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Sympathetic Effects of Manual and Electrical Acupuncture of the Tsusanli Knee Point: Comparison with the Hoku Hand Point Sympathetic Effects

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Sympathetic effects of manual and electrical acupuncture of the Tsusanli knee point were evaluated by thermography in 19 normal subjects under the same procedure used in a previous study using the Hoku hand point. A generalized long-lasting warming (sympathetic inhibition) effect was observed under manual and electrical acupuncture of the Tsusanli point. In addition, a segmentally related short-lasting cooling (sympathetic activation) effect occurred with Tsusanli electrical acupuncture only. The warming effect is consistent with the results of the Hoku study and appears to be a central sympathetic inhibition evoked by acupuncture. The cooling effect was segmentally related to the acupuncture site in both studies. This cooling effect most likely reflects a segmental activation of vasomotor spinal reflexes and not a general emotional arousal. These sympathetic mechanisms may be functionally correlated with central and peripheral mechanisms of acupuncture analgesia.

INTRODUCTION

Although acupuncture analgesia has been studied extensively, very few systematic control studies have examined acupuncture sympathetic effects. Kaada (17) reported widespread vasodilation in the human induced by low-frequency transcutaneous nerve stimulation, an acupuncture-related technique. In pharmacological studies, he demonstrated the participation of cen-

Abbreviations: EA, MA—electrical, manual acupuncture; T_{sk}—skin temperature.

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tral, serotonin-mediated sympathoinhibition, which was not antagonized by conventional low doses of naloxone (17, 18). In addition, the effectiveness of acupuncture analgesia has been reported to correlate with the degree of cutaneous vasodilation (29). Our purpose was to evaluate, in a control study, the sympathetic effects of acupuncture as evidenced by skin temperature changes. The site of stimulation as well as the acupuncture technique were taken into account, because differential effects were expected, as had been suggested by analgesic studies. Manual acupuncture induces long-lasting generalized, opioid-mediated analgesia (3, 8, 26) and transcutaneous electrical stimulation induces transient segmental nonopioid-mediated analgesia (8, 27, 33, 37). The mechanism of footshock-induced analgesia (FSIA) has been shown to be dependent on the body region shocked: front-paw FSIA was opioid-dependent whereas hind-paw FSIA was nonopioid-dependent (38).

In a preliminary report (24) we demonstrated by thermography a skin temperature (T_{sk}) rise in both hands of three subjects after unilateral Hoku manual acupuncture, which suggested a sympathetic inhibitory effect. To verify and further evaluate this effect, a control study of the effect of manual and electrical acupuncture of the Hoku hand point was undertaken in 19 normal subjects (11). The T_{sk} of three different areas of the body—face, hand, and foot—was recorded. The results showed a combination of two sympathetic effects as follows.

(i) A generalized long-lasting warming sympathetic inhibitory effect, which was stronger with MA than with EA, and which predominated in the face area. The participation of a segmentally related sympathetic inhibition could not be ruled out upon the assumed neural connections between the Hoku hand point (C7) and the face area (spinal trigeminal tract) (10).

(ii) An initial short-lasting cooling sympathetic activation effect which appeared in the feet for both MA and EA, and in hands only in EA. This sympathetic activation is consistent with the emotional stress associated with acupuncture initiation, but may also represent a segmentally related sympathetic activation evoked by EA only.

Because that study did not permit differentiation between spinal and central effects, nor did it give any information as to the specificity of action of the acupuncture point, we continued the experiment with a different acupuncture point, nonsegmentally related to the Hoku point. The Tsusanli knee point was selected, because it is, together with the Hoku hand point, one of the most commonly used points in acupuncture treatment (2, 21, 23).

The double sympathetic effect (11), which may partly account for the conflicting results in the literature concerning acupuncture sympathetic effects (7, 21–23, 25), is discussed in light of the mechanisms of action of acupuncture analgesia.

MATERIAL AND METHOD

The procedure was fully described in the previous report (11). Nineteen normal subjects (32 ± 8 years old; 17 female and 2 male) participated in a total of 95 sessions in five conditions: control, Hoku MA, Hoku EA, Tsusanli MA, and Tsusanli EA. The Tsusanli conditions were studied 3 weeks after the Hoku conditions. The Tsusanli sessions were conducted at 1-week intervals, at the same time of day for each subject, and were randomly distributed. The subjects were instructed not to eat, drink, or smoke at least 2 h prior to the session. A specially designed air conditioner maintained the experimental room at a constant temperature of 23°C with minimal air draft. Ambient temperature constancy was monitored every 5 min through temperature probes placed on each side of the subject. The subjects rested in the experimental room for 20 min prior to the initiation of the temperature recordings to stabilize physical and emotional states.

Temperature of hands, feet, and face were recorded by an infrared color thermograph (Inframetrics, model 525). Description of the equipment was detailed elsewhere (24). Each session lasted about 1 h. After the 20-min rest period, color slides of the thermograms of the left and right hand dorsa, the left and right foot dorsa, and the face were taken every 5 min for 30 min.

In the acupuncture sessions, a sterile Chinese acupuncture needle was inserted in the left Tsusanli point (motor point of the tibialis anterior muscle, 1 cm below and 2 cm outside the tibial crest) (2) immediately after the first temperature recordings and was removed 15 min later (t_{15}). This is similar to the Hoku sessions, during which the acupuncture needle was inserted in the first intermetacarpal space of the left hand. Temperature recordings were continued for another 15-min period (t_{30}). MA consisted in twirling the needle between temperature recordings, until the subject experienced a painful sensation. EA was delivered to the acupuncture needle by a constant current stimulator. Rectangular, 0.8-ms pulses at 1-Hz frequency were delivered at an intensity strong enough to evoke muscle twitching and the sensation of tapping or pounding just below pain threshold (7 to 15 mA).

In the control sessions, the subjects were resting quietly during the entire period of temperature recordings (30 min).

Data were collected by the method described elsewhere (11). The mean T_{sk} of each body segment was calculated by averaging the temperature of standardized smaller areas, including forehead, nose, and cheeks for the face, phalangeal, metacarpal, and carpal areas for the hand, and phalangeal, metatarsal, and tarsal areas for the foot (11). Left and right hands, as well as left and right feet, did not show any statistical difference in T_{sk} changes. Therefore, they were analyzed, respectively, as a unit by averaging the temperatures of the left and right extremities.

TABLE 1

Face, Hand, and Foot Basal (t_0) Skin Temperatures in Five Conditions[a]

	Control	Hoku MA[b]	Hoku EA	Tsusanli MA	Tsusanli EA	Average of five conditions
Face	33.9 ± 0.4	33.5 ± 0.6	33.6 ± 0.6	33.8 ± 0.5	33.8 ± 0.4	33.7 ± 0.1
Hand	32.6 ± 1.5	32.2 ± 1.7	32.0 ± 1.5	32.5 ± 1.4	32.1 ± 1.7	32.3 ± 0.2
Foot	30.6 ± 1.6	30.4 ± 2.1	30.4 ± 1.5	30.2 ± 2.4	30.1 ± 2.3	30.3 ± 0.2

[a] Values are $\bar{x} \pm$ SD degrees Celsius. $N = 19$ in each condition.
[b] Abbreviations: MA, EA—manual, electrical acupuncture.

T_{sk} changes were calculated at 5, 10, and 15 min by subtracting values carefully determined previously in a control session (11).

A three-factor analysis of variance compared T_{sk} changes in the face, hand and foot, in 19 subjects, at 5 min (t_5), 15 min (t_{15}), and 30 min (t_{30}) after the beginning of the T_{sk} recording, and within five conditions (control, Hoku MA, Hoku EA, Tsusanli MA, and Tsusanli EA). Planned paired comparisons (LSD Fischer test) were made among the means (20). The criterion of significance was set at $P < 0.05$.

RESULTS

Table 1 shows that the initial mean T_{sk} (t_0) of hands, feet, and face in the Tsusanli sessions was similar to those in the Hoku sessions (11). This indicated that the 20-min rest period was adequate to establish homogenous basal T_{sk} within all five conditions, and supported the validity of the comparisons between the Hoku and the Tsusanli acupuncture effects. The analysis of variance indicated that all effects and interactions were significant within the five conditions (Table 2). The effects of Tsusanli acupuncture are shown

TABLE 2

Significances of Effects and Interactions of the Three-Factor Analysis of Variance within All Five Conditions

	F	*P*
Type × cond.	2.312	0.024
Type × cond.	6.678	0.001
Type × cond.	4.546	0.001
Type × cond × time	1.570	0.016

Note. Type: Hand, foot, face. Time: t_5, t_{15}, t_{30} minutes. Cond: Control, Hoku MA, Hoku EA, Tsusanli MA, Tsusanli EA.

in Fig. 1. Tsusanli MA induced a generalized warming effect which was maximum at the end of the session (15 min after acupuncture termination) and was most significant in the face (LSD = 0.70; $P < 0.001$), less significant in the hands (LSD = 2.0; $P < 0.01$), and did not attain the criterion of significance in the feet (LSD = 1.2; NS).

Tsusanli EA induced two kinds of T_{sk} changes: a T_{sk} increase in the face which was maximum at the end of the session (LSD = 0.70; $P < 0.001$), and a segmentally related T_{sk} decrease in the feet which was maximum initially (LSD = 0.91; $P < 0.01$).

Comparison of the mean T_{sk} changes between Tsusanli MA and EA indicated that MA induced a significantly greater T_{sk} increase in the face and hands than did EA. Opposite T_{sk} changes were produced in the feet, i.e., increased T_{sk} with MA and decreased T_{sk} with EA.

T_{sk} changes with Hoku hand point acupuncture and Tsusanli knee point acupuncture are compared in table 3. The MA of both points induced a T_{sk} increase that was maximal at the end of the session (t_{30}), most significant for the face ($F(4,72) = 24.26$; LSD = 0.70; $P < 0.001$), less significant for the hands ($F(4,72) = 3.07$; LSD = 2.01; $P < 0.01$), and did not attain the criterion of significance for the feet ($F(4,72) = 2.77$; LSD = 1.3; NS); the only statistical difference between the MA effects of both points appeared in the magnitude of the T_{sk} increase in the face, with the Hoku hand point yielding the strongest effect. The EA of both points induced a T_{sk} increase that was maximal at the end of the session and most significant for the face; again the face T_{sk} increase was significantly greater with the Hoku point than with the Tsusanli point ($F(4,72) = 24.26$; LSD = 0.70; $P < 0.001$). Opposite T_{sk} changes were found in the hands and feet: in the hands, Hoku point EA decreased T_{sk}, whereas the Tsusanli point EA increased T_{sk} (at t_5); in the feet, the Hoku point EA increased T_{sk}, and the Tsusanli point decreased the T_{sk} (at t_{30}).

DISCUSSION

Compared with the control condition, Tsusanli MA and Tsusanli EA induce a generalized long-lasting warming (sympathetic inhibition) effect, which is distributed according to a craniocaudal gradient, i.e., a maximum effect in the face, which is stronger with MA. In addition, Tsusanli EA induces an initial segmentally related cooling effect (sympathetic activation) in the feet that decreases during the remainder of the session.

The finding of a central warming sympathetic inhibition (generalized warming effect) confirms previous results with the Hoku point (11). The similar temporal course and spatial distribution after stimulation of either a knee point or a hand point is consistent with the hypothesis of the activation of a central inhibitory system. The somatotopicity of this system is unrelated to

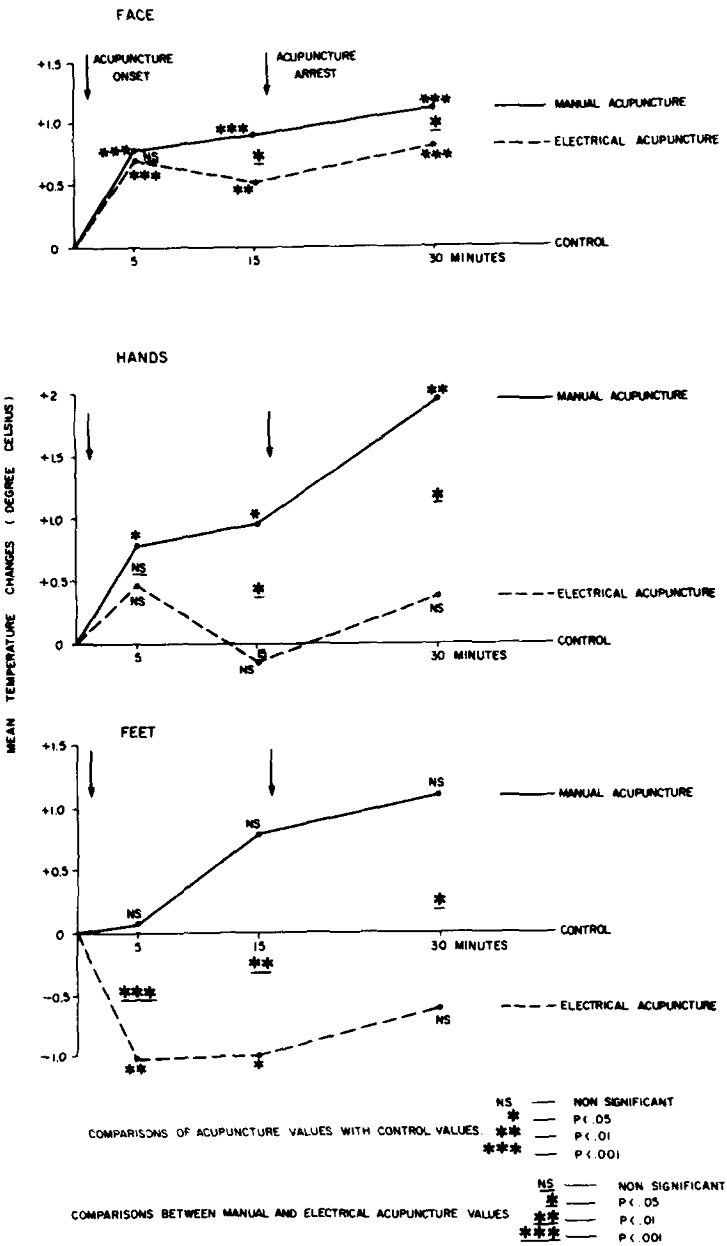

FIG. 1. Mean skin temperature changes during manual and electrical acupuncture of the Tsu-sanli knee point, with the control values as reference ($N = 19$).

TABLE 3

Mean Skin Temperature Changes[a]

	Hoku point	Tsusanli point	Comparison of points
Manual acupuncture			
Time: 5 min			
Face	+0.9 ± 0.06***	+0.8 ± 0.5***	NS
Hands	+0.4 ± 0.3	+0.8 ± 0.2*	NS
Feet	−0.4 ± 0.2	+0.1 ± 0.3	NS
Time: 30 min			
Face	+1.6 ± 0.1***	+1.2 ± 0.6***	**
Hands	+2.3 ± 0.8**	+2.0 ± 0.6**	NS
Feet	+1.1 ± 0.6	+1.1 ± 0.7	NS
Electrical acupuncture			
Time: 5 min			
Face	+0.6 ± 0.09***	+0.7 ± 0.2***	NS
Hands	−0.9 ± 0.4*	+0.5 ± 0.2	***
Feet	−0.9 ± 0.3*	−1.1 ± 0.3*	NS
Time: 30 min			
Face	+1.5 ± 0.11***	+0.8 ± 0.6***	***
Hands	+1.5 ± 0.7*	+0.4 ± 0.5	NS
Feet	+1.5 ± 0.7*	−0.6 ± 0.4	**

[a] Control values (°C were) used as reference for comparisons by the *t* test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

the site of peripheral stimulation. This effect was suggested by the Hoku study (11), but a segmentally related inhibition could not be ruled out on account of the putative segmental connections between the innervation of the hand point and the face (10). However, a segmental reinforcement of this inhibitory effect is suggested as the Hoku acupuncture yields greater T_{sk} changes than the Tsusanli acupuncture. It is of interest that the strong sympathetic effect observed in the face after the Hoku point acupuncture is in agreement with the well known specific cephalic target action of this point (2), which has been used in numerous studies of orofacial analgesia in man and in other animals (35).

Our results also clarify the mechanism of EA sympathetic activation observed in the Hoku study, i.e., Tsusanli knee point EA decreases selectively the foot T_{sk}, indicating a segmentally related response and not an effect mediated by a generalized emotional arousal. This sympathomimetic effect probably represents the activation of segmental vasomotor reflexes (5). This is consistent with Procacci's findings (31) that demonstrated a segmental sympathetic reflex by inhibiting skin potential response in both ipsilateral and contralateral limbs, after unilateral sympathetic block. Bilateral connections on a segmental level of the sympathetic innervation are also evidenced by the well known "mirror image" of reflex sympathetic dystrophy, which shows a contralateral extension of the dystrophic process. The short duration of the sympathomimetic effect may be accredited to the development of an adaptation to repetitive stimulation. However, adaptation of the preganglionic reflex to repetitive stimulation of afferent fibers in spinal nerves is reported to begin at a stimulation frequency of 1/s and to be completed at rates above 5/s (5). An alternative possibility involves a depressive effect exerted by the "specific" sympathetic inhibitory effect of acupuncture. It is interesting to note that the segmentally related spinal activation of the sympathetic nervous system explains the observation found in the literature reporting an exacerbation of the sympathetic dystrophy symptoms after transcutaneous electrical stimulation treatment (1). The relation between segmental sympathetic activation and segmental spinal analgesic system (27, 37) has not yet been investigated in the literature.

Although the literature relative to interactions between pain control and the sympathetic nervous system is limited, there is evidence of a relationship between both functions. The sympathetic nervous system has been shown to play a significant role in the stabilization of the cutaneous pain threshold in normal man (14). Several analogies between peripheral sympathetic vasomotor tone and pain sensation have been reported: thermography studies identify chronic pain areas as cold spots and acute pain areas as warm spots (12, 36, 39). Dorsal column stimulation has been associated with a T_{sk} increase in the region of analgesia (13). The somatotopically organized analgesic system found in the midbrain region of the rat mirrors the cephalocaudal gradient distribution of the sympathetic inhibitory effect observed in our study (32).

Acupuncture analgesia may be mediated at least partly by the central endogenous opioid system (3, 8, 26, 30). Anatomical, physiological and pharmacological observations indicate common features of the central endogenous analgesic system and the thermoregulatory function. Opiate receptors are found in high concentration in the brain stem and in the hypothalamic thermoregulatory centers (4, 16, 34). Electrical stimulation of the nucleus raphe magnus has been shown to produce analgesia (28) as well as to inhibit thermogenesis (9). Serotoninergic systems implicated in acupuncture analgesia (15) are believed to be part of the central thermoregulatory pathway (6). Administration of morphine and endorphin intravenously and intraventricularly has produced hypothermia secondary to peripheral vasodilation and depression of the thermoregulatory center (19, 40). This strongly suggests that endogenous opioid systems may be involved in acupuncture sympathetic inhibitory effect.

In conclusion, the sympathetic effects of acupuncture found in this study are temporally and spatially similar to two separate acupuncture analgesic mechanisms: (i) the finding of a long-lasting generalized sympathetic inhibitory effect with MA is correlated with the generalized endogenous opioid analgesia found after MA (3, 8, 26); (ii) the short-term segmentally related sympathetic excitatory effect associated with EA is correlated with the segmental spinal analgesia found with transcutaneous electrical stimulation (27, 37).

REFERENCES

1. ABRAM, S. E. 1976. Increased sympathetic tone associated with transcutaneous electrical stimulation. *Anesthesiology* **45:** 575–577.
2. Academy of Traditional Chinese Medicine. 1975. An outline of chinese acupuncture. Foreign Language press, Peking, 102.
3. AKIL, H., D. J. MAYER, AND J. C. LIEBESKIND. 1976. Antagonism of stimulation produced analgesia by naloxone, a narcotic antagonist. *Science* **191:** 961–962.
4. ATWEH, S. F., AND M. J. KUHAR. 1977. Autoradiographic localization of opiate receptors in rat brain. I. Spinal cord and lower medulla. *Brain Res.* **124:** 53–67.
5. BEACHMAN, W. S., AND E. R. PERL. 1964. Characteristics of a spinal sympathetic reflex. *J. Physiol. (London)* **173:** 431–448.
6. BRUCK, K., AND P. HINCKEL. 1982. Thermoafferent systems and their adaptive modifications. *Pharmacol. Ther.* **17:** 357–381.
7. CHAN, C. S., AND S. P. CHOW. 1981. Electroacupuncture in the treatment of post-traumatic sympathetic dystrophy (Sudeck's atrophy). *Br. J. Anaesth.* **53:** 899–901.
8. CHENG, R. S. S., AND B. POMERANZ. 1979. Electroacupuncture analgesia could be mediated by at least two pain relieving mechanisms, endorphin and non-endorphin systems. *Life Sci.* **25:** 1957–1962.
9. CRISTANTE, L., P. HINCKEL, AND K. BRUCK. 1981. Inhibition of thermogenesis by electrical stimulation of the nucleus raphe magnus. *Pflügers Arch.* **391:** R45.
10. DENNY-BROWN, D., E. J. KIRK, AND N. YANAGISAWA. 1973. The tract of Lissauer in relation to sensory transmission in the dorsal horn of spinal cord in the macaque monkey. *J. Comp. Neurol.* **151:** 175–200.
11. ERNST, M., AND M. H. LEE. 1985. Sympathetic vasomotor changes induced by manual and electrical acupuncture of the Hoku point visualized by thermography. *Pain* **21:** 25–33.
12. FISCHER, A. A. 1981. Thermography and pain. *Arch. Physic. Med.* **62:** 542.
13. FRIEDMAN, H., B. S. NASHOLD, AND G. SOMJEN. 1974. Physiological effects of dorsal column stimulation. *Adv. Neurol.* **4:** 769–773.
14. GALETTI, R., AND P. C. PROCACCI. 1966. The role of the sympathetic nervous system in the control of somatic pain and associated phenomena. *Acta Neuroveg.* **28:** 495–500.
15. HAN, J. S., AND L. TERENIUS. 1982. Neurochemical basis of acupuncture analgesia. *Annu. Rev. Pharmacol. Toxicol.* **22:** 193–220.
16. HOLADAY, J. W. 1983. Cardiovascular effects of endogenous opiate systems. *Annu. Rev. Pharmacol. Toxicol.* **23:** 541–594.
17. KAADA, B. 1982. Vasodilation induced by transcutaneous nerve stimulation in peripheral ischemia (Raynaud's phenomenon and diabetic polyneuropathy). *Eur. Heart J.* **3:** 303–314.
18. KAADA, B., AND O. EIEBEN. 1983. In search of mediators of skin vasodilation induced by transcutaneous nerve stimulation. II. serotonin implicated. *Gen. Pharmacol.* **14:** 635–664.

19. KAVALIERS, M. 1982. Pineal mediation of the thermoregulatory and behavioral activity effects of β-endorphin. *Peptides* **3:** 679–685.
20. KIRK, R. E. 1968. *Experimental Design: Procedures for the Behavioral Sciences.* p. 87. Wadsworth, Belmont, CA.
21. LEE, M. O., D. C. LEE, S. KIM, AND D. H. CLIFFORD. 1975. Cardiovascular effects of acupuncture at Tsusanli (St 36) in dogs. *J. Surg. Res.* **18:** 51.
22. LEE, D. C., M. O. LEE, AND L. E. MORRIS. 1976. Inhibition of the cardiovascular effects of acupuncture (moxibustion) by propanolol in dogs during halothane anesthesia. *Can. Anaesth. Soc. J.* **23:** 307–318.
23. LEE, G. T. C. 1974. A study of electrical stimulation of acupuncture locus Tsusanli (St36) on mesenteric microcirculation. *Am. J. Chin. Med.* **2:** 53–66.
24. LEE, M. H., AND M. ERNST. 1983. The sympatholytic effect of acupuncture as evidenced by thermography: a preliminary report. *Orthop. Rev.* **12:** 62–72.
25. MATSUMOTO, T., AND M. F. HAYES. 1973. Acupuncture, electric phenomenon of the skin and postvagotomy gastrointestinal atony. *Am. J. Surg.* **125:** 176–180.
26. MAYER, D. J., AND D. D. PRICE. 1976. Central nervous system mechanisms of analgesia. *Pain* **2:** 379–404.
27. MELZACK, R., AND P. D. WALL. 1965. Pain mechanisms: a new theory. *Science* **150:** 971–979.
28. OLIVERAS, J. L., F. SIERRALTA, V. FARDIN, AND J. M. BESSON. 1981. Implication des systèmes serotoninergiques dans l'analgésie induite par stimulation éléctrique de certaines structures du tronc cérébral. *J. Physiol. (Paris)* **77:** 473–482.
29. OMURA, Y. 1975. Patho-physiology of acupuncture treatment: effects of acupuncture on cardiovascular and nervous systems. *Acupunct. Electrother. Res.* **1:** 51–140.
30. POMERANZ, B., R. CHENG, AND P. LAW. 1977. Acupuncture reduces electrophysiological and behavioral responses to noxious stimuli: pituitary is implicated. *Exp. Neurol.* **54:** 172–178.
31. PROCACCI, P., F. FANCINI, M. MARESCA, AND M. ZOPPI. 1979. Skin potential and EMG changes induced by cutaneous electrical stimulation. *Appl. Neurophysiol.* **42:** 125–134.
32. ROSENFELD, J. P., AND P. KERESZTES-NAGY. 1980. Differential effects of intracerebrally microinjected enkephalin analogs on centrally versus peripherally induced pain, and evidence for a facial versus lower body analgesic effect. *Pain* **9:** 171–181.
33. SJOLUND, B., AND M. ERIKSSON. 1979. The influence of naloxone on analgesia produced by peripheral conditioning stimulation. *Brain Res.* **173:** 295–301.
34. SNYDER, S. H., AND S. R. CHILDERS. 1979. Opiate receptors and opioid peptides. *Annu. Rev. Neurosci.* **2:** 35–64.
35. TODA, K., H. SUDA, M. ICHIOKA, AND A. IRIKI. 1980. Local electrical stimulation: effective needling points for suppressing jaw opening reflex in rat. *Pain* **9:** 199–207.
36. UEMATSU, S., AND D. M. LONG. 1976. Thermography in chronic pain. Pages 52–68 in *Medical Thermography. Theory and Applications.* Brentwood.
37. WALL, P. D., AND W. H. SWEET. 1967. Temporary abolition of pain in man. *Science* **155:** 108–109.
38. WATKINS, L. R., D. A. COBELLI, P. FARIS, M. D. ACETO, AND D. J. MAYER. 1982. Opiate vs non-opiate footshock-induced analgesia (F.S.I.A.): the body region shocked is a critical factor. *Brain Res.* **242:** 299–308.
39. WEXLER, C. E., AND FAANOS. 1979. Lumbar, thoracic and cervical thermography. *J. Neurol. Orthop. Surg.* **1:** 37–41.
40. WONG, T. M., A. KOO, AND C. H. LI. 1981. B-endorphin. Vasodilating effect on the microcirculatory system of hamster cheek pouch. *Int. J. Peptide Protein Res.* **18:** 420–422.