THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 7, Supplement 1, 2001, pp. S-121–S-127
Mary Ann Liebert, Inc.

Research on the Neurophysiological Mechanisms of Acupuncture: Review of Selected Studies and Methodological Issues

JOANNIE SHEN, M.D., M.P.H., Ph.D.

ABSTRACT

This presentation reviews studies that contribute to an understanding of the neurophysiological mechanisms of acupuncture. A 1973 study, using volunteer medical students, looked into acupuncture's analgesic effect on experimentally induced pain and suggests that humoral factors may mediate acupuncture-induced analgesia. In a study of the possible role of the cerebrospinal fluid transmission of pain suppression effects of acupuncture, cerebrospinal fluid from acupuncture-treated rabbits was infused into recipient rabbits. The analgesic effect was observed in the recipient rabbits, suggesting that acupuncture-induced analgesia may be mediated by substances released in the cerebrospinal fluid. Studies of electroacupuncture in rats revealed that both low-frequency and high-frequency stimulation could induce analgesia, but that there are differential effects of low- and high-frequency acupuncture on the types of endorphins released. In another study, low-frequency electroacupuncture, given as median nerve stimulation in cats, was shown to protect the myocardium by inhibiting sympathetic pressor response and increasing myocardial oxygen demand. The development of neuroimaging tools, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), make noninvasive studies of acupuncture's effects on human brain activity possible. Studies using PET have shown that thalamic asymmetry present among patients suffering from chronic pain was reduced after the patients underwent acupuncture treatment. Other studies, using fMRI, have pointed to relationships between particular acupoints and visual-cortex activation. These powerful new tools open the possibility to new scientific studies of this ancient therapy.

I am very honored to be here, and thank you for having me. My challenge this afternoon is to tell you, within a short time and in an oversimplified version, about the search for the basis of acupuncture, and how it might work.

Acupuncture is one component of health care in Asia that can be traced back for almost 3000 years. The practice of acupuncture in American medicine was rare until the visit of President Nixon to China in 1972. Since that time, there has been an explosion of interest in the United States and Europe in the application of acupuncture to Western medicine. In 1996, the U.S. Food and Drug Administration promoted acupuncture needles from the investigational and experimental medical device category to the regular medical-device category. In 1997, a National Institutes of Health (NIH) consensus statement on acupuncture supported the treatment efficacy of acupuncture

National Institutes of Health Clinical Center, Bethesda, Maryland.

for specific conditions. Particularly, it was found useful in pain, and in nausea and vomiting control.

Given widespread and increasing use worldwide, why are we expending effort in studying its mechanism? Gaining an understanding of the biological basis for acupuncture may provide novel insights into neuroendocrine, immunologic, and other physiological processes. Such insights will broaden our approach and understanding of these disciplines, and potentially lead to improvement in treatment.

There are two general viewpoints of how acupuncture works. In the classical "qi" model, health is related to the balanced flow of vital energy, or qi, through a network of meridians in the body. It is believed by some that interruptions or imbalances in this flow lead to illness. A series of specific points along the meridians can be used to alter the flow of qi by needle insertion or other manipulations that we would recognize as acupuncture. In the neurophysiological model, the mechanical action of needling or its electrical equivalent, what we call electroacupuncture, triggers a chain of events that can be understood through controlled experiments. For example, needling may cause receptors to send neural impulses to the spinal cord or act on ascending pathways to the brain, and cause the release of neurotransmitters that subsequently modulate central functions in the brain.

Realizing that neither model completely explains all the findings that we have observed to date, and that both models have their shortcomings and limitations, I will focus on the neurophysiological model of acupuncture only. In the next half an hour, we will embark on a journey toward understanding how acupuncture works, from a historical perspective and by following study subjects through various studies. First, a group of medical student volunteers will provide a quantitative measure of the acupuncture effect on raising pain threshold. Then, two rabbits will show that such an analgesic effect can be transferred, therefore implicating a humoral basis of such an acupuncture effect. And following that, a study of mice will reveal that endorphins are the mediators of acupuncture's analgesic effect. Similar studies will then tell us that continued acupuncture can lead to tolerance. As acupuncture stimulated with different electrical frequencies appears to have different clinical effects, a group of rats will demonstrate such differential effects at the level of gene expression. Beyond pain treatment, acupuncture has been used in many other conditions, such as cardiovascular disease. A cat receiving electroacupuncture will illustrate how acupuncture can exert cardio-protective effect by regulating the autonomic nervous system. Finally, we will look at some preliminary studies utilizing the recent advances in neuroimaging methods.

The first study using experimentally induced pain to measure acupuncture's analgesic effect quantitatively was conducted in 1973, involving medical students as normal volunteers (Research Group, 1973). Some of the research questions were: Does acupuncture really work? If it works, does it work immediately, as soon as you put the needle in? And does it stop working as soon as you take the needles out? In this study, pain was induced by modified potassium iontophoresis with gradual increase of currents (0.1–0.3 milliampere each step) passing through the skin on the head, thorax, back, abdomen, and leg of a group of 60 participants. The pain threshold was measured by the current (milliampere) needed to produce pain. Measurement was taken every 10 minutes for 100 minutes after the insertion of the needles into two acupuncture points, LI-4 and ST-36, and the needles were manipulated for 50 minutes. Data were expressed as average percent changes in these skin areas compared to their baseline. This method was validated by using morphine as a control; 10 mg of intramuscular morphine increased the pain threshold by approximately 80%–90% on average in this group. Manual acupuncture was given for 50 minutes. Researchers observed that the pain threshold increased gradually, with the peak increase occurring about 20 to 40 minutes after needle insertion. The threshold then gradually returned toward baseline. If procaine, a local anesthetic, was injected prior to needling, then the effect was prevented. Several related studies were performed. First, the onset of acupuncture's analgesic effect onset is slow, with 30 minutes of induction time and the effect had an exponential decay with a half-life of about 16 minutes. Second, whether the needle was placed in acupuncture points located on the left side or the right side of the body made no difference. Different points might have different potency in increasing the pain threshold, but a greater effect was observed when multiple points were used together. Local anesthetics, such as procaine injected locally, blocked this effect. This indicated that sensory input plays an important role. One study, also involving medical students as normal volunteers, found that direct stimulation of peripheral nerve sensory fibers yields a similar response in raising the pain threshold when compared to standard acupuncture (Lim et al., 1977). The gradual increase and delayed return of pain appreciation suggest that acupuncture-induced analgesia may be mediated by humoral factors.

The next experiment of cerebrospinal fluid transmission of pain suppression follows these observations (Research Group, 1974). The hypothesis was that neurochemical factors responsible for acupuncture analgesia may be produced and released from the central nervous system. If this is true, then infusion of the cerebrospinal fluid taken from one animal—the donor rabbit, which has undergone acupuncture—to a recipient animal, might produce a similar effect, and therefore result in an increase of the pain threshold in this recipient animal. In this study, a standard noxious stimulation was applied and the rabbits' avoidance response was measured and compared to their baseline levels. Researchers gave the donor rabbit 30 minutes of acupuncture that produced an analgesic effect in the donor rabbit. The cerebrospinal fluid from the donor rabbit was then infused into the lateral ventricle of a naive recipient rabbit. The analgesic effect was observed in the recipient rabbit.

One methodological issue is that the handling of experimental animals may be stressful for the animals and thus caused the observed analgesic effect. To control for this effect, the research team infused normal saline in another group. Profusion of the ventricle with artificial cerebrospinal fluid had no significant effect in either donor or recipient animals. These results demonstrated that acupuncture-induced analgesia is likely to be mediated by substances released in the cerebrospinal fluid.

These studies were conducted before the discovery of endorphins. When endorphins were discovered, people hypothesized that perhaps endorphins were mediating the delayed effects of acupuncture analgesia. An endorphin antagonist, naloxone, was used in another series of studies. This following experiment used parallel groups of mice (Pomeranz and Chiu, 1976). There are groups of mice, 10 in each group and parallel design. This study set out to test the acupuncture-analgesia-endorphin hypothesis. A standard noxious stimulus was applied to the mouse, and the mouse's response was measured and compared to its baseline. Robust analgesia was observed using this setup. The delayed onset of the acupuncture analgesic effect is similar to what we observed in previous studies—gradual peaking and gradual return. The important message here is that naloxone blocks such effect. This design also used other control groups. Sham acupuncture in mice did not produce an analgesic effect. And using saline injection as a control for naloxone demonstrated no effect in blocking acupuncture analgesia. In summary, naloxone blocks acupuncture analgesia, thereby implicating the role of endorphins in acupuncture analgesia in mice.

An important issue here is the comparison across species. Can the same mechanism be generalized from mice to humans? A study in 1977 observed the same phenomenon in humans (Mayer et al., 1977). In the subsequent years, other independent studies further supported this endorphin hypothesis. The effect is enhanced by protecting endorphins from enzyme degradation. Such effect is suppressed by reducing the pituitary endorphins. Also, mice that are genetically deficient in opiate receptors show poor acupuncture–analgesic effect. As more members of the endorphin family were discovered, studies demonstrated that different opiate antagonists also block acupuncture analgesia.

We will next examine tolerance to acupuncture. Most traditional practitioners are aware of this phenomenon and they refrain from overtreatment of patients. Research studies were able to demonstrate this in a quantitative fashion. If one were to stimulate continuously for 2 hours or longer, acupuncture has diminished analgesic effect. Eventually, the level returns to a baseline level. This might in part be due to desensitization or downregulation of the receptors involved. An exciting discovery was endogenous antiopiates. They were peptides that counteract the pain-modulation action of the endorphins. As Dr. Kim mentioned earlier in the seminar regarding the sphincter of Oddi study, one of the antiopiates was found to be cholecystokinin octapeptide. It was found that this peptide produces a dose-dependent suppression of the analgesic effect induced by either morphine or by acupuncture. This was demonstrated by an experiment in which acupuncture tolerance was reversed by an intracerebral ventricular injection of a cholecystokinin octapeptide antiserum (Han et al., 1985). In this study, the cholecystokinin octapeptide antiserum reversed the tolerance effect and the control serum failed to do so. In summary, prolonged acupuncture induces tolerance to its analgesic effect, and such tolerance is caused in part by the release of antiopiates. However, the story is more complicated. There is more than one type of antiopiate, just as there are different members of the endorphin family. Clearly, more studies are needed.

Next we will discuss the various parameters of acupuncture. Acupuncture has various parameters: traditional practitioners may manipulate a needle in very different ways according to their treatment intention. It may be very rapid, with twirling and spinning of the needle, or it may be a slow and rhythmic maneuver, or electrical stimulation may be applied.

The use of electroacupuncture in research studies revealed that acupuncture analgesia could be induced by low-frequency stimulation, such as 2 Hz, 4 Hz, or high-frequency stimulation, such as 100 to 200 Hz. Based on a variety of studies, it was found that low-frequency acupuncture releases some specific types of endorphin (enkephalin and β-endorphin), and high-frequency acupuncture releases yet another type (dynorphin). Differential effects of low- and high-frequency electroacupuncture have also been demonstrated at the level of gene expression in various parts of the brain in rats (Guo et al., 1996a, 1996b). In a series of experiments in rats, *fos*-like immunoactivity was used as a marker for gene expression. Simply by altering the frequency of electroacupuncture, researchers found that the same nucleus can have a marked difference in its neuronal activation. Using the arcuate nucleus of the hypothalamus as an example, when given low-frequency stimulation, results revealed much higher expression compared to when given high-frequency stimulation. *In situ* hybridization studies revealed that 2-Hz stimulation increased the expression of the messenger RNA for the one particular type of endorphin precursor (the enkephalin precursor protein, pre-pro-enkephalin), with little effect on the mRNA for another type (the dynorphin precursor, pre-pro-dynorphin). In contrast, stimulation at 100 Hz selectively increased another type of messenger RNA expression (pre-pro-dynorphin), particularly so at the parabrachial nucleus of the brainstem.

Some researchers may raise questions about the validity of using *fos*-like immunoreactivity as a surrogate in this type of study, because nonspecific causes may result in its expression. Some researchers might prefer evidence beyond the messenger RNA level and demonstration of such effects at the actual peptide level. Realizing the current limitations in research methodology would prompt the research community to plan future studies with novel methods and approaches. In summary, there are differential effects of low versus high frequency electroacupuncture on *c-fos* expression in the rat brain. And there are also differential effects of low-versus high-frequency electroacupuncture on the expression of messenger RNAs on various kinds of opioid genes. More research is needed in order to demonstrate such effects at the peptide level.

Although acupuncture was regarded as a treatment for pain by many people, clinical observations have suggested that acupuncture may have therapeutic effects on hypertension, coronary heart disease, and certain disrhythmias. The next study uses low-frequency median nerve stimulation as a surrogate for electroacupuncture (at P-6 point) in a feline model (Li et al., 1998).

In this experiment, bradykinin was applied to a cat's gallbladder to induce a reflex pressor effect expressed as both elevated diastolic and systolic pressure. The cat's left anterior descending artery was artificially occluded to create an ischemic condition. Under usual circumstances, when bradykinin is applied, the reflex pressor effect will increase the oxygen demand of the heart; but in this case, the oxygen supply is limited by the artificially occluded artery. When this happened, a pathologic–physiologic response manifested as thinning of the ischemic myocardium was observed. This is indeed observed in the control group. What happens when you give 30 minutes of median nerve stimulation to the cat? This study showed that the expected pathologic wall thinning of the myocardium was not found in the group that received the median nerve stimulation. What protected the heart from ischemia? Data showed that the reflex pressor effect was inhibited in the acupuncture group, which was demonstrated by relatively lowered blood pressure compared to the control group. As a result, the acupuncture group's heart was protected from ischemia, and therefore did not display pathological wall thinning compared to the control. This protective effect persisted for hours in the follow-up period. In summary, low-frequency electroacupuncture given as median nerve stimulation protects the myocardium by inhibiting sympathetic pressor response and decreases myocardial oxygen demand. Such protective effect persisted long after the removal of acupuncture needle stimulation.

Some may question that the invasive form of direct median nerve stimulation is nothing like acupuncture used in clinical situation. Will a less invasive form of electroacupuncture produce a similar effect as an open-median-nerve stimulation that we saw in this study? Indeed, the study has been repeated in less invasive form, as if in real acupuncture treatment; and the effect is still the same as demonstrated in the following study. Furthermore, what might be the central mechanism of such an observed cardioprotective effect? (Chao et al., 1999) Researchers found that the inhibitory effects of electroacupuncture on blood pressure and myocardium were reversed by intravenous injection of naloxone, or microinjection of naloxone into the rostral ventrolateral medulla. These results indicated that the inhibitory effect of electroacupuncture on the bradykinin-induced pressor response and the consequent improvement of ischemic dysfunction were dependent on the activation of opioid receptors, specifically receptors located in the rostral ventrolateral medulla.

Thus far, I have covered a series of landmark studies. These provide evidence for the real effect of acupuncture on pain and autonomic nervous system regulation, as well as indications of how acupuncture might work. The effects appear to be mediated centrally in the brain. I now turn to less invasive methods that will allow study of acupuncture effects in humans without invasive measurements.

Many remarkable developments have occurred in the last decade in the arena of neuroscience. The technological developments of neuroimaging, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), have made it possible to visualize not only the anatomy, but also the functional activation of a brain in noninvasive ways. Using these techniques has provided useful information revealing acupuncture's effect on human brain activation.

Using PET, scientists observed that the thalamic asymmetry present among patients suffering from chronic pain was reduced after they underwent acupuncture treatment for pain condition in a pre-treatment and post-treatment design. Their subjects' rating of pain was also reduced (Alavi et al., 1997). And using PET, scientists observed that the acupuncture "de qi" state was associated with activation of the hypothalamus and the cerebellar vermis when compared to superficial needling (Hsieh, 1998). Meanwhile, localization of the central nervous pathway for acupuncture stimulation has been developed utilizing fMRI (Wu et al., 1999). Using fMRI, scientists observed signal changes in the limbic circuitry, implicating a possible intermediary effect of acupuncture in addiction treatment (Hui et al., 1998).

Also using fMRI are the observations of acupuncture-point stimulation and corresponding cortical responses. Particularly worth mentioning is a collaborative study by scientists from Korea and the United States lead by Dr. Z.H. Cho, who is present in the audience today. Researchers reported a correlation between visual cortex activation and corresponding acupoint stimulation (Cho et al., 1998). In this study, stimulation on the visual-related acupoint VA1—it is also called BL67—located on the foot was found to be correlated to the fMRI signal-intensity changes in the visual cortex in 12 normal subjects. Four of those subjects had increased signal intensity, and eight had decreased signal intensity. In Dr. Cho's unpublished follow-up study, GB37 and GB43 points were used. The point GB37 is indicative for the treatment of vision diseases in ancient medical textbooks. Using fMRI, stimulation of this point was observed to be associated with visual cortex activation. On the other hand, stimulation of GB-43 point, which reportedly is indicated for the treatment of hearing disease, was observed to be associated with auditory cortex activation.

An unexplained observation in the above study was that stimulation of the visual related point correlated with signal intensity change in the visual cortex, as revealed by increased signal in 4 subjects, but decreased signal in 8 of the 12 participants. However, as a controlled condition, visual stimulation always produced positive signal in all participants. According to the researchers, a traditional practitioner consultant saw these subjects and classified them into two groups, yin and yang. Those with traditional yin characteristics were regarded to have deficient blood circulation, and those with yang characteristics had an overabundance. The researchers reported that the yin subjects, when they receive acupuncture, had an increase in signal in response, and the yang subjects ended up having a decrease in signal in response. Acupuncture is known by some to have a homeostatic effect. It was thought by some that the traditional classification of yin–yang characteristics might have a physiological basis. fMRI might provide an interesting new opportunity to look at this and perhaps observe acupuncture's homeostatic effect at work. This will be a new test of an ancient paradigm, but certainly a provocative new paradigm for Western medicine.

As we have seen, there are powerful new tools to examine the effects of acupuncture in a noninvasive manner. Along with the new opportunities come new challenges as well. The questions that might be addressed may include whether acupuncture points really exert point specificity in their therapeutic effect. An important aspect of clinical research should also consider the placebo effect.

I believe that as modern biomedical research seriously and enthusiastically explores this ancient therapy, horizons will be broadened and we will see improved clinical applications in expanded areas. Finally, I would like to acknowledge those people who have given me their help and advice in preparing for this lecture, whose material I have borrowed liberally from. Mistakes are my responsibility, not theirs. In alphabetical order: Zang-Hee Cho, Ph.D., Song-Ping Han, Ph.D., Richard Hammerschlag, Ph.D., William Harlan, M.D., Daniel Hommer, M.D., and John Longhurst, M.D., Ph.D. Thank you.

ACKNOWLEGMENTS

The author thanks National Institute on Alcoholism and Alcohol Abuse, National Center for Complementary and Alternative Medicine, and Office of the Directors at the National Institutes of Health for their intramural support.

REFERENCES

Alavi A, LaRiccia P, Sadek AH, Newberg AB, Lee L, Reich H, Lattanand C, Mozley PD. Neuroimaging of acupuncture in patients with chronic pain. J Altern Complement Med 1997;3:S41–S53.

Chao DM, Shen LL, Tjen-A-Looi S, Pitsillides KF, Li P, Longhurst JC. Naloxone reverses responses. Am J Physiol 1999;276:H2127–2134.

Cho ZH, Chung SC, Jones JP, Park JB, Park HJ, Lee JH, Wong EK, Min BI. New findings of the correlation between acupoints and corresponding brain cortices using functional MRI. Proc Natl Acad Sci USA 1998;95:2670–2673.

Guo HF, Tian J, Wang X, Fang Y, Hou Y, Han J. Brain substrates activated by electroacupuncture (EA) of different frequencies (II): Role of Fos/Jun proteins in EA-induced transcription of preproenkephalin and preprodynorphin genes. Brain Res Mol Brain Res 1996;43:167–173.

Guo HF, Tian J, Wang X, Fang Y, Hou Y, Han J. Brain substrates activated by electroacupuncture of different frequencies (I): Comparative study on the expression of oncogene c-fos and genes coding for three opioid peptides. Brain Res Mol Brain Res 1996;43:157–166.

Han JS, Ding XZ, Fan SG. Is cholecystokinin octapeptide (CCK-8) a candidate for endogenous anti-opioid substrates? Neuropeptides 1985;5:399–402.

Hsieh J. Brain activation by acupuncture with "de-Qi": A PET study. Proceedings of the 4th International Conference on Functional Mapping of the Human Brain, Montreal, Canada, July 7–12, 1998: Abst S440.

Hui KKS, Liu J, Chen AJW, Makris N, Kennedy DN, Moore CI, Gollub RL, Rosen BR, Kwong KK. Acupuncture modulates the limbic system and subcortical gray structures of the human brain: direct evidence by fMRI. Proceedings of the 4th International Conference on Functional Mapping of the Human Brain, Montreal, Canada, July 7–12, 1998:Abst S441.

Li P, Pitsillides KF, Rendig SV, Pan HL, Longhurst JC. Reversal of reflex-induced myocardial ischemia by median nerve stimulation: A feline model of electroacupuncture. Circulation 1998;97:1186–1194.

Lim TW, Loh T, Kranz H, Scott D. Acupuncture—Effect on normal subjects. Med J Aust 1977;1:440–442.

Mayer DJ, Price DD, Rafii A. Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. Brain Res 1977;121:368–372.

Pomeranz B, Chiu D. Naloxone blockade of acupuncture analgesia: Endorphin implicated. Life Sci 1976;19:1757–1762.

Research Group of Acupuncture Anesthesia, P.M.C. The effect of acupuncture on the human skin pain threshold. Chin Med J 1973;3:151–157.

Research Group of Acupuncture Anesthesia, P.M.C. The role of some neurotransmitters of brain in finger-acupuncture analgesia. Scientia Sinica 1974;117:112–130.

Wu MT, Hsieh JC, Xiong J, Yang CF, Pan HB, Chen YC, Tsai G, Rosen BR, Kwong KK. Central nervous pathway for acupuncture stimulation: localization of processing with functional MRI imaging of the brain—preliminary experience. Radiology 1999;212:133–141.

Address reprint requests to:
Joannie Shen, M.D., M.P.H., Ph.D.
National Institutes of Health
Building 10/6S Room 240
10 Center Drive
Bethesda, MD 20892