Human acupuncture points mapped in rats are associated with excitable muscle/skin–nerve complexes with enriched nerve endings

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Abstract

As part of our ongoing investigation into the neurological mechanisms of acupuncture, we have tried to correlate the distribution of afferent nerve endings with acupuncture points (AP) in the rat hind limbs. In vivo extracellular microfilament recordings of A\textsubscript{a}/A\textsubscript{h}/A\textsubscript{y} fibers were taken from peripheral nerves to search for units with nerve endings or receptive fields (RF) in the skin or the muscles. The location of the RFs for each identified unit was marked on scaled diagrams of the hind limb. Noxious antidromic stimulation-induced Evans blue extravasation was used to map the RFs of C-fibers in the skin or muscles. Results indicate that, for both A- and C-fibers, the distribution of RFs was closely associated with the APs. In the skin, the RFs concentrate either at the sites of APs or along the orbit of meridian channels. Similarly, the majority of sarcous sensory receptors are located at the APs in the muscle. Results from our studies strongly suggest that APs in humans may be excitable muscle/skin–nerve complexes with high density of nerve endings.

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1. Introduction

For more than 2000 years, acupuncture has been widely used to treat various functional disorders and certain types of intractable pain [13,14,23]. Acupuncture in pain relief is also demonstrated in animal models of persistent pain (e.g., ankle sprain pain) [8]. The outcome of acupuncture therapy is largely dependent on accurate localization and penetration of the acupuncture points (APs). “DeQi” is a Chinese word describing the sensation when the needle is properly inserted into the APs. It is an unpleasant sensation mixed with soreness, numbness, and tingling. Although considerable efforts have focused on explaining the essence of APs anatomically or physiologically [5,6,9–12,16], the physiological and morphological characteristics of APs remain unknown. In the present study, we hypothesized that the mixed sensation of “DeQi” may be mediated by simultaneous activation of various types of cutaneous or sarcous sensory receptors within the APs.

In previous studies, we have shown that in the APs, peripheral sensory receptors, especially muscle receptors, are activated by acupuncture-induced contraction of the muscle compartment. The longer the needle puncture lasts, the more receptors that are activated. The elicited neuronal activity then invades the motoneurons innervating homonymous or synergistic muscles. Thus, puncture of an AP may elicit a reflex loop consisting of a muscle compartment of AP, afferent fibers, motoneurons, and efferent fibers, which works in an input–output–input fashion. This reflex loop is believed to be the underlying neurophysiological mechanism of the propagation of needle feeling along the meridian [24]. This theory is consistent with the fact that the specific needle feeling “DeQi” propagates along its meridian channel with extremely low conduction velocity, usually in the ranges of centimeter per second (cm/s), which is much lower than the
normal conduction velocity of peripheral nerve fibers. The reflex activities can be explained by the monosynaptic localization and motoneuron recruitment theory [1,3,4]. Since “DeQi” can be obtained only by stimulating the APs, it is possible that APs may possess some special physiological or morphological characteristics. Here, we report for the first time that the density of peripheral nerve endings in the skin or muscles is much greater in the APs as compared to areas beyond the APs, and thus, an AP may be an excitable complex with high-density nerve endings/receptive fields (RFs).

2. Materials and methods

Sprague–Dawley rats of either sexes weighing between 250 and 300 g were anesthetized with pentobarbital sodium (40 mg/kg; i.p.). Supplemental dose (10 mg/h) was provided via a cannula in the jugular vein to maintain the anesthesia. Rectal temperature was monitored and maintained at 37 ± 1 °C via a self-controlled heating blanket. The protocols used in this study were approved by the Animal Use and Care Advisory Committees of the Chinese Academy of Medical Sciences.

2.1. Mapping the cutaneous receptive fields

Under general anesthesia, an incision along the hind limb was made and the tibial, peroneal, saphenous, and sural nerves were exposed and bathed in a paraffin oil pool formed by skin flaps. Microfilament recording was made from each nerve to search for single fibers, as reported previously [15]. To avoid any bias in sampling, we divided the whole nerve into four to five bundles of similar size. About three to six fibers in each bundle were recorded to cover the entire innervated area. Once an afferent fiber unit was isolated, its RF was located by brushing the skin with a cotton swab, gently stroking with fingers, and noxious/unnoxious poking with von Frey filaments. A pair of bipolar stimulating electrode was then inserted into the center of the RF followed by a single current pulse delivered to the skin to measure the conduction velocity (CV) of the recorded unit. The location of each identified unit was marked proportionally on a foot chart. The recorded units were identified as Aβ- or Aδ-fibers based on their CVs.

2.2. The distribution of sarcoptic sensory receptors

The anterior tibialis (TA) or rectus femoris muscle was dissected with blood supplies preserved. The tibia or femur was positioned horizontally and fixed by iron clamps; and the distal tendon of the muscle was cut and attached to a strain gage to measure the muscle tensions. Microfilament activities (Aα) of deep peroneal or femoral nerve were recorded; and the RFs of the muscle receptors were identified with von Frey filaments [21]. The receptors were classified as muscle spindles (MSs), Golgi tendon organs (GTOs), and pressure receptors based on the criteria described previously [18]. The location of each receptor was marked on a scaled diagram of the selected muscle.

2.3. Mapping C-fiber-innervated territories by Evans blue technique

It is known that repeated antidromic electrical stimulation of peripheral nerve at C-fiber strength causes an increase in the vascular permeability in its territory and leads to plasma extravasation that can be visualized by intravenous injection of Evans blue [7,20]. The same technique was used to examine C-fiber distribution in the skin or muscle. Great care was taken to prevent any damage to the skin or muscles in the targeted territories during isolation of the tibial, superficial peroneal, deep peroneal, saphenous, and sural nerves. In order to eliminate the interference from efferent reflex, the nerves were cut at the mid-thigh level before receiving peripheral stimulation.

Evans blue (50 mg/kg, 50 mg/ml in 0.9% saline) was administered intravenously followed by an electrical stimulation of the nerves (500 μs in pulse width, 10 Hz, and 10 V), which was applied to the caudal cut end of the nerve for 10 min for the skin nerves and 30 min for the muscle nerve. Ten minutes after the end of electrical stimulation, rats were perfused transcardially with 250-ml saline to flush the dye from the intravascular compartment and to display the extravasated Evans blue.

3. Results

3.1. Distribution of cutaneous RFs

A total of 421 fibers were recorded from the tibial (130), common peroneal (71), saphenous (134), or sural nerves (86) from 27 rats (9 female and 18 male), out of which 320 were identified as Aβ- and 76 were Aδ-fibers. The remaining 25 fibers were unidentified.

The distributions of all RFs were marked proportionally on sketched limb charts. It was found that the densities of the nerve endings varied at different regions of the skin. Some regions had high density of nerve endings, and some had only a few RFs. A great number of nerve endings were found at the tiptoes, where the sites of EX-LE 12 APs are located (Fig. 1B). On the dorsal surface of the paw, most afferent terminals ended at the areas between digit tendons, where the loci of APs or/and the orbit of meridians were located. Also, there was a high density of nerve endings along the boundary line of glabrous and hairy areas (i.e., the lateral and medial edges of the paw), which were perfectly accordant with the routes of the urinary bladder and the spleen meridians (Fig. 2).
3.2. RF distribution in the muscle

A total of 16 rats (5 female and 11 male) were used in this series of experiments. One hundred and fourteen afferent units were isolated from deep peroneal nerve innervating the TA. Of the 114 units, 101 were MSs, 7 were GTOs, and 6 were pressure receptors. An additional 143 units including 117 MSs, 16 GTOs, and 10 pressure receptors were obtained from the femoral nerve innervating the rectus femoris muscle. The distributions of nerve endings were not even in the muscle, similar to that in the skin. The majority of the nerve terminals located in the proximal portion of TA, whereas the caudal portion possesses only a few nerve endings (Fig. 3B, bottom). Consistently, the receptors in the rectus femoris muscle located primarily in the proximal and middle-distal portion of the muscle (Fig. 3B, top). The results obtained from the TA and the rectus femoris muscle demonstrated a close correlation between nerve endings and the sarcous APs. No gender differences of cutaneous or sarcous RF distributions were found in this study.

3.3. C-fiber innervation in the skin and muscles

A total of 12 rats were used in this study. The innervating territories of C-fibers in superficial peroneal, deep peroneal, tibial, sural, and saphenous nerve were visualized through Evans blue extravasation method. As shown in Fig. 3C, the blue coloration mainly appeared in the proximal region of the TA, which also had high density of A-fiber endings. Evans blue was also shown on
the tiptoes and the distal part of the sole, which are innervated by the tibial nerve (Fig. 1C). We compared these results with human atlas of plantar APs (Fig. 1A) and found that the colored loci perfectly match the major APs such as the KI 1 on the sole and the EX-LE 12 on the tiptoes. Similar to the tibial nerve, the area with dye extravasation after antidromically stimulating the saphenous nerve, the peroneal nerve, or the sural nerve at C-fiber strength represented a narrow zone that is consistent with the orbit of a meridian in human (Fig. 4).

4. Discussion

In the present study, combining single fiber recordings with the Evans blue extravasation, we have found that A-/C-fiber terminals in the skin and muscles of the rats are distributed in close association with the loci of APs in humans. Using rats in this study to examine the neurological mechanisms of the APs in humans was based on the fact that rat hind limbs are anatomically identical to those of humans, and it is expected that the location of the APs and the travel routes of the meridians in rats should resemble those of humans.

On the dorsum of the paw, high density of cutaneous nerve endings or sensory receptors is presented on the tiptoes, the distal region of the sole, and the areas between the digit tendons. The nerve terminal-enriched areas are in accordance with either the APs (e.g., EX-LE 12, KI 1) or the orbit of meridians on the dorsum of the paw. A similar correlation is identified in the muscles, for example, nerve endings in TA are concentrated in the proximal portion, where four key APs, the ST 36–39, are located. There is no AP in the caudal portion of the TA, where only scattered nerve endings are recorded. In the rectus femoris muscle, the nerve endings concentrate at the proximal and middle-distal sections but not the middle section of the muscle. In agreement with the fiber distribution, there is one AP (e.g., ST 31) in the proximal and three APs (e.g., ST 32–34) in the distal portion of the rectus femoris muscle, while there is no AP in the middle portion. Thus, based on the data obtained from this and our previous studies, we propose that the sarcous AP may be an excitible muscle/skin–nerve complex, in which a series of reflex activation occurs in response to needle puncture.

Our data from the TA indicates that the sarcous nerve endings are concentrated at the nerve entrance points where many APs are located. These findings are supported by previous reports that most of the muscle spindles distribute around the point of nerve entrance and many of the APs locate at the sites where nerves enter the muscle [6,16]. Functionally and anatomically, sarcous APs

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Fig. 3. Spatial distribution of muscle sensory receptors and the sites of the sarcous APs. In A, the solid lines illustrate the orbit of the Stomach meridian. The majority of the nerve endings are found in the proximal portion of the TA (bottom of B) and in the proximal and middle-distal regions of the rectus femoris muscle (top of B), which are also the sites of many sarcous APs. C shows the areas with Evans blue extravasation on the surface of the TA (arrowhead) after noxious antidromic stimulation of the deep peroneal nerve. The dye extravasation area is in the proximal portion of the TA that overlaps with the nerve endings as shown in the bottom of B.

Fig. 4. Noxious stimulation of the cutaneous nerves reveals the innervated territories as indicated by Evans blue. Each of the colored area following electrical stimulation of the saphenous (A), the common peroneal (B), or the sural nerve (C) corresponds with the route of the Three-Yin meridian of foot, the Stomach meridian, or the Bladder and Gallbladder meridian, respectively. These meridians are shown in Figs. 2C, 3A, and 2A, respectively.
resemble a motor point, which is the most excitable point of a muscle and receives high concentration of nerve endings. It is located in the skin over the muscle and corresponds approximately to the level at which the nerve enters the muscle belly [2]. However, the difference between APs and motor points is obvious. Motor points are defined as separate points with excitable muscle zone and are functionally independent from each other. APs are more complex than motor points. The number of APs is greater than that of motor point; and there is a close communication between individual APs. It is our understanding that APs with homogenous function form a meridian and that each AP is also involved in the function of an internal organ.

Our previous study indicates that the excitability of different regions within a muscle is variable. Some portions of the muscle are sensitive to mechanical stimulation and may serve as excitable trigger points. Moreover, only in some selected regions of the muscle can needle puncture cause a vermicular contraction (but not a simple twitch), which propagates along the longitudinal axis of the same muscle [17]. Repeated needle punctures in a single AP can initiate needle feeling that is associated with electromyogram (EMG) bursting and propagating along the meridian and can be maintained for a while after the needle withdrawal [25]. The needle feeling or vermicular contraction can be, at least, partially explained by certain electrophysiological observations, i.e., the recruitment of the motoneurons and after-discharges of afferent fibers. It was found that the afferent discharges from a given AP can be elicited not only by mechanical stimulation of the AP, but also by inputs from the homonymous meridional points located in heterogeneous but synergistic muscles. The latter phenomenon can be completely eliminated by muscle immobilization [24], suggesting that a complex reflex may have been initiated by an excited AP via an input–output–input circle occurring in the spinal cord.

The distribution of cutaneous nerve endings was consistent with either the location of non-sarcous APs (on the sole) or the orbit of the meridian. On the dorsum of the paw, the nerve endings form a line along the meridian channels. It has been known that activation of cutaneous afferents from the hind limb can produce either excitatory or inhibitory effects on the associated motoneurons [19]. In our own laboratory, it was found that stimulation of the cutaneous nerve over the sarcous AP could enhance the activity of sarcous APs within the same meridian [24]. Further investigation is necessary to understand the mechanisms underlying the modulation of sarcous APs by the cutaneous ones.

In summary, based on the findings from animal study, it suggests that APs in humans may be excitable muscle/skin–nerve complexes with high density of nerve endings. Needle feeling may result from the activation of multireceptors within an AP or from the communication between non-sarcous APs and sarcous APs.

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References


