# Cutaneous analgesia, hemodynamic and respiratory effects, and β-endorphin concentration in spinal fluid and plasma of horses after acupuncture and electroacupuncture

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**Objective**—To determine cutaneous analgesia, hemodynamic and respiratory effects, and β-endorphin concentration in spinal fluid and plasma of horses after acupuncture and electroacupuncture (EA).

**Animals**—8 healthy 10- to 20-year-old mares that weighed between 470 and 600 kg.

**Procedure**—Each horse received 2 hours of acupuncture and 2 hours of PAES at acupoints Bladder 18, 23, 25, and 28 on both sides of the vertebral column as well as sham needle placement (control treatment). Each treatment was administered in a random order. At least 7 days elapsed between treatments. Nociceptive cutaneous pain threshold was measured by use of skin twitch reflex latency (STRL) and avoidance to radiant heat (≤ 50°C) in the lumbar area. Skin temperature, cardiovascular and respiratory variables, and β-endorphin concentration in spinal fluid (CSF-EN) and plasma (plasma-EN) were measured.

**Results**—Acupuncture and EA significantly increased STRL and skin temperature. The CSF-EN was significantly increased from baseline values 30 to 120 minutes after onset of EA, but it did not change after acupuncture and control treatments. Heart and respiratory rates, rectal temperature, arterial blood pressure, Hct, total solids and bicarbonate concentrations, base excess, plasma-EN, and results of blood gas analyses were not significantly different from baseline values after acupuncture, EA, and control treatments.

**Conclusions and Clinical Relevance**—Administration of EA was more effective than acupuncture for activating the spinal cord to release β-endorphins into the CSF of horses. Acupuncture and PAES provided cutaneous analgesia in horses without adverse cardiovascular and respiratory effects. (*Am J Vet Res* 2002;63:1435–1442)

Acupuncture has been used for more than 3,000 years to prevent or modify the perception of pain (acupuncture analgesia), normalize physiologic functions, and treat clinical disorders or dysfunction (acupuncture therapy) in humans and domestic animals.[1] Techniques of acupuncture include conventional fine-needle insertion (baizeh), hemoacupuncture (xuezhen), fire needling (huozhen), and injection of air into an acupoint (qizhen).[2] Acupuncture points may be stimulated by needles manually (ie, acupuncture) or electrically (**electroacupuncture [EA]**). Although acupuncture has been used extensively in horses, only a few controlled studies[3-7,a] exist on the use of EA in horses and ponies. To our knowledge, direct comparative effects of acupuncture and EA in horses have not been reported.

Mechanisms of action of acupuncture and EA for producing analgesia have been reviewed elsewhere.[8-14] Analgesia may be produced by endogenous opioid peptides of pituitary origin (endorphins, enkephalins, and dynorphins) after electrical stimulation of certain receptor sites in the dorsal horn of the spinal cord[15-16] or by peripheral electrical stimulation of large sensory afferent nerves that modulate nociceptive input in the dorsal horn of the spinal cord (ie, the gate control theory of pain).[17-20]

Evidence exists to suggest that pro-opio melanocortin-derived peptides (ACTH, β-endorphin) are released in young horses during training[21] and in adult horses during anaerobic exercise[22] and periods of stress and pain,[23] in response to experimentally induced pain,[24] or when restrained with or without a nasogastric tube.[25,26] Such release produces a phenomenon known as stress-induced analgesia.[27,28] In addition, studies[7,29] in horses have revealed that inadequate tissue perfusion and hypoxia are major stimuli to the release of β-endorphin into plasma during anesthesia, similar to release during anaerobic exercise. Electroacupuncture increases plasma concentrations of β-endorphin and cortisol in horses, but the increase does not consistently correlate with the degree of cutaneous analgesia.[4]

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Electroacupuncture increases the content of immunoreactive β-endorphin in brain regions of rats[30] and in CSF of humans without pain[31-33] or humans with recurrent chronic pain[34] or heroin addiction.[35] To our knowledge, the effects of acupuncture and EA on β-endorphin concentrations in CSF of horses have not been evaluated. Our hypothesis was that EA produces greater cutaneous analgesia and higher β-endorphin concentrations in CSF and plasma of horses than does acupuncture alone. For this reason, the purpose of the study reported here was to evaluate cutaneous thermal nociception, hemodynamic and respiratory effects, and β-endorphin concentrations in CSF and plasma of resting conscious horses treated by use of acupuncture and EA.

## Materials and Methods

**Animals**—Eight healthy mares (5 Standardbreds, 3 Thoroughbreds) that were between 14 and 20 years of age and weighed between 470 and 600 kg were included in the study. At least 8 weeks before the study, the left carotid artery of each horse was surgically elevated to a subcutaneous position. Other surgery was not performed. The study protocol and experimental design were approved by The Ohio State University Laboratory Animal Care and Use Committee.

**Preparation of horses**—Each horse was restrained in a standing position in a stock in a quiet, climate-controlled room from 9:00 AM to 5:00 PM. Horses were considered healthy on the basis of results of physical examination, WBC count, and ECG. They were allowed to eat hay during the experiment. Prior to each experiment, hair over the area of the left carotid artery, right jugular furrow, and **lumbosacral (LS)** area was clipped, and the skin was prepared for aseptic placement of catheters. The skin and subcutaneous tissues in these areas were infiltrated with a 2% solution of lidocaine hydrochloride.[b] An 18-gauge, 30-cm catheter[c] was inserted into the left carotid artery for measurement of systemic **arterial blood pressure (ABP)** and for collection of arterial blood samples. Proper positioning of the arterial catheter was confirmed at the time of insertion and prior to blood pressure determinations by observing characteristic pressure waveforms on an oscilloscope. Arterial pressure waveforms were obtained by use of a calibrated strain-gauge transducer.[d] The point of the shoulder was used to determine the zero pressure point. Mean blood pressure in the carotid artery was obtained by electronic integration of the signal obtained from the blood pressure transducer. Surface ECG leads were placed for continual monitoring of a base-apex ECG, and a thermometer probe[e] was positioned approximately 20 cm into the rectum for determination of deep rectal temperature. A 30-cm-long piece of 240-polyethylene tubing was inserted into the right external jugular vein to enable collection of blood samples for radioimmunoassay of β-endorphin concentrations.

**Catheterization of the subarachnoid space**—A 17-gauge, 19.5-cm Huber point needle with stylet[f] was advanced along the median plane of the vertebral column until the LS subarachnoid space was reached, as identified by a free flow of CSF from the needle hub.[36] Distance from the skin to the lumen of the subarachnoid space was calculated by measuring the distance between the needle hub and skin puncture site and subtracting this value from the length of the needle. Approximately 0.5 mL of 2% lidocaine solution[b] was injected into the LS subarachnoid space during a 30-second period to prevent pain caused by impingement of the catheter tip and spring guide at the conus medullaris. The bevel of the needle was directed cranially, and a variable length (15.5 to 53 cm) of a 90-cm section of polyethylene tubing[g] with a spring guide[h] was threaded through the needle until resistance was felt, which generally was at the level of L1 to L6. The vertebral location of the catheter tip was determined by measuring the distance between the free end of the catheter and the surface of the skin as well as the distance between the skin and the LS subarachnoid space and then subtracting these values from the total length of the catheter.[36] The distance between the LS and thoracolumbar intervertebral spaces was obtained by palpation and measurement of the LS, lumbar, and thoracic intervertebral spaces, respectively.

**Acupuncture points and stimulation**—Acupuncture points Bladder 18, 23, 25, and 28 on both sides of the vertebral column were selected on the basis of our experiences when treating horses with musculoskeletal pain (**Fig 1**). These points were identified on the basis of anatomic landmarks that have been described by the International Veterinary Acupuncture Society.[37] In addition, each acupoint was verified by measuring the skin's resistance using the search electrode of an electroacupuncture stimulator[i] that displayed detection of a point by sound and meter. Hair over each acupuncture site was clipped, and the skin was prepared for aseptic placement of sterile, disposable, single-use acupuncture needles (28 gauge, 10 cm).[j] The needle sleeve guide was used to tap the needle tip through the skin. The guide was then removed, and approximately 9 cm of the needle was advanced vertically into the soft tissues. We were careful to avoid bending the needles. Passage of a straight needle was confirmed by palpation of minimal resistance to needle rotation. Needles were left in position during the entire 2-hour experiment.

For **percutaneous acupoint electrical stimulation (EA)**, 8 acupuncture needles were placed at the same locations as in the aforementioned acupuncture treatment (Fig 1). Needles then were connected to 4 bipolar leads (with each lead connected to 1 positive and 1 negative probe) connected to an EA stimulator.[k] Needles were stimulated by use of 4 volts, 1 milliamp, and 0.1 millisecond in biphasic square pulses and with 15 and 30 Hz alternating frequencies so that

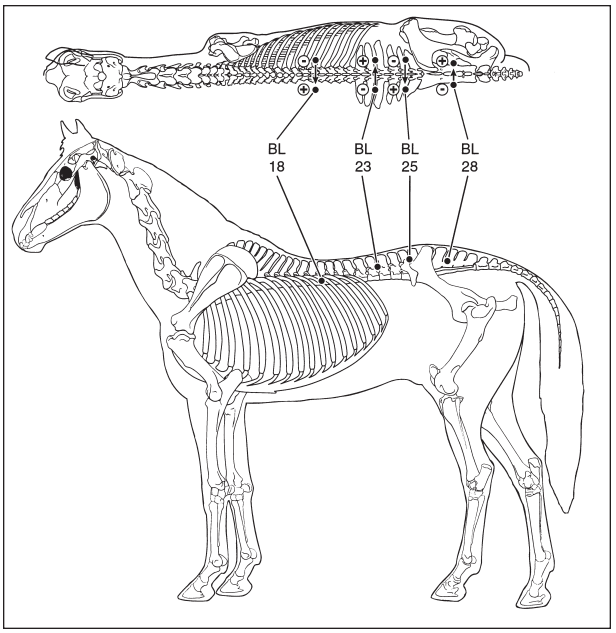


Figure 1—Illustration of the dorsal (top) and lateral (bottom) views of the equine skeleton indicating location of acupuncture points Bladder (BL) 18, 23, 25, and 28. Location for placement of a catheter at the lumbosacral junction (L6-S1) also is an acupuncture point (Governing Vessel 20; Bai hwi).

the current flowed across the vertebral column between the opposite leads throughout the 2-hour experiment. The current was adjusted as needed to produce small vibrations in the stimulated muscles.

Acupuncture needles were not inserted into horses for the control treatment. During the control treatment, horses were treated in a similar manner to during the acupuncture treatment. Horses were randomly assigned to the order in which they would receive control, acupuncture, and EA treatments. Horses were allowed at least 7 days between subsequent treatments.

**Experimental protocol**—After placement of catheters, rectal thermometer probe, and ECG leads, each horse was allowed to stand undisturbed for at least 60 minutes prior to initiation of treatment. Baseline measurements were obtained immediately before (time 0) and after insertion of acupuncture needles at Bladder 18, 23, 25, and 28 on both sides of the vertebral column of horses and immediately prior to electrical stimulation (EA treatment). Hemodynamic and respiratory measurements, scores of nociceptive pain threshold of the skin, and samples of CSF and venous blood were obtained immediately before (time 0) and 30, 60, 90, and 120 minutes after insertion of acupuncture needles (acupuncture treatment), electrical stimulation (EA treatment), and onset of control treatment. Time 0 for the control treatment was designated at a point at least 60 minutes after IV and subarachnoid catheterizations.

Catheters and needles were removed after completion of each treatment, and the horses walked back to their stalls. All horses were examined for neurologic deficits and evidence of infection at needle and catheter puncture sites 24 hours after insertion of acupuncture needles and catheters.

**Assessment of hemodynamic and respiratory effects**—**Heart rate (HR)** and rhythm were determined from the ECG. **Respiratory rate (RR)** was determined for each horse by counting thoracic and abdominal excursions during a 1-minute period. Arterial blood samples (2 mL/sample) were collected anaerobically into heparin-coated plastic syringes, using the catheter inserted in the carotid artery. Syringes were capped and placed in an iced water bath, and samples were analyzed within 20 minutes after collection for determination of $Pa_{O_2}$, $Pa_{CO_2}$, and pHa by use of a microprocessor blood gas analyzer.[l] All blood gas values were corrected on the basis of rectal temperature, which was measured immediately before collection of each blood sample. The Hct and concentration of **total solids (TS)** were measured in arterial blood samples by use of a microhematocrit method[m] and refractometer,[n] respectively. Standard bicarbonate concentration and **base excess (BE)** were calculated. Electrocardiograms and systolic, diastolic, and mean ABP were monitored on the oscilloscope and recorded simultaneously by use of a photographic recorder[o] at a rate of 10 mm/s at each sample collection time.

**Assessment of analgesia**—A specially designed instrument[p] with a heat device was used to determine response to thermal stimulation. The instrument consisted of a linear ramped-intensity incandescent bulb housed in a metal cylinder.[38] The heat device was manually held perpendicular to the center of a temperature probe[q] placed on the skin of the paralumbar fossa of the lumbar area (L1 to L5) from which hair had been clipped. Distance between the skin and heat device was 2.5 cm, and intensity of the lamp at maximal output was 36.4 watts. Analgesia was defined as an increase in response threshold, compared with baseline values, to application of noxious thermal stimuli at the lumbar site. Thermal stimulus was applied until a **skin twitch reflex (STR)** and **avoidance response (AR)** were noticed; AR included STR and any abrupt movement of the tail, limbs, or torso or turning the head toward the stimulus coincident with application of the stimulus. **Latency of radiant heat-evoked STR (STRL)** and lumbar skin temperature after thermal application were displayed on the heat-lamp analgesia meter and precision thermometer,[r] respectively, and values were recorded. The precision thermometer was calibrated to 37°C (standard calibration of the manufacturer) for rectal and skin temperature before each experiment. A stimulus of ≤ 50°C for < 20 seconds was used. This stimulus does not result in visible epidermal damage.[38] To avoid observer-dependent subjective differences, the same investigator (RTS) performed scoring of threshold responses in all horses.

**Collection of CSF and plasma samples**—Five milliliters of CSF and 8 mL of venous blood were collected via catheters into chilled syringes and transferred into chilled polypropylene tubes[s] containing 0.5 and 0.8 mL, respectively, of 1.0 trypsin inhibitory units of aprotinin,[t] 0.5% bacitracin,[u] and 80mM EDTA, to achieve final concentrations of 0.10 trypsin inhibitory units of aprotinin, 0.05% bacitracin, and 8mM EDTA per milliliter of CSF and venous blood. Samples were centrifuged[v] at 7,000 × *g* for 7 minutes at 4°C, and supernatants were removed and stored at –70°C. Opioid peptides were extracted by use of 1% trifluoroacetic acid (buffer A) and 60% acetonitrile in 1% trifluroacetic acid (buffer B), as described elsewhere.[39-41]

**Extraction of β-endorphin from CSF and plasma**—A column[w] containing 200 mg of $C_{18}$ was equilibrated by washing it once with 1 mLof buffer B followed by 3 washes with buffer A (3 mL/wash). Vacuum pressure was not applied to the column during these washes.

The CSF and plasma samples were diluted by addition of an equal amount of buffer A. Samples were centrifuged at 10,000 × *g* for 20 minutes at 4°C. The CSF or plasma samples diluted with buffer A were loaded onto the washed $C_{18}$ column. The column was slowly washed twice with buffer A (3 mL/wash), and the wash solutions were discarded. Opioid peptides were slowly eluted with 3 mL of buffer B, and the eluant was collected in a polypropylene tube. A light vacuum pressure was applied to the column during these washes and elution. Eluant was evaporated to dryness by use of a lyophilizer. Lyophilized residue was analyzed by use of **radioimmunoassay (RIA)**.[39-41] Recovery rates for β-endorphin from CSF and plasma were ≥ 90%.

**Assay of β-endorphin concentrations**—The β-endorphin concentrations were quantitated by comparison with β-endorphin standards. Anti-β-endorphin antisera were produced in rabbits and were extremely sensitive. Cross-reactivity of antisera was 100% for β–h-endorphin and β–c-endorphin, 36% for β–h-lipotropin, and < 0.01% for β-endorphin, β-melanocyte stimulating hormone, met-enkephalin, and leu-enkephalin. Lyophilized residue was dissolved in RIA buffer containing 100mM phosphate buffer (pH 6.0), 50mM NaCl, 5mM EDTA, 0.1% gelatin, 0.1% Triton X-100, and 0.025% thimerosal. Samples of β-endorphin standards (1 to 1,000 pg in assay buffer) were incubated for 24 hours at 4°C in polypropylene tubes with 100 µL of antiserum diluted in assay buffer. This was followed by addition of 100 µL of $^{125}$I-β-endorphin (approx 5,000 counts/min/tube), and tubes were incubated for an additional 16 to 18 hours. Separation of free and antibody-bound endorphin was accomplished by adding 0.5 mL of assay buffer containing 1.6% charcoal and 0.16% dextran T-70 to each tube. Samples were centrifuged,[x] and radioactivity of the supernatant was counted in a gamma counter[y] for 2 minutes. Intra- and interassay coefficients of variation were 5 and 12%, respectively. All samples from each horse were analyzed in a single assay.

**Statistical analysis**—Nociceptive skin pain threshold (ie, STRL), skin temperature before and after thermal stimulation, hemodynamic and respiratory variables, and β-endorphin concentrations in CSF and plasma were expressed as mean ± SD for each treatment. Mean values were compared among and within treatments over time by use of a 2-way ANOVA with repeated measures on 2 factors (time and treatment) and a Dunnett *t* test.[z] Values of $P < 0.05$ were considered significant.

## Results

All of the horses tolerated the procedures well. None of the horses developed neurologic deficits or infections at needle or catheter puncture sites 24 hours after IV or subarachnoid placement of catheters or insertion of acupuncture needles.

Distance from the skin to the lumen of the subarachnoid space of the 8 horses for the 24 experiments varied between 12.0 and 14.5 cm (mean ± SD, 13.0 ± 0.6 cm). The subarachnoid space was readily catheterized in all horses. On the basis of differences in resistance, the catheter was easily threaded within the subarachnoid space for a variable distance, extending 34 cm from the LS intervertebral space to the thoracolumbar (T18-L1) intervertebral space in 1 horse during acupuncture treatment, 25 to 28 cm from the LS intervertebral space to L2 in 3 horses during acupuncture treatment and 4 horses during EA and control treatments, 15 to 19 cm from the LS invertebral space to L3 in 4 horses during acupuncture and EA treatments and 3 horses during control treatments, and 15 cm from the LS intervertebral space to L4 in 1 horse during acupuncture treatment.

Baseline mean ± SD latency of radiant heat-evoked (≤ 50°C) STRL in the lumbar area of 8 horses before acupuncture, EA, and control treatments was 14.1 ± 2.0, 14.3 ± 1.1, and 14.6 ± 1.8 seconds, respectively. The STRL significantly ($P < 0.001$) increased to 17.6 ± 2.5 and 19.8 ± 0.5 seconds 30 minutes after beginning treatment with acupuncture and EA, respectively (**Fig 2**). Mean STRL was also significantly ($P < 0.001$) increased 30 to 90 minutes after onset of acupuncture and EA treatments, compared with values for the control treatment. Mean STRL did not change significantly throughout the 2-hour control treatment.

Horses tolerated significantly ($P < 0.001$) higher mean skin temperature in the lumbar area during acupuncture and EA treatments, compared with baseline values or the control treatment (**Fig 3**). During control treatment, horses did not tolerate thermal stimuli above mean baseline value throughout the 2-hour period. Mean ± SD skin temperature of horses at onset of STR after thermal application (maximum, 50°C) was 40.7 ± 2.7, 41.4 ± 2.7, 40.3 ± 2.7°C before and 45.3 ± 2.7, 48.6 ± 2.5, and 38.9 ± 2.9°C after acupuncture, EA, and control treatments, respectively.

Similarly, mean difference of skin temperature in the lumbar area before and after thermal application was significantly ($P < 0.001$) greater in horses 30 to 120 minutes after acupuncture and EA treatments, compared with baseline values (**Fig 4**). Mean difference of skin temperature did not change significantly during the 2-hour control treatment.

Mean β-endorphin concentration in CSF of horses

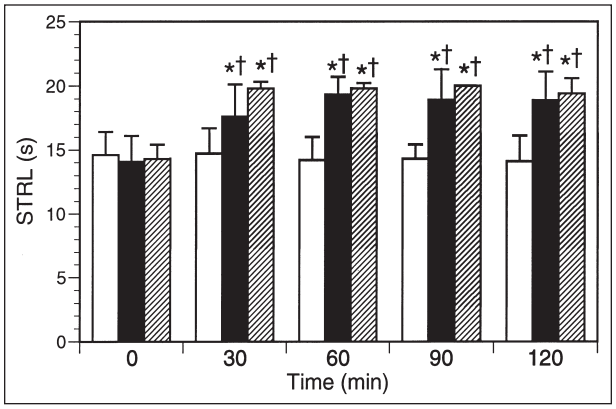


Figure 2—Mean ± SD latency of radiant heat-evoked skin twitch reflex (STRL) in 8 mares immediately before (time 0) and 30, 60, 90, and 120 minutes after onset of acupuncture (black bars), percutaneous acupoint electrical stimulation (EA; striped bars), or no needle placement (control treatment; white bars). A thermal stimulus of ≤ 50°C was applied to the paralumbar area; STRL was defined as the stimulation time required to induce an avoidance response. *Within a treatment, value is significantly ($P <$ 0.001) different from baseline (ie, time 0) value. †Within a time period, value is significantly ($P < 0.05$) different from value for control treatment.

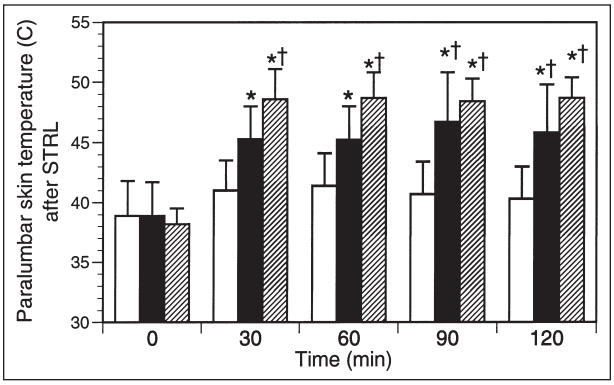


Figure 3—Mean ± SD paralumbar skin temperature in 8 mares before (time 0) and 30, 60, 90, and 120 minutes after onset (time 0) of acupuncture (black bars), EA (striped bars), or control treatment (white bars). A thermal stimulus of ≤ 50°C was applied for < 20 seconds to the paralumbar area until STRL was defined. *See* Figure 2 for key.

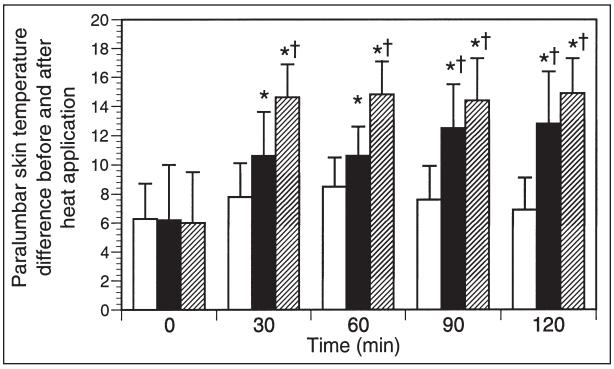


Figure 4—Mean ± SD difference in paralumbar skin temperature before and after thermal application in 8 mares before (time 0) and 30, 60, 90, and 120 minutes after onset of acupuncture (black bars), EA (striped bars), or control treatment (white bars). A thermal stimulus of ≤ 50°C was applied for < 20 seconds to the paralumbar area until STRL was defined. *See* Figure 2 for key.

was 18.9 ± 4.0, 17.5 ± 3.9, and 20.4 ± 4.5 pg/mL before and 21.0 ± 2.8, 23.4 ± 4.5, and 19.8 ± 4.1 pg/mL 30 minutes after initiation of acupuncture, EA, and control treatments, respectively. The β-endorphin concentrations in CSF were significantly increased from baseline values 30 to 120 minutes after onset of EA, and the peak concentration (26.1 ± 6.2 pg/mL) 120 minutes after onset of EA was significantly different from the peak concentration for the control treatment (**Fig 5**). The β-endorphin concentrations in CSF did not change significantly after initiation of acupuncture and control treatments.

Mean concentration of β-endorphin in venous plasma of horses was 41.2 ± 9.2, 41.0 ± 38.8, and 38.9 ± 8.9 pg/mL before and 42.2 ± 9.4, 44.3 ± 9.3, and 37.8 ± 7.7 pg/mL 60 minutes after initiation of acupuncture, EA, and control treatments, respectively. Plasma β-endorphin concentrations did not change significantly from baseline values or over time for the control treatment (**Fig 6**).

Mean HR of 8 mares before acupuncture, EA, and control treatments was 34.1 ± 3.5, 34.4 ± 2.9, 35.7 ± 5.9 beats/min, respectively, whereas mean RR was 25.7 ± 5.0, 22.7 ± 5.0, 26.0 ± 4.8 breaths/min, respectively, and mean rectal temperature was 37.6 ± 0.2, 37.6 ± 0.2, and 37.6 ± 0.2°C, respectively. Mean ABP before acupuncture, EA, and control treatments was 123 ± 11, 119 ± 10, and 127 ± 8.6 mm Hg, respectively, whereas mean Hct was 33 ± 3, 32 ± 2, and 33 ± 3%, respectively, mean $Pa_{O_2}$ was 100 ± 7, 103 ± 5, and 101 ± 8 mm Hg, respectively, mean $Pa_{CO_2}$ was 42 ± 1.3, 43 ± 2.8, and 40 ± 5 mm Hg, respectively, and mean pHa was 7.42 ± 0.02, 7.42 ± 0.02, and 7.43 ± 0.02, respectively. Mean bicarbonate concentration before acupuncture, EA, and control treatments was 26.5 ± 2.5, 25.7 ± 2.0, and 26.1 ± 2.4 mEq/L, respectively, whereas BE was 2.4 ± 2.3, 2.4 ± 2.2, and 2.0 ± 2.1 mEq/L, respectively, and TS concentration was 6.5 ± 0.3, 6.4 ± 0.2, and 6.4 ± 0.2 mEq/L, respectively. None of the values for these variables differed significantly from baseline values after onset of acupuncture, EA, and control treatments.

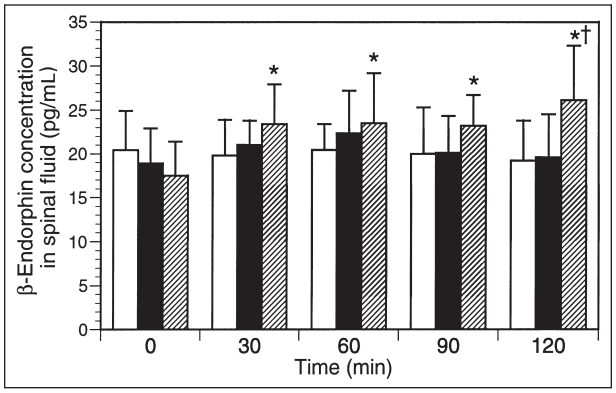


Figure 5—Mean ± SD β-endorphin concentration in CSF of 8 mares before (time 0) and 30, 60, 90, and 120 minutes after onset of acupuncture (black bars), EA (striped bars), or control treatment (white bars). *See* Figure 2 for key.

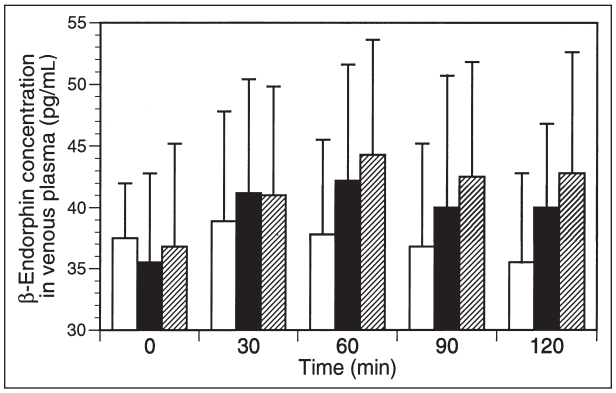


Figure 6—Mean ± SD β-endorphin concentration in venous plasma of 8 mares before (time 0) and 30, 60, 90, and 120 minutes after onset of acupuncture (black bars), EA (striped bars), or control treatment (white bars). *See* Figure 2 for key.

## Discussion

Acupuncture and EA applied at acupoints Bladder 18, 23, 25, and 28 on both sides of the vertebral column of horses significantly increased the cutaneous pain threshold in the lumbar area and minimally affected hemodynamic and respiratory variables. The β-endorphin concentrations in CSF of horses were significantly increased from baseline values at 30 to 120 minutes after onset of EA but did not change after onset of acupuncture and control treatments. Plasma β-endorphin concentrations of horses tended to increase after onset of acupuncture and EA treatments; however, the changes were not significant because of large variations and differences in responses within and among horses. It is apparently more useful to measure endorphin concentrations in cerebral structures known for their abundance of opioid-sensitive receptors, compared with measurement of concentrations in plasma.

A radiant-heat analgesia meter was able to detect analgesia in the lumbar area of all horses during treatment with acupuncture and EA. The use of a slow (20 seconds) ramping of thermal intensity was used to induce subtle increases in stimulation and make the pain threshold more obvious. We did not know whether horses reacted after or at the exact moment at which the thermal stimulus became painful. We accounted for the wide range of pain tolerance or perception among horses by comparing the pain threshold values before and after acupuncture, EA, and control treatments in the same horse at each time period. Application of EA, compared with acupuncture, produced greater STRL on the basis of stimulation time required to induce an avoidance response, thereby causing greater skin temperature after thermal stimulus (maximum, 50°C) and greater difference in skin temperature before and after thermal application.

Although our data did not permit an unequivocal definition of the mechanism of cutaneous analgesia induced by acupuncture and EA, it is probable that analgesia was produced by opioid and nonopioid endocrine mechanisms.[8,42,43] Increased STRL and skin surface temperature of horses developed within 30 minutes after onset of EA stimulation and before significant increases of β-endorphin concentrations in CSF samples were detected, indicating that nonendorphinergic mechanisms were involved in the early phase of increased cutaneous pain threshold. Similarly,

increased STRL and associated increased skin temperature of horses 30 to 120 minutes after initiation of acupuncture stimulation were not accompanied by significant changes in β-endorphin concentrations in CSF, indicating that acupuncture involves nonendorphinergic mechanisms to produce increased cutaneous pain threshold in horses.

Other endogenous opioids (met-enkephalin, dynorphin), neurotransmitters, and neuromodulators, including serotonin, noradrenaline, dopamine, γ-amino butyric acid, glutamate, and vasoactive intestinal peptides, were not assessed in the study reported here. They could have been involved in the analgesic effect of acupuncture and EA and require further investigation.[8,33,43-45]

Because inserting a needle anywhere in the body may involve some degree of afferent sensory stimulation, it is necessary to define the sites of tissue and neural elements involved and the frequency, intensity, or waveform modulation of stimulation. The location, intensity, and frequency of electrical stimulation are important determinants of analgesic efficacy in humans and domestic animals. Several studies[42,46,47] have documented that electrical stimulation with differing frequencies (Hz) at traditional acupuncture sites induces the release of various opioid peptides (β-endorphin, met-enkephalin, dynorphin) in the spinal cord of rats and results in antinociceptive effects for several methods of pain measurement (tail-flick latency to radiant heat, hot plate, hot water, paw pressure) via various opioid receptors.

We stimulated acupoints Bladder 18, 23, 25, and 28 to stimulate the release of β-endorphin from corresponding spinal nerves T13, L2, L5, and S2, respectively. Other investigators have stimulated several acupoints on the dorsum of horses by use of acupuncture with dry needles,[48] acupuncture with moxibustion (warm-needling acupuncture),[49] injection of saline (0.9% NaCl) solution in acupoints (aquapuncture),[50] EA,[51] and low-powered infrared lasers[52,53] with varying degrees of success in the management of back pain in horses, although there is little scientific information available from well-controlled clinical studies to support such treatments.

Acupuncture needles were inserted to almost full depth (9 cm) bilaterally at acupoints Bladder 18, 23, 25, and 28 of horses to stimulate the cutaneous branches and underlying tissues supplied by the thirteenth thoracic (Bladder 18), second lumbar (Bladder 23), fifth lumbar (Bladder 25), and second sacral (Bladder 28) spinal nerves, respectively. Because thermal stimuli were applied between L2 and L5, which is also where acupuncture points Bladder 23 and 25 were stimulated, it is possible that the needles with and without electrical current stimulated A-β fibers and inhibited small C fibers, thereby interrupting (or gating) pain input into the CNS.[17,18,20] In addition, CSF was collected via a catheter from spinal cord segments L1 to L4 that matched the region in which heat (≤ 50°C) was applied. We believe that electrical stimulation at the specific vertebral levels corresponding to the local pathologic process (ie, thermal application) produces greater short-term improvements in pain control than electrical stimulation at sites distal to local pathologic processes. Acupuncture and EA treatments at the site of thermal application could have been responsible for the consistent increase in STRL in horses.

In the study reported here, a mixed pattern of 15 and 30 Hz of alternating frequencies (ie, dense-disperse mode electrical stimulation) was used in horses. Varying the frequency of the electrical stimulus allegedly can decrease accommodation (ie, adaptation) and improve analgesic effects produced by peripheral electrical stimulation.[11] A similar mixed pattern (15 and 30 Hz; dense-disperse model) of electrical stimulation in humans has produced the greatest decrease in pain, improvement in physical activity, quality of sleep, and decrease in requirements for orally administered medications at the end of a 2-week treatment period in patients with chronic lower lumbar back pain when compared with use of low (4 Hz) or high (100 Hz) frequencies alone.[54]

In the study reported here, acupuncture and EA treatments shared the similarity of stimulating the same acupoints (Bladder 18, 23, 25, and 28) and produced cutaneous analgesia in horses without adverse hemodynamic and respiratory effects. However, significant differences were detected between the 2 neuromodulation techniques in that EA stimulated a field or a particular segmental distribution rather than a unique acupoint and was more effective than acupuncture in activating a horse's pain modulation system at the spinal cord to release β-endorphin into the CSF. Studies[55] of experimentally induced pain in human volunteers confirm that the use of needles alone will provide some relief of pain and that the addition of electricity to needles is twice as effective in producing analgesic effects.

Plasma concentrations of β-endorphin increased slightly in horses in the study reported here, indicating minimal activation of the pituitary ACTH-adrenocortical axis, which could have been related to stress.[23] However, the horses were calm and cooperative, and HR, RR, and ABP were within reference ranges throughout the 2-hour acupuncture and EA treatments, which is inconsistent with the possibility that acupuncture- and EA-induced analgesia were induced by stress.

Our findings are in agreement with those of a study[7] in which 5 adult ponies were given EA at the Lung 1 (Qiang Feng), Bladder 23, and Stomach 36 (Tsu San Li) acupoints, using 9 Hz for 30 minutes followed by 55 Hz for 20 minutes. In that study, significant changes were not detected in plasma concentrations of β-endorphin, met-enkephalin, dynorphin, arginine vasopressin, ACTH, cortisol, dopamine, adrenaline, noradrenaline, glucose, and lactate, which was attributed to large variations within ponies and differences in response among ponies.

Electroacupuncture in horses reportedly can increase the cutaneous pain threshold and plasma concentration of β-endorphin.[4] However, we did not detect a good correlation between the plasma concentration of β-endorphin and amount of cutaneous analgesia produced. In another study,[5] variations in plasma concentrations of β-endorphin depended on sex of horses and locus of stimulation. In our study, only adult mares

were used, and the same acupuncture loci were stimulated during acupuncture and EA, producing consistent cutaneous analgesia at the lumbar area in all horses without significant increases in plasma concentrations of β-endorphin.

Horses of the study reported here appeared relaxed and drowsy after subarachnoid catheterization and during acupuncture and EA, and relaxation after EA was more obvious than during acupuncture and control treatments. The spinal needles used for catheterization were inserted on the dorsal midline at the LS junction, an important acupoint (Governing Vessel 20; Bai hui), which may have triggered the unique sedative behavior commonly seen in horses after acupuncture at the Bai hui acupoint. Acupuncture stimulation for 10 minutes at the Bai hui acupoint in 14 horses has been used to produce sedation and a decrease of serum cortisol concentration (from 2.6 ± 1.9 to 1.9 ± 1.7 mg/100 mL; 27%) 60 minutes after initiation of the acupuncture treatment.[56]

Horses in our study also had higher concentrations of β-endorphin in the CSF during EA treatment, compared with concentrations during acupuncture and control treatments. We did not determine the plasma cortisol concentrations in horses in this study. Because β-endorphin and ACTH are derived and concurrently released from the pituitary gland from the same precursor (ie, pro-opiomelanocortin[57]), and because secretion of cortisol is mainly under regulation of ACTH, it is understandable that excitement of horses during exercise, restraint, and use of a nasogastric tube has been associated with increased plasma concentrations of β-endorphin and cortisol.[4,25,26]

To our knowledge, concentrations of β-endorphin in CSF and plasma necessary to produce analgesia and the pharmacokinetics and pharmacodynamics of β-endorphin in CSF and plasma in horses have not been reported. In the study reported here, we documented that EA activated, at least in part, CNS endorphinergic circuitry, which resulted in an increased β-endorphin concentration in the CSF.

Acupuncture and EA (15 and 30 Hz; dense-dispersion model) produced cutaneous analgesia with minimal circulatory and respiratory disturbances in conscious, standing horses. Application of EA was more effective than acupuncture in activating the pain modulation system at the level of the spinal cord to release β-endorphin into the CSF. Additional studies are necessary to determine the critical concentrations of β-endorphin in the CSF and plasma that are necessary to produce analgesia in horses with pathophysiologic conditions.

[a]Xie H, Ott EA, Harkins JD, et al. Influence of electroacupuncture stimulation on pain threshold and neuroendocrine responses in horses, in *Proceedings*. 24th Annu Int Cong Vet Acupunct, 1998;167.

[b]Lidocaine 2% injectable, Phönix, Scientific Inc, St Joseph, Mo.

[c]Vialon polymer resin radiopaque intracath, Deseret Medical Inc, Sandy, Utah.

[d]Gould Statham Instruments Inc, Hato Ray, Puerto Rico.

[e]YSI 400 probe tele-thermometer, Yellow Springs Instrument Co, Yellow Springs, Ohio.

[f]Tuohy needle, Becton-Dickinson Co, Rutherford, NJ.

[g]Formocath, Becton-Dickinson Co, Rutherford, NJ.

[h]USCI, CR Bard Inc, Covington, Ga.

[i]ITO 4107, M. E. D. Servi-Systems Canada LTD, Stittsville, ON, Canada.

[j]Cherry handy acupuncture needle, M. E. D. Servi-Systems Canada LTD, Stittsville, ON, Canada.

[k]ITO IC 4107, M. E. D. Servi-Systems Canada LTD, Stittsville, ON, Canada.

[k]ABL 500-K pH and blood gas analyzer, Radiometer-Copenhagen, Copenhagen, Denmark.

[l]Criticaps, micro-hematocrit capillary tube reader, Monoject Scientific, St Louis, Mo.

[m]10436 Veterinary refractometer, Cambridge Instruments, Buffalo, NY.

[o]Simultrace recorder model VR-12, Electronics for Medicine, Pleasantville, NY.

[p]Heat lamp analgesia meter, Columbus Instruments International Corp, Columbus, Ohio.

[q]408 Banjo surface probe, Yellow Springs Instrument Co, Yellow Springs, Ohio.

[r]YSI Precision 4000 A thermometer, Yellow Springs Instrument Co, Yellow Springs, Ohio.

[s]Cryotubes, Nunc Brand Products, Naperville, Ill.

[t]Aprotinin, Sigma Chemical Co, St Louis, Mo.

[u]Bacitracin, Sigma Chemical Co, St Louis, Mo.

[v]Juan Laboratory Equipment, Juan Inc, Winchester, Va.

[w]SEPCOL1, Peninsula, San Carlos, Calif.

[x]Beckman TJ-6 centrifuge, Beckman, Palo Alto, Calif.

[y]Gammacounter, Beckman, Palo Alto, Calif.

[z]Systat for Windows, version 5.2, Statistics, Evanston, Ill.

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