Neurophysiological Mechanisms of Acupuncture Analgesia in Experimental Animal Models

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Introduction

Acupuncture and transcutaneous electrical nerve stimulation (TENS) are two different procedures which result in analgesia by stimulation of peripheral tissues. Acupuncture, using either the traditional technique of manual rotation of needles [3, 35, 36, 62] or electroacupuncture [2, 3, 36], has been used successfully as an anesthetic [5, 32] and as a treatment for chronic pain [6, 24, 42, 61]. TENS has been widely used in the West to produce analgesia clinically, as well as in experimental animal models [31, 44, 50, 56, 63, 73]. Like TENS, the effects of acupuncture appear to be due to the activation of peripheral nerve fibers [11, 22, 25, 43, 53, 59, 69]. Although acupuncture is effective in producing analgesia, the reported effectiveness varies greatly between studies. For example, in studies performed in the West alone, analgesia produced by acupuncture ranges from a mild effect to the one spectacular enough to allow performance of open heart surgery [32]. Not only the effectiveness but also the methodology of stimulation varies between laboratories. For example, frequency of stimulation for electroacupuncture ranges from several pulses per second [2, 26] to 10 KHz [43, 48]. The lack of a standard method of application and the inconsistency of the effects between studies are primarily due to incomplete understanding of the mechanisms of acupuncture (and TENS) analgesia, which greatly hampers further development of the technique.

In an attempt to study the mechanisms of acupuncture and TENS analgesia, experimental animal models were developed. Using these models, some important factors determining analgesic effectiveness were investigated. The basic approach has been electrophysiological recording of neuronal activity in the spinal cord, which can be used as a nociceptive index, and its modulation by electrical stimulation applied directly to a peripheral nerve in an anesthetized animal.

Experimental Animal Models

Development of Experimental Cat Model. It is important to study the mechanisms of acupuncture and TENS for technical improvement of the analgesic procedures as well as for improving understanding of the pain control mechanisms in general. To study the underlying mechanisms, it is vitally important to develop a good experimental animal model. I attempted to develop animal models which permit not only a wide range of experimental manipulations but also the measurement of objective indices that reflect levels of pain.
First developed was an experimental cat model using the flexion reflex as the pain index. The cat was anemically decerebrated by ligation of the carotid arteries bilaterally and the basilar artery under ketamine anesthesia. The flexion reflex was recorded as a compound action potential in the hamstring nerve while evoking the reflex by electrically stimulating the sural nerve with an intensity high enough to activate unmyelinated C fibers (20 V, 0.5 ms). The flexion reflex consisted of two components: early and late. The early and late components have latencies of about 10 and 200 ms and durations of about 10 ms and 1 s, respectively [29]. To test the viability of the flexion reflex as a pain index in this preparation, its sensitivity to systemically injected morphine was examined [17]. Although IV injection of morphine tended to depress the early component, the results were highly variable between experiments. The late component was reliably depressed by systemic injection of morphine in a dose-related fashion. The late component was very sensitive to IV injections of morphine: it was depressed to 50% and 25% of the original size with doses of 1 and 2 mg/kg, respectively. Furthermore, this depressant effect by morphine was reversed by a small dose of naloxone (0.05–0.1 mg/kg). From the results of these studies, in conjunction with the fact that the flexion reflex has classically been known as a common nociceptive reflex [65], I felt that the late component of the flexion reflex could be used as a reasonably good pain index.

Using this model (flexion reflex in anemically decerebrated cat), testing of the analgesic effect of acupuncture was begun. Electroacupuncture was performed using a 28-gauge hypodermic needle on the St.36 Zusanli point in the lateral upper tibial region of the ipsilateral hindlimb (tibialis anterior muscle) [53]. Stimulation was applied with high intensity pulses (20 V, 2 ms) repeated at a rate of 2 Hz for 1 h. This electroacupuncture procedure produced depression of the late component of the flexion reflex to less than half the size of the prestimulus control, which is comparable to the effect produced by systemic injection of morphine 1 mg/kg. The depression of the flexion reflex produced by electroacupuncture was reversed by a systemic injection of naloxone. When all peripheral nerves (except the sural nerve) in the ipsilateral hindlimb were cut, the effect of electroacupuncture was no longer observed, while direct electrical stimulation of the common peroneal nerve at the proximal end with the same stimulus parameters mimicked the effect of electroacupuncture. In these results, electroacupuncture applied in an experimental animal produced an analgesic effect which was apparently initiated by afferent nerve activity. The effect seems to be mediated by the release of opiate substances.

Since it is difficult to perform a quantitative analysis on recordings of compound action potentials, the model was then refined, based on the recording of the flexion reflex as single unit activity from motor axons [12]. The activity in single motor axons was recorded from filaments of the L7, S1, or S2 ventral roots in the cat. The reflex activity in the motor axons was elicited either by electrical stimulation of a hindlimb nerve or by natural forms of stimulation applied to the foot. As in recordings of compound action potentials, electrical stimulation of an afferent nerve elicited early and late components of the flexion reflex, the late component representing activation of Aδ and C afferent fibers. When natural forms of stimuli were applied to the foot, sustained activity in the motor axons could only
Fig. 1A, B. Effects of peripheral nerve stimulation on the flexion reflex in a spinal cat. A Reflex was elicited by electrical stimulation of the common peroneal nerve (upper histogram). Inhibition (to 52% of control) of the reflex was observed immediately after stimulation of the tibial nerve at a rate of 2 Hz for 15 min (middle histogram). The reflex gradually recovered with time, reaching 101% of control 40 min after the termination of the stimulus (lower histogram). B After the reflex recovered from the inhibition, another control reflex discharge was recorded (upper histogram). Stimulation of the tibial nerve a second time produced a greater inhibition (to 41% of control, middle histogram). Then naloxone hydrochloride 0.05 mg/kg was given intravenously and the reflex recovered to 92% of control 10 min later (lower histogram). All histograms were compiled from responses to 10 consecutive stimuli, and bin widths are 2 ms. P.N. STIM., peripheral nerve stimulation. (Reproduced from [12]).
be elicited by intense noxious mechanical or thermal stimuli. This fact reinforces the contention that the flexion reflex could be used as a pain index. Since the results of an earlier study indicated that the analgesic effects produced by electroacupuncture and direct peripheral stimulation are comparable [53], conditioning stimulation was applied to the common peroneal or tibial nerve at a suprathreshold intensity for C fibers at a rate of 2 Hz for 15 or 30 min. This produced an inhibition of the flexion reflex late discharges which outlasted the conditioning stimuli. Maximum inhibition on average was about 40% and 43% of the control reflex value in decerebrate and spinal cats, respectively. In decerebrate cats, the duration of inhibition varied from less than 10 min to over 1 h beyond termination of the conditioning stimuli, depending on the unit. However, inhibition lasted over 20 min for all units tested in spinal animals. The long-lasting inhibition of the flexion reflex was reversed by a systemic injection of naloxone hydrochloride (0.05 mg/kg). Figure 1A shows an example of this analgesic effect recorded in the spinal cat and Fig. 1B shows its reversal by naloxone.

Development of Experimental Monkey Model. Although the flexion reflex appears to be a good pain index, the reflex is a pain reaction (motor reflex) rather than a phenomenon related to pain perception per se. Therefore, it seems desirable to develop a model based on a more direct indicator of pain sensation such as the activity of nociceptive tract neurons. The spinothalamic tract (STT) is one of the best known nociceptive tracts in primates, including humans [40, 52, 71, 72]. Primate STT cells are well suited to transmit pain information to the brain since they respond well to various forms of noxious stimuli applied to the periphery [14, 23, 37]. Furthermore, various analgesic manipulations inhibit their activity [27, 28, 30]. Hence, it was decided to use the activity of monkey STT cells evoked by unmyelinated C fibers in the sural nerve as a pain index to test the analgesic effect produced by peripheral conditioning stimulation [13].

Identified STT cells were recorded from the lumbosacral spinal cord or intact, anesthetized monkeys. In addition, presumed STT cells were recorded from both unanesthetized/decerebrated, and decerebrated/spinal monkeys. Presumed STT cells were identified by antidromically activating them from the contralateral, ventral, lateral funiculus of the cervical spinal cord. Both C fiber activity evoked by electrical stimulation of the sural nerve and the activity evoked by noxious heat were greatly inhibited by repetitive conditioning stimuli applied either to the common peroneal or tibial nerve with a strong enough intensity for activation of C fibers at 2 Hz for 15 min. The inhibition was maintained during the period of conditioning stimulation and often outlasted it by 20–30 min as shown in Fig. 2. The inhibition of cells produced by peripheral nerve stimulation was observed in decerebrate and spinalized animals as well as in intact anesthetized monkeys, although the mean recovery time in the decerebrate group was faster. This indicates that anesthetism does not interfere with the inhibitory mechanisms. Furthermore, the presence of inhibition in spinalized animals suggest that the inhibition must depend in part on spinal cord neuronal circuitry.

Although direct peripheral nerve conditioning stimulation produced inhibition of STT-cell activity, it was interesting to try a commercially available TENS unit on a monkey to test clinical applicability. The C-fiber-evoked, STT-cell activity
was compared before, during, and after application of TENS for 5 min while monitoring the current delivered by the TENS unit [41]. Application of TENS produced inhibition of C-fiber-evoked, STT-cell activity. Powerful inhibition of STT-cell activity occurred during application of TENS, and the inhibition gradually recovered after termination. Therefore, using this experimental monkey model, an analgesic effect was demonstrated not only by direct conditioning stimulation of a peripheral nerve but also by application of a commercially available TENS unit.

Animal Preparation. In these animal models, three different preparations were used: animals with intact brains and anesthetized with anesthetic drugs; unanesthetized, decerebrate animals; and unanesthetized, decerebrate/spinal animals. In most cases, the animal was prepared by performing a decerebration followed by a spinalization, after which the anesthetic drug could be discontinued. The spinal animal provides several advantages. First, the flexion reflex, a pain index which was recorded, is severely depressed in the presence of a common anesthetic drug [51]. While the flexion reflex is extremely unstable in unanesthetized/decerebrate preparations, it is strong and stable in spinal animals [12].
Second, analgesic mechanisms can be studied without the potential complications of anesthetic drugs. Third, the antinociceptive effect that was observed mainly depends on spinal mechanisms, as evidenced by the lack of a significant change of the observed effect after spinalization [13]. Fourth, although spinalization eliminates any potential contribution of supraspinal structures for the production of analgesic effects, it was thought important to study the spinal mechanisms first without the complication of supraspinal influences. After the spinal mechanisms are fully unveiled, the search can then be extended to supraspinal mechanisms.

**Analgesic Stimulation.** In these experimental animal models, a peripheral nerve was stimulated directly with electrical pulses (rather than application of acupuncture or TENS) because they are more controllable. Since both acupuncture and TENS are forms of peripheral nerve stimulation, direct stimulation of a peripheral nerve with proper parameters should mimic these procedures. To be able to manipulate with a variety of combinations of stimulus parameters, including a high enough intensity to activate C fibers, a model using an anesthetized and paralyzed animal was chosen. Partly because a behavioral pain index cannot be used in an anesthetized animal and partly to obtain an objective index, neuronal activity that reflects the level of pain was recorded as a pain index.

**Components of Analgesic Effect**

It is controversial as to whether or not the analgesia produced by acupuncture is naloxone reversible [4, 49, 58]. It has been reported that both TENS [67, 68] and electroacupuncture [9] with low-frequency stimulation produce naloxone-reversible analgesia, whereas high-frequency stimulation produces analgesia that is not naloxone reversible. However, Woolf et al. [74] were able to produce naloxone-reversible analgesia with high-frequency, percutaneous, nerve stimulation. Therefore, it is not clear whether or not the production of naloxone-reversible analgesia is stimulus-frequency dependent.

Results from this laboratory indicate that low-frequency stimulation of a peripheral nerve, in fact, produces both the naloxone-reversible and non-naloxone-reversible components of analgesia simultaneously [13, 15]. As shown in Fig.2, analgesic effects in the monkey start to appear within a few seconds after the beginning of repetitive conditioning stimulation and gradually but rapidly build up so that the maximum effect can be seen within minutes. The maximum effect is generally maintained throughout the duration of stimulation. At the termination of the stimulation, the antinociceptive effect gradually fades away after dozens of minutes. When identical conditioning stimulation is repeated following systemic injection of naloxone, as shown in Fig.3, no significant difference can be seen during stimulation, but recovery from the analgesic effect after termination of the stimulation occurs slightly but significantly faster. This phenomenon was interpreted as evidence indicating that the analgesic effect produced by peripheral conditioning stimulation consists of two different components, naloxone reversible and non-naloxone reversible.
Figure 3. Effect of naloxone on the inhibition produced by peripheral nerve stimulation in the monkey. After demonstrating good inhibition by repetitive conditioning stimulation of the tibial nerve for 5 min (intensity was suprathreshold for Aδ fibers, and frequency was between 2 and 20 Hz, depending on the unit), the identical conditioning stimuli were repeated 5 min after intravenous injection of naloxone hydrochloride (0.05 mg/kg). Data were collected from 13 spinothalamic tract cells. The C-fiber evoked responses measured during the first 50 s of conditioning stimulation (STIM), during the period between 100 and 150 s of conditioning stimulation, and during the first and second 50-s periods following termination of conditioning stimulation are expressed as a percentage of the average prestimulus control values. Each bar represents 1 SEM. The dot represents a significantly ($P < 0.05$) different value from the one obtained before naloxone injection. (Reproduced from [15])

Figure 4 illustrates a hypothetical development and recovery of analgesia produced by peripheral nerve conditioning stimulation. The concept of this figure is based partly on personal experimental results and partly on an attempt to explain controversial published results from other laboratories. The analgesic effect produced by peripheral nerve conditioning stimulation is the summed effect of the naloxone-reversible and non-naloxone-reversible components. The non-naloxone-reversible component is much more powerful, develops quickly, and is short lasting. On the other hand, the naloxone-reversible component is weaker and develops slowly, but lasts for a long period of time. To be able to observe the naloxone-reversible component, the duration of the conditioning stimulation has to be long. During conditioning stimulation, the naloxone-reversible component is difficult to detect due to the dominance of the powerful non-naloxone-reversible component. However, at the tail end of the recovery period, it should be easy to detect the naloxone-reversible component if one employs a method that is sensitive enough. It is further hypothesized that the magnitude of each of the two components depends on multiple variables such as animal preparation (types of anesthesia, decerebration or spinalization, etc.), types of afferent nerves being stimulated, patterns of conditioning stimulation (frequency, patterned bursts, etc.), and species. Therefore, it is possible to observe, either completely or partially, naloxone-reversible or non-naloxone-reversible analgesia produced by peripheral nerve conditioning stimulation, depending on the time of observation, the type of
animal preparation used, and the method of conditioning stimulation. However, it is still not clear what the exact conditions are which favor the production of one or the other type of analgesia.

Although it is reasonably clear that the naloxone-reversible component is mediated by opiates, the chemical mediators for the non-naloxone-reversible component are not known. The tens of seconds required for the development of the non-naloxone-reversible component seem to be too slow for purely electrical events. Attempts were made to interfere pharmacologically with this component [13], but none of the agents tested changed the inhibition to any appreciable degree. Drugs tested included GABA blockers (picrotoxin and bicuculline) and the glycine blocker strychnine, since these are well-known to have an action on spinal inhibitory mechanisms. Serotonin and catecholamine blockers (metergoline and phentolamine) were also tested since these monoamines have been implicated in acupuncture analgesia [29a]. It is possible that another known chemical agent, such as one of the neuropeptides other than enkephalin, or accumulation of K⁺ [39] mediates the non-naloxone-reversible analgesia.

Although the chemical mediators are not known, this component is powerful and develops quickly, making it easy to study and allowing versatile experimental manipulations. Therefore, most of my subsequent work was focused mainly on the non-naloxone-reversible component of analgesia.
Some Factors Influencing Analgesic Effects

It is important to note that all the findings made in this laboratory concerning factors influencing analgesia are for non-naloxone-reversible analgesia (except in the cat studies described above), which is powerful but has a short onset latency and quick recovery. It is possible that the naloxone-reversible component is determined by completely different factors than those described here.

Size of Afferent Fibers that Produce Analgesia. Acupuncture analgesia is reported to be related to the activation of Aβ or group II fibers [3, 35, 59, 69], but evidence for this is not definitive. Woolf et al. [74] found that strong percutaneous stimulation which excited Aδ fibers elicited a more powerful analgesia than did weak stimulation. In addition, several conflicting reports appear in Chinese abstracts. Two groups of workers [8, 45] claimed that acupuncture analgesia is transmitted mainly by large A fibers, while another group [7] concluded that small fibers (including unmyelinated fibers) contribute more to the analgesia than do large fibers. Therefore it is not clear what size of afferent fibers triggers the peripheral nerve stimulation-produced analgesia.

In one study [15], the C-fiber-evoked, STT-cell activity was compared before, during, and after repetitive conditioning stimuli were applied to the tibial nerve for 5 min. As shown in Fig. 5, very little analgesic effect was produced by the conditioning stimulation at a strength that was sufficient to activate most of the Aβ fibers but none of the Aδ fibers of the tibial nerve. However, when the conditioning stimulus strength was increased to a level that activated many of the Aδ fibers, there was a powerful analgesic effect. When the strength of the conditioning stimulus was increased still further to include C fibers, the inhibition became even more powerful. Since the largest increment of the analgesic effect occurred when Aδ fibers were recruited, it can be concluded that the Aδ fiber group is the most important for producing the analgesic effect, although significant additional effects were also produced by the Aαβ and C fiber groups. When TENS was applied in the experimental monkey model while monitoring the current delivered by a commercially available TENS unit, inhibition of C-fiber-evoked, STT-cell activity occurred only when the intensity of TENS exceeded the threshold of the Aδ fibers [41]. The result obtained from the cat experiment is consistent with the above findings [66]. Conditioning stimulation applied to the tibial nerve at an intensity that excites only Aαβ fibers produced weak inhibition of the flexion reflex; increasing intensity above the threshold for Aδ fibers produced much greater inhibition. Results of the above studies suggest that the critical afferent fiber group that triggers an analgesic effect upon activation is the Aδ fiber group.

Most Effective Stimulus Frequency. A wide range of frequencies has been used to produce analgesic effects by peripheral nerve stimulation. It ranges from several pulses per second [2, 26] to 10 KHz [43, 48]. Although all of these stimulation frequencies seem to produce analgesia, it is not known which is the most effective. For low-frequency stimulation, Cheng and Pomeranz [9] reported that 4 Hz gives a better result than 0.2 Hz. Also while 4 Hz analgesia was blocked by naloxone, 100 Hz was not. Eriksson et al. [21] used short trains of pulses (100 Hz internal fre-
Fig. 5 A–D. Inhibition of a spinothalamic tract cell produced by peripheral nerve stimulation with graded strengths in the monkey. A The peristimulus time histogram was compiled after 10 consecutive stimuli (at the time indicated by arrow) applied to the sural nerve (bin width, 8 ms). The C-fiber evoked responses indicated by the bracket were used to form histograms in B–D. B–D While collecting C-fiber evoked responses to test stimuli applied to the sural nerve every 10 s throughout the recording period, graded strengths of conditioning stimuli were applied to the tibial nerve at a frequency of 2 Hz for 5 min. Intensities of conditioning stimuli for B, C, and D were 2.3 times Aβ fiber threshold, 20 times Aδ fiber threshold, and 3 times C fiber threshold, respectively. Waves shown in insets were recorded from the tibial nerve 2 cm distal to the stimulating electrodes. Arrows in C and D indicate the Aδ and C waves, respectively. Bin widths in B–D, 2 s. (Reproduced from [15])
Fig. 6A-F. Inhibition of a spinthalamic tract cell produced by peripheral nerve stimulation at different frequencies in the monkey. In A the evoked A and C responses are shown in a peristimulus time histogram which was compiled from 10 consecutive stimuli applied to the sural nerve at the time indicated by the arrow (bin width, 8 ms). The C-fiber evoked responses indicated by the bracket were used to form histograms in B-F. B-F Data collection was similar to that in Fig. 5, except that conditioning stimuli were delivered with a strength of 10 times Aδ fiber threshold and with the frequency indicated above each histogram. P.N.STIM., peripheral nerve stimulation. (Reproduced from [15])

frequency, 700-ms duration) repeated at a rate of 2 Hz to improve both the effectiveness of analgesia and the patient tolerance over either 100-Hz or 2-Hz fixed rate stimulation.

The results of personal study [41] confirmed this in that TENS with bursts of pulses at a low rate ("comfort bursts"; 3 bursts/s, 7 pulses/burst, internal frequency 85 Hz) was more effective in producing inhibition of STT-cell activity in the monkey than that with a fixed rate of high-frequency pulses (85 Hz) at a given intensity. However, when the different frequencies of fixed rates were compared, it was found that the higher the frequency, the more powerful was the analgesia which resulted within the range (0.5–20 Hz) tested [15]. In this study, the C-fiber-evoked, STT-cell activity was compared during conditioning stimuli of varying frequencies applied to the tibial nerve for 5 min. As shown in Fig. 6, the conditioning stimulus was set at a strength above threshold for Aδ fibers, and the stimulus frequency was varied from 0.5 to 20 Hz. There was weak inhibition with the
frequency at 0.5 Hz, and the inhibition increased progressively as the stimulus frequency was increased. Stimulus frequency beyond 20 Hz could not be tested because the STT-cell activity is generally completely suppressed at 20 Hz, making further comparison difficult. Therefore, it seems likely that higher frequency stimulation is more effective than lower frequency in producing an analgesic effect within a reasonable range, at least for the non-naloxone-reversible component. (But frequencies above 4 Hz at intensities sufficient to activate Aδ fibers would cause intolerable muscle spasm due to tetanic contractions.)

**Stimulation Point Specificity.** Acupuncture can be applied to any of several hundred known acupuncture points all over the body. It has been reported that the application of acupuncture to a particular point is critical for analgesia to develop in a given region of the body [18, 26, 38, 69]. Although the old Chinese acupuncture theory of a hypothetical system of energy channels or meridians was considered the basis for acupuncture specificity [3, 36], the current scientific explanation of acupuncture does not support this. Since acupuncture is a form of peripheral nerve stimulation, the exact point of stimulation should not make much difference as long as the innervating nerve is stimulated effectively. Furthermore, particular acupuncture points on the body are believed to exert effects on specific areas some distance away from the points [10, 70]. However, the result of a carefully controlled experiment showed that acupuncture in a given point decreased pain sensitivity by the same degree in “target” and “non-target” areas [46]. On the other hand, TENS is generally most effective when electrodes are placed within the painful dermatome [21, 44].

To test stimulation point specificity in this experimental animal model, conditioning stimulation was applied to nerves innervating different parts of the body [15]. The most effective nerve in producing inhibition of activity of STT cells recorded from the monkey lumbar spinal cord was the ipsilateral tibial nerve. The contralateral sciatic nerve, the ipsilateral median nerve, and the contralateral median nerve were less effective, in that order. This result suggests that the inhibition of STT-cell activity produced by peripheral nerve stimulation is segmentally organized. A similar result was obtained by applying TENS with a commercially available TENS unit [41]. Furthermore, no systematic difference in analgesic effect produced by stimulation of the tibial or common peroneal nerve has been noted [13]. Therefore, no evidence for stimulation point specificity for the production of analgesia was seen in these studies, at least for the non-naloxone-reversible component. It is possible that the powerful non-naloxone-reversible analgesic effect produced here is equivalent to the TENS effect which is reportedly organized segmentally without precise stimulation point specificity [31, 34, 56].
Fig. 7. Effect of peripheral conditioning stimulation on the activity of dorsal horn cells (DH) and ventral root motor axons (VR) evoked by mechanical and thermal stimuli in the spinal cat. The activity of the dorsal horn cells was evoked by application of innocuous mechanical (repetitive brushing with a camel’s hair brush), noxious mechanical (pinching a fold of the skin with a pair of serrated forceps), and noxious heat (application of noxious thermal stimulation with a contact petier thermode) stimuli applied to the skin within the receptive fields. The reflex activity in motor axons could only be evoked by noxious stimuli. The evoked activity obtained immediately after conditioning stimulation of the tibial nerve at 2 Hz for 5 min was compared with the prestimulus control evoked activity. Data are expressed as mean percentage of the control value (bars indicate SEM). Dots indicate values significantly different from the controls. Asterisks indicate values significantly different from those obtained after conditioning stimulation at Aβ fiber strength. Note that the dorsal horn cell activity evoked by pinching was inhibited more than that evoked by brushing when the conditioning stimulation was applied at C fiber strength. Note also that the evoked activity in the motor axons was inhibited more by conditioning stimulation than that in the dorsal horn cells in general. (Modified from [54])

**Analgesic Effect Tested Using Pain Responses to Natural Stimuli**

The experimental animal models described above seem to be useful for studying peripheral conditioning stimulation produced analgesia. However, these models are based primarily on the inhibitory effect of peripheral nerve conditioning stimulation on electrically-evoked responses to peripheral nerve stimulation. Therefore, it is not absolutely certain that the elicited responses are “pain responses” and that the effects produced by peripheral conditioning stimulation are “analgesic effects”. A study was recently conducted to test the effects of conditioning stimulation of a peripheral nerve on the activity of dorsal horn cells evoked by natural forms of stimuli applied to the receptive fields in the unanesthetized, decerebrate/spinal cat [16, 54]. The responses of spinal neurons were evoked by noxious and innocuous mechanical stimuli. Conditioning stimulation of a peripheral nerve produced a powerful inhibition of the responses elicited by noxious stimuli, suggesting that this inhibition is an analgesic effect. Furthermore, the inhibition was differentially greater on the responses to noxious than to innocuous stimuli as shown in Fig. 7. Selective reduction of nociceptive responses of dorsal horn cells were observed earlier by Pomeranz and Cheng [57].
Sensitivity Difference Between Analgesic Tests

Indices commonly used to determine levels of pain in experimental animals include measurement of various types of behavioral signs (e.g., vocalization), autonomic motor reactions (e.g., pupil size, blood pressure), somatic motor reactions (e.g., flexion reflex), and activity of nociceptive tract neurons (e.g., dorsal horn cells, STT cells). My tests mainly depend on two of the above indices, the flexion reflex and the activity of nociceptive tract neurons. In an attempt to determine the sensitivity for detecting analgesic effects between two tests, a study was conducted to compare the effects of conditioning stimulation of a peripheral nerve on the activity of dorsal horn neurons and motor axons evoked by noxious stimuli applied to the receptive fields [16, 54]. Comparison was also made of activities evoked by noxious mechanical and thermal stimuli. The reflex activity recorded in motor axons was found to be more sensitive than the activity of dorsal horn cells. The magnitude of the analgesic effect was bigger for the responses to noxious thermal than to mechanical stimuli (Fig. 7). Therefore, among the combination of methods tested, the most sensitive one for detecting analgesic effects produced by conditioning stimulation of a peripheral nerve seems to be the recording of reflex activity of motor neurons elicited by noxious thermal stimuli. Perhaps because of this high sensitivity, many analgesic effects have been successfully demonstrated using the tail flick test [19, 33, 60, 75], a form of reflex motor activity elicited by noxious thermal stimuli.

Problems Associated with Acupuncture Research

Acupuncture is methodologically defined as a procedure, and yet there is no standard procedure. Traditional acupuncture can be defined as a procedure that involves the insertion of acupuncture needles into acupuncture points and the application of mechanical stimulation by manually rotating and moving the needles in and out to treat a disease or to relieve the symptoms of a disease. A number of questions arise from this definition. Is a procedure that applies the same stimulation with a nonacupuncture needle still acupuncture? Is a procedure that applies the same stimulation into nonacupuncture points still acupuncture?

The problem is much worse in electroacupuncture. Electroacupuncture is a procedure that stimulates acupuncture points electrically by passing current through acupuncture needles inserted into the points. If one applies the electrical stimulation through hypodermic needles instead of acupuncture needles, is this procedure electroacupuncture? What should the parameters (intensity, frequency, and patterns) of electrical stimulation be to qualify as electroacupuncture? How deeply do the electrodes have to be inserted into the tissue? Can stimulation through a surface electrode be called electroacupuncture? What would then be the critical difference between electroacupuncture and TENS?

There have been some attempts to define acupuncture effects. One notable example is a proposal made by Andersson [1], when comparing the effects of TENS and acupuncture. He proposed that acupuncture be defined as needle manipulation producing the “De Qi” sensation and electrical stimulation at low
frequencies (below 10 Hz) given at an intensity producing more than local muscle contractions. TENS was proposed to be defined as stimulation at higher frequencies. According to Andersson [1], TENS produces a rapidly developing, short lasting, and segmentally distributed analgesia, whereas acupuncture results in an analgesia that has a slow onset, is long lasting, and may be nonsegmentally distributed. However, there are a number of exceptions to this in published results, as reviewed elsewhere [15]. For example, TENS with high frequency (70–200 Hz) reportedly resulted in analgesia lasting longer than 2 h [9, 47]. Pertovaara et al. [55] reported that stimulation with bursts of pulses repeated at 2.5 Hz produced analgesia that could be observed only during stimulation.

Since acupuncture is a methodologically defined term, acupuncture applied with different methods may produce analgesia through different mechanisms. It is most likely that peripheral nerve stimulation produces analgesia through multiple mechanisms. A particular method of stimulation may favor one mechanism, whereas other methods may elicit other mechanisms. Therefore, it is very important to review the published literature while keeping in mind the particular method employed for each study. Part of the confusion in the acupuncture literature may be due to the production of analgesia through different mechanisms in different studies, depending on the methods employed. For example, frequently cited literature for the central structures involved in acupuncture analgesia include studies by Du et al. [20] and Shen et al. [64]. These studies observed a fast onset, short lasting, inhibitory effect of a viscerosomatic reflex by electroacupuncture at high frequency (25–100 Hz). The results of these studies may not apply to acupuncture effects produced at low frequency (2–4 Hz) since the mere act of acupuncture does not necessarily produce an analgesic effect by the same mechanism that underlies typical acupuncture effects.

Summary

This is a brief review of attempts to investigate the peripheral and spinal mechanisms underlying acupuncture analgesia. The basic approach has been the electrophysiological recording of neuronal activity from the spinal cord, which can be used as a nociceptive index, and its modulation by electrical stimulation of a peripheral nerve in experimental animals. Two experimental animal models have been developed. In one, the late component of the flexion reflex in spinal cats was used as a nociceptive index. In the other, activity of STT cells in monkeys evoked by unmyelinated fibers in a peripheral nerve was used as a nociceptive index. In both models, prolonged repetitive stimulation of a peripheral nerve was shown to produce an antinociceptive effect which is largely dependent on spinal mechanisms. The antinociceptive effect produced consists of two components, naloxone reversible and non-naloxone reversible. The non-naloxone-reversible component is very powerful and develops quickly, but it lasts only a short period, whereas the naloxone-reversible component is less powerful, develops slowly and lasts a long time. My effort has been concentrated mainly on the non-naloxone-reversible component of the antinociceptive effect because I was attracted by its powerful effect. The most important afferent fibers responsible for the antinociceptive effect

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were found to be in the Aδ group. Within the range tested (0.5–20 Hz), higher frequencies of stimulation were more effective in producing the antinociceptive effect than lower frequencies. The inhibition produced by peripheral conditioning stimulation was differentially greater on the responses to noxious than to innocuous stimuli. The magnitude of the antinociceptive effect is larger for the responses to noxious thermal than to mechanical stimuli. Furthermore, the reflex activity recorded in motor axons seems to be more sensitive than the response of dorsal horn cells.

Finally, lack of a precise and widely accepted definition of “acupuncture” and “acupuncture effect” has been pointed out and is discussed as a problem in the study of acupuncture mechanisms.

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