minutes of collection and stored at -20 ° C until it was assayed. Radioimmunological assay (RIA) was used to measure plasma concentration of β-endorphin [14], cortisol [15] and ACTH [16]. The β-endorphin kits were purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA). The coat-a-count cortisol kits were purchased from the Diagnostic Products Corporation (Los Angeles, CA). The ACTH kits were from Diagnostic Systems Laboratories, Inc. (Webster, TX). The plasma hormone concentration at -15' and -5' was considered as the baseline: The baseline = (value at -15' + value at -5') ÷ 2.

Design and Analysis of Data

This is a parallel arm design with 2 factors. Data are presented as mean±s.e. Data were analyzed using the general linear models (GLM). The significant level was 0.05 when the Duncan's Multiple Range Test was used to compare data for the different time periods of the same group. The significant level was 0.01 when Least Squares Means were used to compare data for the different treatment groups at each measuring time. The statistical analyses were performed using SAS for Windows (version 6.12).

Results

Pain Threshold

<u>HWRL in left limb</u>: The data are summarized in table 2. For the bupivacaine treatment group(PCG), pain threshold increased significantly by 66-86% from 15 minutes up to 120 minutes after treatment ($P<0.0001$). For the Acu-II group, pain threshold increased by 79% at during, 111% at 0' and 85% at 15' time periods ($P<0.01$). For the Acu-IV group, pain threshold increased ($P<0.01$) by 74% at during and 97% at 0 time period. For the groups Acu-I, Acu-III and NCG, there was no significant change ($P>0.05$) among different time periods. This result suggests that different acupuncture treatments induced different responses to painful stimulus in horses. The differences in assignment of treatment among the 4 acupuncture groups were either acupoints or frequency of electrical stimulation. The same acupoints were used in Acu-II and Acu-III, but different frequencies (80-120Hz for Acu-II and 20 Hz for Acu-III) were used. The different results of these two groups indicated that the high frequency of 80-120 Hz may be better for electro-acupuncture analgesic effect.

Even though the same frequency of electrical stimulation (80-120 Hz) was used in the both Acu-I and Acu-II group, the different acupoints were used in these 2 groups. The different results between these 2 groups indicated that acupuncture analgesic effect depends upon a specificity of acupoints.

The same frequency (80-120 Hz) was used for both Acu-II and Acu-IV group. The only difference in these two groups was that only 2 of 4 acupoints used in Acu-II group were assigned in Acu-IV group. Acu-II induced a stronger and longer analgesic effect than Acu-IV. This result suggest that when more acupoints are involved in acupuncture treatment, a stronger analgesic effect results.

The right limb: HWRL in the right front limb in horses is listed in table 3. For PCG, NCG, Acu-I, Acu-II and Acu-IV groups, HWRL did not change significantly ($P>0.05$) among the different time periods. However, there was a significant increase in pain threshold in different time periods in the Acu-III. It is of interest that HWRL in left limb (where local acupuncture stimulation took place) did not increase significantly ($P>0.05$) in Acu-III group, while pain threshold in right limb increased significantly by 37% at during time period ($P<0.05$). This may indicate that Acu-III (low frequency of 20 Hz) treatment induced a stronger analgesia in other body areas than the local areas which was close to the stimulated acupoints.

Plasma Concentration of β-endorphin

Plasma concentration of β-endorphin is summarized in table 4. Plasma concentration of β-endorphin did not change significantly ($P>0.05$) among different time periods in Acu-I, Acu-III, Acu-IV, PCG and NCG. But, β-endorphin increased significantly by 211.9% ($P<0.001$) at 0' time period in Acu-II group, and continued to increase by 63.3% at 15', 60.3% at 30', and 43.4% at 60' time periods. The plasma β-endorphin level returned to the baseline at 90', and decreased by 24% at 180' time period. These results coincide with the significant increase in the pain threshold (HWRL) in the Acu-II group. The endorphin release may be one of the pathways by which the acupuncture stimulation induces analgesic effect.

Even though the HWRL increased significantly at 0' time period in the Acu-IV group, the plasma concentration of β-endorphin did not change significantly using this acupuncture treatment. This may be related to different responses due to different acupuncture treatment, or to the other mechanisms by which acupuncture relieves pain.

Plasma concentration of ACTH

Plasma concentration of ACTH is summarized in table 5. Plasma concentration of ACTH in all groups did not change significantly after treatment (P>0.05). This may indicate that the release of ACTH is not the pathway by which acupuncture treatment relieves pain. However, the plasma ACTH level decreased numerically by 25.5% and 23.1% at 30 minutes and 60 minutes after treatment in Acu-II group. For PCG group, plasma ACTH decreased by 28.5%, 29.0%, 24.2%, 40.9%, 35.1% and 38.4% respectively at 0, 15, 30, 60, 90 and 120 minutes after treatment. It is unclear if this decrease is related to the local administration of bupivacaine.

Plasma Concentration of Cortisol

Plasma concentration of cortisol is summarized in table 6. There was no difference (P>0.05) in % change in plasma cortisol levels in Acu-IV, PCG and NCG groups. However, plasma concentration decreased (P<0.005) by 20.4%, 27.4%, 33.1%, 29.6% and 20.7% respectively at 30, 60, 90 120 and 180 minutes after treatment in the Acu-I group. For the Acu-II group, acupuncture induced at first a significant 23% increase in plasma cortisol level at 0 time period, and then a decrease by 20.2% at 15, 20.9% at 30, 26.1 % at 90 and 21% 180 minutes after treatment ($P<0.001$). The Acu-III induced a decrease in plasma cortisol concentration from 60 to 180 minutes after treatment ($P<0.001$).

Discussion

Pain Threshold

Pain threshold (PT) has been used to evaluate acupuncture analgesic effect in horses[11], cattle [17], sheep [18], pigs [19], dogs [20], and rabbits [21]. Heat, pinprick and pinch were the most commonly used noxious stimuli for measurement of PT in horses.[11, 12] An increase in PT was interpreted as analgesia induced by treatment. In this study, the focused radiant light beam was used to produce noxious heat which was directed onto the pastern to elicit the classic flexion-withdrawal reflex. The hoof withdrawal reflex latency (HWRL) was used to measure PT. PT determination was conducted in the both front limbs. Four different acupuncture treatments (different acupoints and frequency of electrical stimulation) were used.

On the basis of the data analysis of the left front limb, the Acu-II group (80-120 Hz of frequency and 4 local acupoints including left Qiang-feng, left San-yang-luo, left Qian-chan-wan and left Qian-jiu) induced a significant increase in PT of the left front limb ($P<0.01$). PT of the left limb increased by 79% at during, 111% at 0' and 85% at 15' time periods ($P<0.01$), and continued to maintain the numeric increase up to 180 minutes after the termination of acupuncture treatment. This result coincides with other data and the general acceptance of acupuncture analgesic effect.[1, 11, 18] EA stimulation in Qiang-feng and San-yang-luo induced an increase in PT across the 5 trunk areas in horses ($P<0.05$).[11] EA stimulation in Qiang-feng and San-yang-luo induced an analgesia strong enough to perform laparotomy in a goat.[22] CO_2 Laser stimulation in Qiang-feng and San-yang-luo induced a sufficient acupuncture analgesia for standing laparotomies to be performed in 50 ruminants (including 12 yellow cattle, 8 water buffalo and 30 goats).[23]

In the Acu-I group, 2 acupoints in the back, Bai-hui and Duan-xue, and 2 acupoints in the left front limb, Qiang-feng and San-yang-luo were used with a frequency of 80-120 Hz of electrical stimulation. This acupuncture treatment did not change the PT in the left front limb ($P>0.05$). Qiang-feng and San-yang-luo, Bai-hui and Duan-xue are generally considered as two pairs of acupoints for analgesia for abdominal surgeries in TCVM's perspective.[4,25] Failure to induce analgesia using this acupuncture treatment may be related to locus of noxious stimulation. EA in Bai-hui and Duan-xue or in Qiang-feng and San-yang-luo has been shown to induce a strong analgesia in the flank, back, lumber areas and abdomen but not in limbs and heads in sheep, goats and pigs.[19] Zhou also found that acupuncture analgesia was sufficient for 10 abdominal surgeries, but not for 4 limb surgeries in horses.[26]

In the Acu-III group, PT in the left limb increased inconsistently by 44-46% from during to 0 time period, 13-18% from 15 to 30', by 36% at 60', by 3-4% from 90 to 120' time period and then dropped by 11% at 180' time period, but this change in PT was not significant ($P>0.05$). In the Acu-III group, the same 4 acupoints as used in group Acu-II were used but with 20 Hz frequency. The results indicate that high frequency (80-120 Hz) electrical stimulation of acupoints may induce a stronger local analgesic effect than a low frequency (20 Hz). This result did not coincide with Chen and Wang's findings that low frequency (2-3 Hz) electrical stimulation had a stronger and longer analgesic effect in cattle and sheep than high frequency (50-300 Hz).[24] On the basis of clinical analysis of 575 cases associated with acupuncture analgesia in cattle and sheep, they found that the EA with high frequency induced the greatest increase in PT within 5-15 minutes, but this acupuncture analgesia declined very quickly after the termination of EA stimulation and returned to the baseline within 1.5 hours. The EA with low frequency induced the highest peak 30 minutes after the beginning of stimulation and this acupuncture analgesia could last up to 2-4 hours after the termination of acupuncture stimulation.[24] The different results may be a species effect. The location of PT test could also be a factor as the trunk (especially in flank) is generally considered a better analgesia area than the limb (especially in pastern and hoof).[11, 19] The flank was used for locus of noxious stimulation in Chen and Wang's research [11] while the pastern was used the locus of noxious stimulation in this study.

In the Acu-IV group, either 2 acupoints Qiang-feng and San-yang-luo, or Qian-chan-wan and Qian-jiu were used with 80-120 Hz of frequency of electrical stimulation. PT in the left limb increased by 74% at during and 97% at 0 time periods ($P<0.01$), and then returned to the baseline level at 15 minutes after the termination of acupuncture stimulation. The only difference between Acu-II and Acu-IV groups was the acupoints. Only 2 acupoints (1 pair) of the 4 acupoints (2 pairs) used in Acu-II were selected for Acu-IV. The longer acupuncture analgesic effect from the Acu-II group suggests that some acupoints could potentiate the other acupoints. This result coincides with clinical application of 4-6 acupoints for each treatment.[25] Even though the PT increased at during and o time periods in the Acu-IV group, the PT started to decline by 8% at 90' and 120' and 11% at 180' time periods. The reasons for this declination remain to be determined. Bossut et al found that acupuncture analgesic efficacy varied between sexes in horses.[11] Analgesia was induced equally well in both castrated males and intact females by the EA stimulation in Yao-pang-san-xue, Bai-hui and Ba-shan, however, EA stimulation in Bai-hui and

Ba-shan caused a significant analgesia in females only.[11] No difference in acupuncture analgesic effect was found in our study, however, only 5 males (7 times) but 17 females (27 times) were used in this study. The bias of sex could have contributed to the different results.

Acupuncture treatments, bupivacaine and saline injection were conducted only in the left limb. The positive control bupivacaine injection induced a significant increase in PT only in the left limb ($P<0.05$) but not in the right limb. Thus, the contralateral, or the non-treated limb was considered as a controlled limb. In this study, the left limb is the treated limb while the right limb is the controlled limb. For the Acu-II group, PT in the controlled limb increased by 23% at during and 35% at 0 time period without significance ($P>0.05$). However, when significant level α was set as 0.15, the 35% increase at 0 time period was significant. This result indicates that the acupuncture treatment may induce a whole body analgesia, rather than a local analgesia.

Even though the Acu-III acupuncture treatment (low frequency of 20 Hz) did not induce a significant increase in PT of the treated limb, PT in the controlled limb increased significantly by 37% at during time period, and continued to increase by 24% at 0', 18% at 15' and 13% at 30' time periods. This result suggests that low frequency EA stimulation induced a stronger analgesic effect in other body areas than the local areas close to the stimulated acupoints. It suggests that in clinical practice the acupoints close to the painful areas should be stimulated with high frequency EA while the acupoints far from the painful areas should be stimulated with low frequency EA. Janssens et al [28] suggested that segmental analgesia may be better when high frequency (100 to 1000 Hz) and low intensity stimulation is used, while low frequency (2 to 10 Hz) stimulation causes a more typical generalized analgesia.

Acupuncture Effect on β-endorphin

The results showed that the Acu-II acupuncture treatment induced an increase in plasma concentration of β-endorphin by 211.9% ($P<0.001$) at 0 time period and by 63.3% at 15', 60.3% at 30' and 43.3% at 60' time periods. All other acupuncture treatment (Acu-I, Acu-III and Acu-IV) induced a slight increase in plasma concentration of β-endorphin but without significance. As discussed above, the Acu-II caused a stronger analgesic effect. This result coincides with the findings in which the stronger the analgesic effect, the higher the β-endorphin content in the brain.[29] Wu et al. [30] also found that the release of β-endorphin increased significantly when the pain threshold increased significantly after EA treatment. There was a linear correlation between increases in β-endorphin and the body's ability to tolerate pain.[31] It seems that there is a significant correlation between the release of β-endorphin and the increase of pain threshold. The release of β-endorphin may be one of pathways in which acupuncture relieves pain in horses.

In order to probe into whether β-endorphin is involved in descending modulation of the somatosensory area (SII) of the cerebral cortex on the nucleus centrum medianum (CM) of the thalamus and the mechanism of acupuncture analgesia, Dong et al. [32] compared effects of electrical stimulation of SII and EA on β-endorphin content in the perfusate from the nucleus CM. They found that β-endorphin content in the perfusate was increased significantly by electrical stimulation of SII and not by EA, suggesting that the β-endorphin release from the nucleus CM was elevated by the stimulation of SII and was not influenced by EA. It indicates that β-endorphin may not be involved in the regulatory effect of EAA at the thalamus level.[32] However, EA stimulation at the acupoint Zu-san-li induced an increase in the expression of c-fos and poropiomelanocortisn (POMC) in the hypothalamic arcuate nucleus (Arc) in rats.[33] An electrolytic lesion or surgical isolation of the Arc weakened the acupuncture analgesia.[34] EA effect on the depressor-bradycardia was mediated by the β-endorphinergic projections from Arc and nucleus tractus solitarii (NTS) to the rostral ventrolateral medulla.[35] Local application of naloxone into the preoptic area (PA) partially reversed EA analgesic effect in rabbits.[36] These results suggest that Arc, NTS and PA may be involved in EAA.

Li et al. [37] demonstrated that the release of β-endorphin and lue-enkephalin from the reticularis paragigantocellularis lateralis (RPGL) in the EA group was significantly higher than that in the control group ($P<0.05$). The RPGL may be the common relay station in the EAA and morphine analgesia.[37] EA treatment induced an increase in pain threshold and the level of leu-enkephalin and β-endorphin release from PA, but a decrease in release of noradrenaline from PA.[38] The high concentrations of β-endorphin are also found in the hypothalamus, PAG and locus ceruleus.[38] Han et al.[39] demonstrated a rich enkephalinergic innervation, but the absence of β-endorphin containing fibers in the spinal cord. β-endorphin might produce its analgesic effects by suppressing substance P (SP) release in the spinal cord, and met-enkephalin directly blocks release of SP, which may be related to what closed the gate in the spinal cord pain transmission system.[40] Using multimicropipettes for extracellular recording and iontophoresis, He and Dong [41] found that morphine and etrophine produced a strong naloxone reversible inhibition on the spontaneous activity of the PAG neurons. EA induced a similar inhibitory effect on PAG neurons to iontophoretic morphine and etrophine, and the inhibition could be reversed by iontophoretic naloxone. A correlation existed between the effects of EA and opiates ($P<0.0174$).[41] This result indicates that acupuncture signals may activate the central opioid peptidergic system to exert a control over the transmission of pain sensation in the PAG, to block the conveyance of nociceptive impulse at situ and at other relay stations through inhibitory systems.

EAA mediated by β-endorphin was also demonstrated in the cerebrospinal fluid (CSF) studies. Clement-Jones et al.[42] found that EA effectively alleviated recurrent pain and significantly increased β-endorphin level in the lumbar CSF. EA induced an increase in ventricular CSF in patients with brain tumors, and there was a linear correlation between the percentage increase of β-endorphin and of pain threshold.[40] EA also increased the pain threshold and CSF enkephalins in rabbits [44] and monkeys [45]. CSF is formed from the blood by secretory and filtration processes and is absorbed into the blood by way of subdural venous sinuses and the spinal veins.[40] Because the skull forms an inelastic box and the brain-blood-CSF relationship is a reciprocating one, measurement of β-endorphin and enkephalins level in both CSF and peripheral blood may be a future potential approach to evaluate the involvement of neurotransmitters in the acupuncture analgesia.

The results of this study also show that pain threshold increased by 79% at during, 111% at 0' and by 85% at 15' time periods after acupuncture stimulation, while β-endorphin increased only at 0' time period after acupuncture treatment. It is possible that acupuncture induced the release of β-endorphin at first and then bound to the brain and other tissues quickly, thus the peripheral circulation of β-endorphin levels dropped. Based on the fact that EA stimulation resulted in a decrease of plasma β-endorphin, Szcudlik and Lypka [46] suggested that the increase of β-endorphin binding to the tissue receptor sites seem to be responsible for the peripheral (plasma) β-endorphin decrease after acupuncture. Panzer [52] also noticed that EA affected the endogenous opioids in ways that modified gastrointestinal motility and yet did not change plasma (peripheral) β-endorphin levels. Our study result indicated that the release of β-endorphin from the central nervous system induced by EA into the peripheral circulation may initially be responsible for an increase in plasma level of β-endorphin. Then β-endorphin binding to the tissue receptors results in a declination of plasma β-endorphin. This may be the reason pain threshold remains high while plasma β-endorphin tends to decline.

Even though the Acu-IV acupuncture treatment induced a significant increase in pain threshold, it did not change the plasma concentration of β-endorphin ($P>0.05$). This suggests that there may be other mechanisms by which acupuncture relieves pain. Other workers have shown that the naloxone (an opiate antagonist) does not inhibit EAA at high frequency (200 Hz) stimulation even though it completely reverses the EAA with low frequency (4 Hz), while parachlorophenylalanine (serotonin synthesis inhibitor) partially blocks high frequency (200 Hz) EAA but produces no effects on the low frequency (4 Hz) EAA.[47] Another study has shown that intracerebroventricular saralasin (angiotension II antagonist) injection abolishes or blocks EAA at 100 Hz stimulation but not at 15 Hz frequency, while 15 Hz EAA is blocks by intravenous injection of naloxone.[48] Thus, the low frequency EAA may be mediated by endorphins while the high frequency EAA may be due to neuropeptides serotonin and angiotensin II. Serotonin enhancement of acupuncture analgesia was also demonstrated in other studies.[49]

Endogenous Hormones Including ACTH and Cortisol

In this study, plasma levels of ACTH did not change among the time periods in all groups. This indicates that EA may not cause significant ACTH response. This result coincides with another study conducted in healthy volunteer people.[30] As β-endorphin is released in a one-to-one molar ratio with ACTH from the pituitary,[36] why did not ACTH change while β-endorphin increased after EA stimulation? The change of ACTH should parallel with that of β-endorphin if both of them have the same origin (pituitary). The lack of significant change in plasma ACTH concentration after acupuncture stimulation in the present study suggests that β-endorphin may be released from other sources rather than the pituitary into peripheral blood. Some studies have demonstrated that EA stimulation induced the release of β-endorphin from the reticularis paragigantocellularis lateralis (RPGL)[37] and from the preoptic area (PA)[39]. The high concentrations of β-endorphin are found in the hypothalamus, PAG and locus coeruleus.[38]

The striking finding of the present study shows that EA induced a significant change in β-endorphin but not ACTH, which indicates that acupuncture induced-biochemical changes including the release of β-endorphin may not be simply due to an effect of "stress". However, Malizia et al. [51] found that EA induced a significant increase in both β-endorphin and ACTH in healthy volunteer people. The different results may be related to variation of species and subject statues.

In the present study, plasma concentration of cortisol did not change significantly ($P>0.05$) among different time periods in the Acu-IV acupuncture treatment, positive control and negative control groups. However, plasma cortisol concentration decreased significantly by 20.4% to 33.1% at 30, 60, 90 120 and 180 minutes after treatment in Acu-I group. For Acu-II group, acupuncture treatment induced at first a significant 23% increase in plasma cortisol level at 0 time period, and then a significant decrease by 20.2% to 26.1% 15 minutes after acupuncture stimulation. The Acu-III acupuncture treatment induced a significant decrease in plasma cortisol level at 60 up to 180 minutes after treatment ($P<0.001$). The reasons for the decrease of cortisol induced by acupuncture treatment remain to be determined.

References

1. Richardson PH, Vincent CA. Acupuncture for the treatment of pain: a review of evaluative research. *Pain* 1986; 24: 15-40.

2. Fleming P. Acupuncture treatment for musculoskeletal and neurologic conditions in horses. In: Schoen AM, ed. *Veterinary Acupuncture: Ancient Art to Modern Medicine*. Goleta: American Veterinary Publications, 1994; 499-532.

3. Yu C. *Traditional Chinese Veterinary Acupuncture and Moxibustion*. Beijing: China Agriculture Press, 1995; 1-4.

4. Xie H. *Traditional Chinese Veterinary Medicine*. Beijing: Beijing Agricultural University Press,1994; 205-249.

5. Liang HJ. Effect of acupuncture treatment on rheumatic pain (bi-syndrome) in animals. *Chinese Journal of Traditional Veterinary Science* 1982; 1: 36-37. (In Chinese).

6. Li KC. Electro-acupuncture for treatment of lameness in horses. *Chinese Journal of Traditional Veterinary Science* 1993; 1: 13-14. (In Chinese).

7. Steiss JE, White NA, Bowen JM. Electro-acupuncture in the treatment of chronic lameness in horses and ponies: a controlled clinical trial. *Canadian Journal of Veterinary Research* 1989; 53:

239-243.

8. Bao XM. Electro-acupuncture for treatment of stifle arthritis in cattle. *Chinese Journal of Traditional Veterinary Science* 1982; 3: 56-57.

9. Xie H, Asquith RL, Kivipelto J. A review of the use of acupuncture for treatment of equine back pain. *Journal of Equine Veterinary Science* 1996; 7: 285-290.

10. Zhou GL. Electro-acupuncture for treatment of 8 equine cases with colic. *Chinese Journal of Traditional Veterinary Science* 1992; 1: 44. (In Chinese).

11. Bossut DFB, Page EH, Stromberg MW. Production cutaneous analgesia by electro-acupuncture in horses: variations dependent on sex of subject and locus of stimulation. *American Journal of Veterinary Research* 1984; 4: 620-625.

12. Harkins JD, Mundy GD, Stanley S, et al. Determination of highest no effect dose (HNED) for local anaesthetic responses to procaine, cocaine, bupivacaine and benzocaine. *Equine Veterinary Journal* 1996; 1: 30-37.

13. Klide AM, Martin Jr BB. Methods of stimulating acupoints for treatment of chronic back pain in horses. *Journal of The American Veterinary Medical Association* 1989; 10: 1375-1379.

14. Bossut DFB, Leshin LS, Malven PV. Radioimmunological measurement of beta-endorphin in

equine plasma. *Proceedings of the Society for Experimental Biology and Medicine* 1983; 173: 454-459.

15. Rijnberk A, Weees AV, Mol JA. Assessment of two tests for the diagnosis of canine hyperadrenocorticism. *The Veterinary Record* 1988; 122: 178-180.

16. Moore JN, Steiss J, Nocholson WE, et al. A case of pituitary adrenocorticotrophin-dependent Cushing's syndrome in the horse. *Endocrinology* 1979; 3:576-582.

17. Zhang WM, Xiong SY, Xiao JS, et al. Clinical study on Yan-chi acupuncture analgesia. *Chinese Journal of Veterinary Science and Technology* 1981; 9: 6-10. (In Chinese).

18. Bossut DFB, Stromberg MW, Malven PV. Electro-acupuncture-induced analgesia in sheep: measurement of cutaneous pain thresholds and plasma concentrations of prolactin and β-endorphin immunoreactivity. *American Journal of Veterinary Research* 1986; 3: 669-676.

19. Zhu ZY. Acupuncture analgesia in pigs, cattle and sheep. *Chinese Journal of Veterinary Medicine* 1992; 8: 38. (In Chinese).

20. Wu PX, Huang S. Influence of Laser acupuncture on pain threshold and body temperature in dogs. *Journal of Traditional Chinese Veterinary Medicine* 1988; 2:13-17. (In Chinese).

21. Yu C, Zhong XH. Effect of L-tryptophan and p-chloroamphetamine on CO2 laser analgesia in

rabbits. *Chinese Journal of Veterinary Medicine* 1985; 4: 34-35. (In Chinese).

22. Liu WP. Electro-acupuncture analgesia for surgery in a goat. *Chinese Journal of Traditional Veterinary Science* 1987; 1:7-9.

23. Chen CJ, Wang CW, Yang YB, et al. A study of CO2 irradiation analgesia for surgery. *Chinese Journal of Veterinary Science and Technology 1980;* 5: 1-5. (In Chinese).

24. Chen CJ, Wang CW. Veterinary electro-acupuncture parameters and analgesic effect. *Journal of Traditional Chinese Veterinary Medicine* 1983; 3:7-10. (In Chinese).

25. Yu C. *Chinese Veterinary Acupuncture*. Beijing: China Agriculture Press, 1984; 70-107. (In Chinese).

26. Zhou ZD. Acupoint Fu-tu as analgesia for surgery in horses. *Journal of Traditional Chinese Veterinary Medicine* 1984; 4: 27-29. (In Chinese).

27. Wei CB. Effect of acupuncture stimulation on pain threshold and body temperature in rabbits. *Chinese Journal of Traditional Veterinary Science* 1990; 4: 1-3. (In Chinese).

28. Janssens LAA, Rogers PAM; Schoen AM. Acupuncture analgesia: a review. *Veterinary Record* 1988; 122: 355-358.

29. Chen QS, Zhou DF, Wang DY, et al. Radioimmunoassay of β-endorphin in the brain and pituitary as related to the effectiveness of acupuncture analgesia in the rat. *Acupuncture Research* 1982; 7: 36-39. (In Chinese).

30. Wu GC, Zhu JM, Cao XD. Involvement of opioid peptides of the preoptic area during electro-acupuncture analgesia. *Acupuncture & Electro-Therapeutics Research* 1995; 20: 1-6.

31. Hamra JG, Kamerling SG, Wolfsheimer KJ, et al. Diurnal variation in plasma ir-beta-endorphin levels and experimental pain thresholds in the horses. *Life Sciences* 1993; 2:121-129.

32. Dong X, Wang S, Wang Y. Effects of electrical stimulation of SII and electro-acupuncture on beta-endorphin contents in the perfusate from the nucleus centrum medianum of the thalamus in cats. *Acupuncture Research* 1996; 4: 25-27. (In Chinese).

33. Zhang J, Wang MZ, He LF. Coexistence of Fos protein and proopiomelanocortin mRNA in hypothalamic nucleus following electro-acupuncture. *Acupuncture & Electro-Therapeutics Research* 1996; 21: 1-5.

34. Quo SY, Yin WP, Zhang HQ, et al. Role of hypothalamic arcuate region in lip-acupuncture analgesia. *Acta Physiology Sinica* 1982; 34: 71-77.

35. Ku YH, Zou CJ. β-endorphinergic neurons in nucleus arcuatus and nucleus tractus solitarri mediated depressor-bradycardia effect of "Tinggong" 2Hz-electro-acupuncture. *Acupuncture &*

Electro-Therapeutics Research 1993; 18: 175-184.

36. Wu GC, Jiang JW, Cao XD. Effect of naloxone microinjected into rabbit's preoptic area on electro-acupuncture analgesia. *Acta Acad Med Prima* 1984; 11: 134-139. (In Chinese).

37. Li Z, Wu GC, Cao XD. Role of opioid peptides of rat's nucleus reticularis paragigantocellularis lateralis (RPGL) in acupuncture analgesia. *Acupuncture & Electro-Therapeutics Research* 1995; 20: 89-100.

38. Ferrante FM. Opioids. In: Ferrante and VadeBoncouer, ed. *Postoperative Pain Management*. New York: Churchill Livingstone, 1993; 145-209.

39. Han JS, Xie GX, Zhou ZF, et al. Acupuncture mechanisms in rabbits studied with microinjection of antibodies against β-endorphin, enkephalin and substance P. *Neuropharmacology* 1984; 1: 1-5.

40. Lumb WV, Jones EW. *Veterinary Anesthesia*. Philadelphia: Lea & Febiger, 1984; 43-73.

41. He LF, Dong WQ. Activity of opioid peptidergic system in acupuncture analgesia. *Acupuncture & Electro-Therapeutics Research* 1983; 8: 257-266.

42. Clement-Jones V, MacLoughlin L, Tomlin S, et al. Increased β-endorphin but not met-enkephalin levels in human cerebrospinal fluid after acupuncture for recurrent pain. *Lancet* 1980;