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A New Type of Signaling Pathways as Pilomotor Lines along Skin for Transmitting Acupuncture Signals to Produce Acupuncture Effects

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Abstract

In our previous study, we observed a linear system consisted of sympathetic endings in the arrector pili muscles (AP muscles) along the rat skin termed sympathetic substance lines, or SSLs. After shaving the hair of the rats, the first wave of hair re-growth was not evenly distributed, but followed specific hair loop lines (HLLs). The patterns of HLL and SSL correspond with each other and also with the "Meridians" described in Chinese traditional medicine (CTM). Here I investigated in rabbits and rats whether the acupuncture signals are transmitted *via* the SSL/HLL, and whether the acupuncture analgesia (AA) is dependent on any peripheral mechanism. Firstly, when acupuncture was operated or phenylephrine, an agonist for α receptor, was injected into the dermis at an acupoint, a pilomotor line occurred. The course of the pilomotor line coincided with the SSL/HLL. When the skin was incised or regitin, an antagonist for α receptor, was injected into the dermis, the pilomotor line did not cross the site of incision or injection. These results directly demonstrated the process of transmission of acupuncture signals involving the pilomotor line and the sympathetic. Secondly, AA produced by acupuncture at an acupoint was significantly blocked when the skin was incised or regitin was injected into the dermis along the SSL/HLL or the Meridian. These results suggest that the factor that blocked the pilomotor line also blocked the AA and the pilomotor line related to the AA. Lastly, noradrenaline was shown to be released from the skin along the Meridian line after acupuncture; when phenylephrine was injected into an acupoint, AA was strongly simulated. All these results indicate that: 1. the transmission pathway of acupuncture signals exists in the skin, just as the Meridians described in the CTM; 2. these pathways are the SSLs/HLLs and the pilomotor lines; and 3. the pilomotor line is just for the transmission of acupuncture signals and the transmission is dependent on the α receptor in the AP muscles, specifically the contraction of the AP muscles. Moreover, these findings suggest a new system and a new type of signal transmission in the physiology.

Key Words: acupuncture analgesia, meridian, pilomotor line, receptor, single pathway, sympathetic

Introduction

Acupuncture, a physical therapeutic in Chinese traditional medicine (CTM), has been practiced for at least 3,000 years in China, and is gaining acceptance in the west country as the alternative medicine. One of the most consistently supported uses of acupuncture therapy is in pain treatment, called acupuncture analgesia (AA). It is also beneficial for chronic health problems (1-6, 11, 12, 26-28). While it is anticipated that a combination of eastern and western medical practices may lead to better treatments in the future, it is not yet completely understood how acupuncture works. The traditional acupuncture theory involves a mysterious element of "Qi" (chee), said to circulate throughout the whole body along certain pathways, called "Meridians" or Meridian lines, distributed along the surface of the body. Acupuncture involves the insertion of fine needles into "acupoints" along the Meridians to excite Qi, a procedure also called acu-esthesia,

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for adjusting body functions and curing diseases. According to CTM, the acupuncture effects (AE) are achieved only when the excited Qi runs along the Meridian lines and reaches the target organs. There are a small number of people (about 1%) who, when an acupoint is stimulated, are able to feel the transmitted sensation along a Meridian line, called TSM. When experiencing TSM, the sensibility of skin decreased obviously (13), an effect analogous to the transmission of Qi along a Meridian line. It is considered that the transmitted sensation, or Qi, is a type of signal produced by acupuncture. We have observed in four subjects, including the author of this paper, that injection of regitin into the skin along the Meridian line blocked TSM. Therefore, I attempted to objectively repeat this phenomenon in the animals.

My objective is to study whether Meridians or other unknown signaling pathways indeed exist along the skin, and whether signals produced by acupuncture indeed transmit through such pathways. In one of our previous studies (16), using whole-body macro-autoradiography with an iodine-125-labeled tyrosine, we localized the distribution of iodine-125-catecholamine in the skin of rats. The images on the film showed various pairs of symmetrical lines running from the head, through the back of the animal leading to the hind limbs along pathways termed sympathetic substance lines (SSLs). The SSL consisted of a linear loop system around whole body skin. The characteristics of SSL are similar to the Meridians in CTM. Furthermore, we proved that the SSLs come from dense nets of noradrenergic nerve fibers innervating the arrector pili muscles (AP muscles) beneath the line. In another study (14), when the hair of adult rats was shaved, the first wave of hair regrowth did not distribute evenly but along specific craniocaudally oriented lines that ran from the head of the animals, through the body trunk to the limbs, and were symmetrical along the left and right sides of the body. The symmetric hair-lines from both sides of the body converged around the mouth and nose, and at the pubic region or ventral midline, to form a system of hair loop lines (HLLs). The loops can be distinguished into four main patterns. The courses of hair lines coincide with the SSLs, but are more intact than SSLs. What then is the implication of the SSL and HLL? The hair follicles and AP muscles along the SSL and HLL are hypothesized to play a vital, unknown role in the body function. I therefore sought to investigate whether and how acupuncture and Meridians correlate with the SSLs, HLLs and AP muscles.

Materials and Methods

With the exception of two experiments involving continuous injection of drugs performed in rabbits, all other experiments were carried out in rats. The basic animal model of the experiments is that the stimulation (acupuncture) produces effects, and a Meridian line connects or mediates between the acupuncture and the effect. The factor blocking the mediate line must involve the essence of the Meridian (Fig. 1A). In the study, the AE were observed as pilomotor and pain. The effects were blocked by injection of a drug or incision at skin, and were simulated by another drug.

Blocking of Acupuncture Analgesia by Injecting Regitin along a Meridian Line

For testing the relationship between the α receptor of the sympathetic along the Meridian line of skin and the AE, 12 white rabbits, each about 2 kg in weight, equal number of males and females, were selected. The rabbits were hung in a thick bag with their legs and heads exposed. The hairs around the cheek outside the mouth angle were shaved. Six points for acupuncture and injection were located (Fig. 1B). The dotted line corresponds with the Stomach Meridian line of the human body in CTM, which starts at the foot, ascends *via* the anterolateral of the hind limb, and passes across the abdomen and thorax along the mid-clavicular line, ending at the face. The acupoint name is Zusanli (St36), which is located at the depressed dent below the tibial tuberosity and between the anterior border of the tibia and the tibialis anterior muscle.

Measurement of Pain Threshold. The heat-pain threshold (PT) of animals was measured to estimate the effect of acupuncture analgesia. The pain-induced time of latency of head-throwing reflex was recorded when the cheek outside of rabbit's mouth angle is irradiated by focused light-heat from a Heat Radiation Apparatus (the diameter of the focus was about 5 mm). Before acupuncture, the basic value of pain threshold (BPT) was measured 3 times at 5-min interval, and the mean was calculated. After acupuncture, PT was measured immediately as well as at 5-min interval. If the PT was over 200% BPT, irradiation was stopped to avoid serious burns.

Acupuncture. An acupuncture needle was inserted into an acupoint to about a depth of 10 mm and was twisted about 1 Hz for 10 min.

Injection. Regitin (10^{-5} g/ml, Ciba, Basle, Switzerland), or physiological saline (0.9% NaCl solution), was slowly and continuously injected into the dermis of the skin at two points simultaneously, either along the Meridian line (Fig. 1B-3 and -4), or 2 cm away from the Meridian line (Fig. 1B-5 and -6) by a micro-propeller through two entry needles, respectively. Each rabbit accepted three treatments at 5-day intervals: [1] Normal, acupuncture and injecting physio-

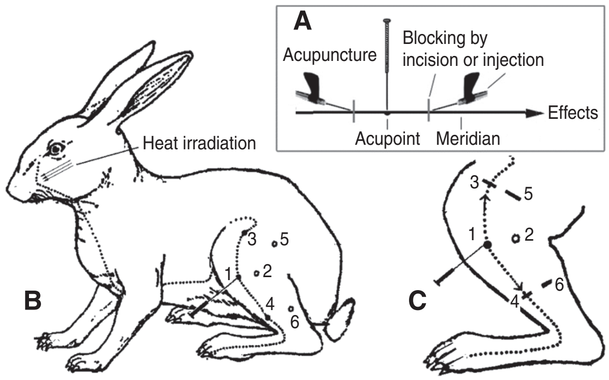

Fig. 1. The basic experimental model (A) and diagram of anatomical sites for acupuncture and injection or incision in rabbits (B) and rat's legs (C). The dotted line is a Meridian line. 1, acupoint (Zusanli, St36); 2, non-acupoint; 3 and 4, two sites for injection in rabbits and incision in rats along the Meridian line, above the knee and in the middle of the crus respectively; 5 and 6, two sites for injection and incision away from the Meridian line in rabbits and rats.

logical saline along the Meridian line; [2] Test, acupuncture and injecting regitin along the Meridian line; [3] Control, acupuncture and injecting regitin away from the Meridian line. The orders of three treatments were equally allocated to every 4 of the 12 rabbits respectively.

Experiments were performed in sequence: [1] the BPT of the rabbit was measured 3 times for 15 min; [2] injection of the physiological saline or regitin was commenced; [3] after 5 min injection with 2 μl, the rabbit received acupuncture for 10 min and another injection of 1 μl simultaneously; [4] after acupuncture, injection continued for 50 min with 4 μl, while the PT was measured at 5-min intervals; [5] after stopping the injection, PT was measured at 5-min intervals for 75 min.

Simulation of Acupuncture Analgesia by Injecting Phenylephrine into an Acupoint

To test whether injection of an α receptor agonist into acupoints can simulate AA, twelve rabbits were used, about 2 kg in weight, equal number of males and females. Phenylephrine (2.5×10^{-6} g/ml, Sigma, St. Louis, USA) was continuously injected into the dermis of the animal at the St36 acupoint (Fig. 1B-1), or 1 cm away from the acupoint (Fig. 1B-2), using a micro-propeller through an entry needle. Each rabbit received both Test injection (on-acupoint) and Control injection (non-acupoint) at 5-day intervals. The order of Test-Control and Control-Test were equally allocated. The injection continued for 30 min with a total injected volume of 10 μl. BPT was measured 6 times for 30 min for observing more normal states. Experiments were performed as follows: [1] the rabbits were hung and the cheek hairs were shaved as described previously; [2] the BPT was measured; [3] injections commenced; [4] after 5 min injection, PT was measured at 5-min intervals for 85 min and the injection continued 25 min.

Producing and Blocking of Pilomotor Zone or Pilomotor Line

Wistar rats, about 200 g, equal number of males and females, were anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal injection). The rats' hair was shaved in discontinuous sections to form approximately ten alternating shaved and unshaved zones from the head to the leg (Fig. 4). The tested Meridian line is located along the scapular midline of the rat's back according to the SSL and HLL.

Group of Acupuncture. Ten rats received acupuncture. A needle was inserted into an acupoint at the lower back of the rats along the scapular midline, about 8 mm in depth, and twisted about 1 Hz for 5 min. The pilomotor was observed and recorded by a camera.

Group of Drug. More than 20 rats were tested. Instead of acupuncture, Phenylephrine (40 μl-60 μl, 0.125 mg/ml, Sigma) was injected into the above acupoint of the rats. Following which pilomotor was observed as above.

Group of Drug-Blocking. Eight rats were tested. Regitin (40-60 μl, 0.1 mg/ml, Ciba) was injected into the dermis on the upper back of the rats along the scapular midline. Five minutes later, phenylephrine was injected into the above acupoint of the rats and pilomotor line was observed as above.

Group of Incision-Blocking. Six rats were used. The entire layer of the skin of the rats was incised on the upper back along the scapular midline, about 2 cm wide. Ten minutes later, the rats were injected with phenylephrine below the incision and the pilomotor line was observed as above.

Blocking of Acupuncture Analgesia by Incising Skin along a Meridian Line

To confirm that the AE was produced depending on specific pathways along the skin, the entire layer of the rat's skin along the Meridian line or SSL was incised. According to the site of incision, a group of Leg-Incision and a group of Back-Incision were divided. In the group of Leg-Incision, 12 rats were selected, 180-200 g in weight, equal number of males and females. Each rat received two treatments of Test and Control at 7-day intervals. Treatment orders of Test-Control and Control-Test were equally allocated.

In the Test treatment, the rats were anesthetized with ether, and incisions at the skin were made 8 mm wide above and 4 mm wide below the acupoint (St36), transversely disconnecting the Meridian line. In the Control treatment, the same incisions were made 5-8 mm away from the Meridian line (Fig. 1C). After incision 30 min, the PT was measured as described in the experiment of rabbits, but the time was the latency of the tail-throwing reflex by irradiating on the middle of the rat's tail. The BPT was measured three times at 7-min intervals. The rats received acupuncture as in the rabbits for 10 min at the acupoint (St36). Afterwards, PT was measured at 7-min intervals for 49 min. In the group of Back-Incision, all treatments were performed as for the Leg-Incision group, with the exception of: [1] the incision above St36 in the Test treatment was 2 cm wide and was located at the upper back of rats, about T8 level along the scapular median line (a SSL always arrays here), and in the Control treatment the incision was also 2 cm wide at the T8 level, but at thorax along the clavicular median line (a Stomach Meridian line passes here in the humans); [2] the PT after acupuncture was measured for 77 min.

Determining Catecholamine Releasing from Skin after Acupuncture

Six Wistar rats, 180-200 g, equal number of males and females, were anesthetized with pentobarbital sodium as above. The hairs around the knee were shaved and the skin was cleaned with ether. An incision at skin was made 8 mm wide above the acupoint (St36) as shown in Fig. 1C-3. The incision was cleaned with physiological saline. After that, 300 μl physiological saline was added into the incision and acupuncture was performed as in the rabbits at the St36 for 10 min. After acupuncture, the liquid in the incision was collected and mixed with 4 M $HClO_4$ (20:1). The mixture was centrifugated at 15,000 rpm at 4°C for 10 min. The supernatant was used for extracting catecholamine by Al_2O_3. The noradrenaline (NA), epinephrine (E) and dopamine (Dopa) of catecholamine were determined by high-performance liquid chromatography (HPLC) with a detector of fluorometry (10). HPLC: Spectra-Physics; Detector: FL2000; Excition: 280 nm; Emission: 316 nm; Mobile phase: 0.1 M NaH_2PO_4 + EDTA 50 mg; Flow rate: Gradient washing. In contrast, 3 samples of liquid from similar incision, remained for 10 min but without acupuncture, were measured. For determining the tissue catecholamine in the skin, three small pieces of skin tissue were crushed using a pestle and mortar. Other treatments were as described above.

Results

Blocking of Acupuncture Analgesia by Injecting Regitin along a Meridian Line

Normal. When the physiological saline was injected along the Meridian line, PT increased immediately after acupuncture, reaching a maximum within 0-15 min, which was always two times higher than the BPT. The PT then decreased and a PT peak was formed. Afterwards, the PT increased and decreased repeatedly, forming 4-5 peaks at 25-30-min intervals before restoring to normal level. Thus, the PT curve undulated regularly or periodically. Comparing with the BPT, all PTs of the post-acupuncture increased significantly ($P < 0.05$ or $P < 0.01$) with the exception of 5 measured points (Fig. 2).

Test. When regitin was injected along the Meridian line, the variations of PT after acupuncture are shown in Fig. 2. During the injection (0-50 min at the X-axis), only the first PT of the post-acupuncture increased significantly ($P < 0.05$), but was only 20% higher than the BPT. The PT values at 25 min and 35 min were remarkably lower than the BPT ($P < 0.05$). After termination of the injections, the PT in some rabbits increased again, sometimes more than 200% BPT. But only at 120 min the mean value was significantly higher than the BPT ($P < 0.05$).

Control. When regitin was injected away from the Meridian line, all PTs of the post-acupuncture increased significantly compared to the BPT ($P < 0.05$, or $P < 0.01$), with the exception of 9 measured points (Fig. 2). The curve of the PT in this group resembled the Normal group and showed more regularly, but tended to descend during the injection of the drug.

The mean of PT of every measured point between the three groups was compared by *t*-test. The PT at all measured points between the Normal and Control groups showed no significant difference ($P > 0.05$). However, the PT at many measured points between the groups of Test and Normal and between the groups of Test and Control were notably different ($P < 0.05$, or $P < 0.001$). During the injection, most of the PT at all measured points in the group of Test were remarkably lower than the other two groups.

Simulating of Acupuncture Analgesia by Injecting Phenylephrine into an Acupoint

The BPT of rabbits were quite steady in the six measured points. In the Test group, when phenylephrine was injected into the acupoint for 5 min, the PT increased rapidly and significantly ($P < 0.01$). The maximum of PT peak appeared at about 10 min after the injection. Then the PT decreased slightly and maintained its higher level. After stopping injection, the PT decreased firstly and finally increased again to reach a peak of higher level (Fig. 3). All PTs both

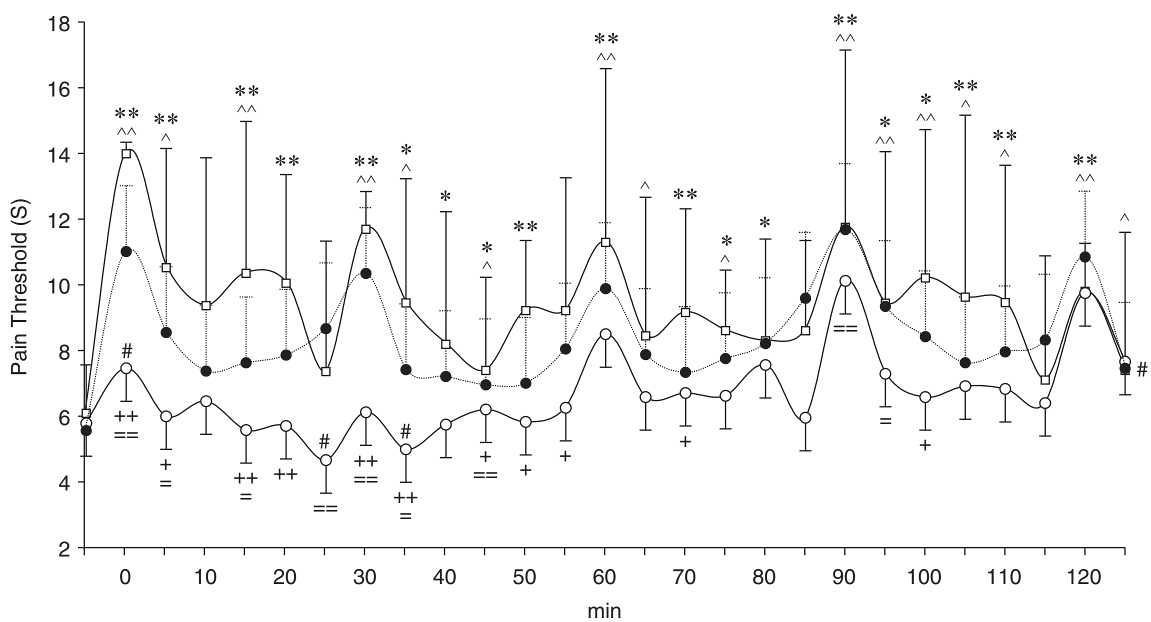

Fig. 2. Variations of pain threshold (Mean ± SD) after acupuncture and injection (n = 12). The most left represents the basic pain threshold (BPT); the injections continued from 0-50 min at the X-axis. □, Normal group; ●, Control group; ○, Drug group. Comparing PT to BPT, $P < 0.05$ (*) or $P < 0.01$ (**) in Normal group, $P < 0.05$ (^) or $P < 0.01$ (^^) in Control group, and $P < 0.05$ (#) in Drug group. Comparing group of Normal to group of Drug, $P < 0.05$ (+) or $P < 0.01$ (++). Comparing group of Control to group of Drug, $P < 0.05$ (=) or $P < 0.01$ (==).

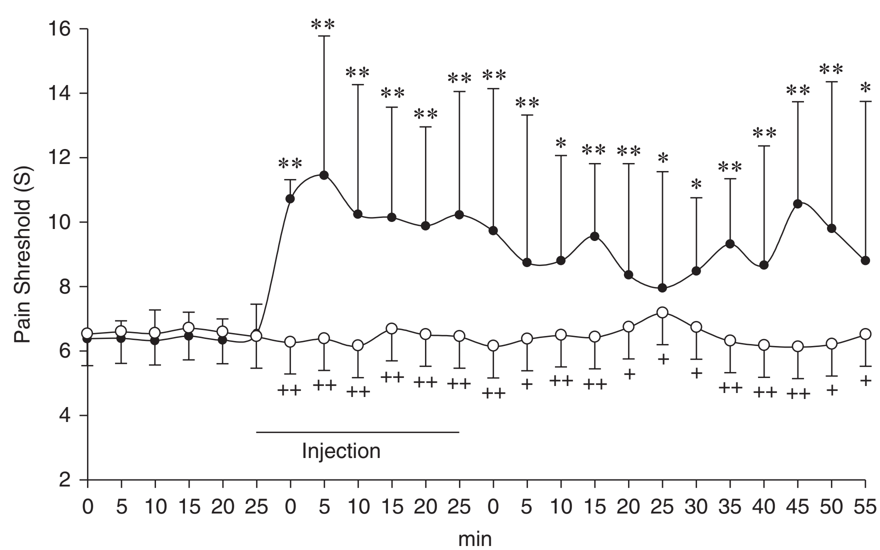

Fig. 3. Variations of pain threshold (Mean ± SD) after phenylephrine was injected into an acupoint (●) or a non-acupoint (○) (n = 12). The 6 points at the left (0-25 at the X-axis) represent the basic pain threshold. The bar between 25-0-25 represents the injection of drugs. Comparing PT to BPT in the Test group, $P < 0.05$ (*) or $P < 0.01$ (**). Comparing the group of Test to the group of Control, $P < 0.05$ (+) or $P < 0.01$ (++).

during and after the injection were remarkably higher than the BPT ($P < 0.05$, or $P < 0.01$). In the Control group, when the same drug was injected into the non-acupoint away from the acupoint, no PT noticeably increased ($P > 0.05$). Comparing the PT between the two groups at all corresponding measured points, there was no difference between the BPT ($P > 0.05$), but all were significantly different between the PT ($P < 0.05$, or $P < 0.01$).

Producing and Blocking of Pilomotor Line

Group of Acupuncture. When acupuncture was performed for one minute at the rat's lower back, the

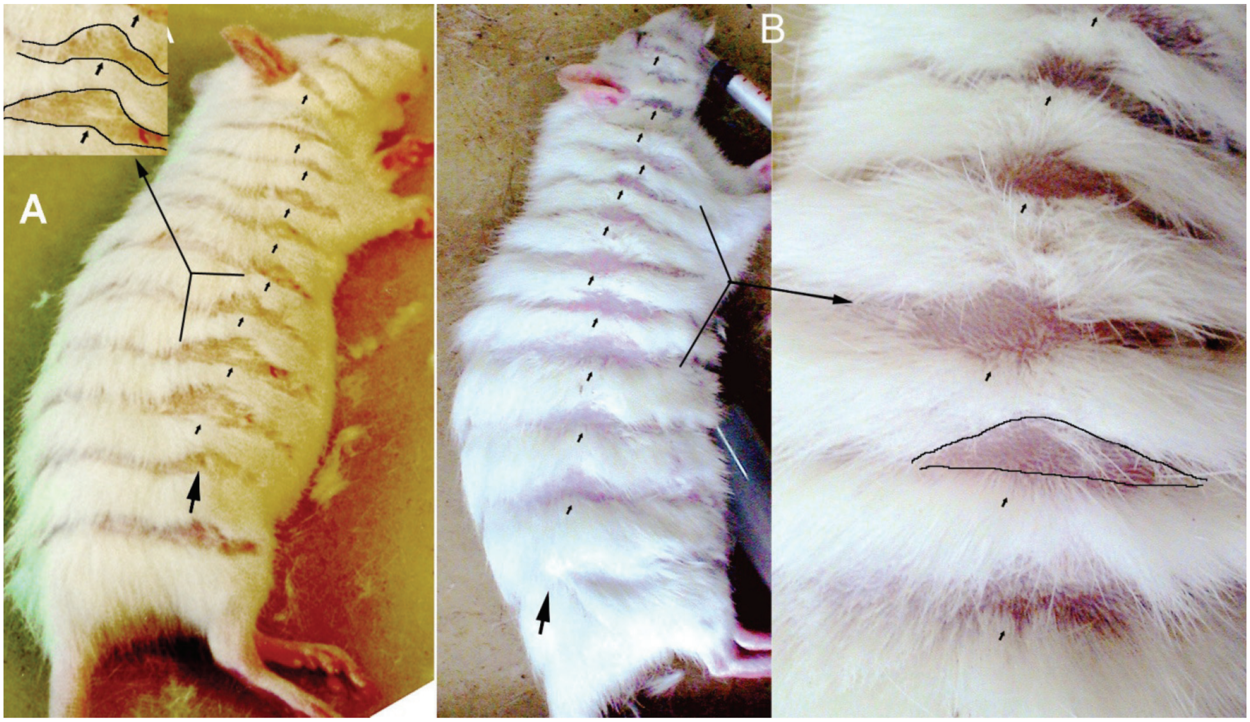

Fig. 4. Pilomotor lines in two rats. A pilomotor line (small arrows) appeared on the lateral back and right cheek of the rat after acupuncture at the lower back (A) or after injection of phenylephrine into the dermis at the lower back (B) (big arrow). The upper left insert and the right photo came from the mid-back of the two rats respectively, showing the outline of the lunate area and the pink skin under the rising hairs. Note the upright hairs in the mid-back of the rat in B.

local hairs around the acupoint always stood up to form a hair cluster. About five minutes after the acupuncture, a pilomotor zone or line appeared from the lower back to the upper back and the cheek of the head (Fig. 4A). The width of the pilomotor line varied largely from 4 mm to 20 mm. The angle of the rising hairs on the upper back and neck varied largely from 30° to 80°, and sometimes erected more than 90°. The length of the pilomotor line always reached the cheek of the head and occasionally extended to another side of the back through the neck or cheek. The pilomotor line usually persisted for about 1 h. When the pilomotor line was formed, the skin under the pilomotor line became pink and flared, almost like hyperaemia.

Group of Drug. When phenylephrine was injected into the acupoint of the rat, the local hairs stood up immediately (mean angle: 22° → 72°), forming a lune around the injected point within half a minute with a 5 mm diameter. The local rising of hairs expanded to 1-2 cm wide in 15 min and could not be flattened. During 4-15 min after injection, a pilomotor zone or line extended up and down from the site of injection (Fig. 4B). These lines maintained for 1-5 hours before disappearing. The pilomotor line produced by drugs was more obvious than that produced by acupuncture.

The course of the pilomotor lines in both the above two groups was coincidental with the course of the SSL in the macro-autoradiography and HLL in the re-growth of shaved hairs.

Group of Drug-Blocking. When regitin was injected into the dermis at the upper back, the injection of phenylephrine at the lower back also produced a pilomotor zone or line. But the line was limited to the lower back of the rat and did not cross the regitin-injected point, and the hairs above the regitin-injected site did not stand up (Fig. 5).

Group of Incision-Blocking. When the incision was made on the upper back of the rat, the pilomotor line induced by injection of phenylephrine below the incision prominently appeared, but did not cross the incision site. The hairs above the incision did not stand up obviously (Fig. 6). It is noteworthy that when the site of injection was just below the incision, the pilomotor line extended downwards to the hip.

Blocking of Acupuncture Analgesia by Incising Skin along a Meridian Line

In the group of Leg-incision, the BPT of the rats was very steady. When the incision was made away from the Meridian line in the Control group, the PT at all measured points after acupuncture increased significantly more than the BPT ($P < 0.05$, or $P < 0.01$).

Fig. 5. Drug-blocked pilomotor line in a rat. When regitin was injected into the dermis at the upper back of the rat (fine arrow), the pilomotor line produced by injection of phenylephrine at the lower back (thick arrow) did not cross the regitin-injected site.

The highest PT appeared immediately after the acupuncture, followed by a gradual decrease. The PT increased again after 21 min, and formed a higher platform. When the incision was made along the Meridian line in the Test group, the PT was as many as the BPT without any increase after the acupuncture (Fig. 7). The comparisons between the Test group and the Control group at all corresponding measured points showed no differences between the BPT ($P > 0.05$), but were significantly different between all PTs after acupuncture ($P < 0.05$, or $P < 0.01$).

In the group of Back-incision, the results of the rats were similar to the group of Leg-incision (Fig. 8). Although the first PT after acupuncture in the Test group increased obviously ($P < 0.05$), there was only one-second increase than the BPT (11.6%).

Determining Catecholamine Releasing from Skin after Acupuncture

The HPLC method in our experiments showed high linearity and precise quantity on the measurement of the standard substances of the catecholamine (NA, E, Dopa). In the homogenate of skin tissues of the rats, NA and E were checked out and E was more than NA, but Dopa was not detected. From the liquid of the incision of the rats after the acupuncture, NA was measured out in all the 6 samples, but E almost did not appear. There had an element with a high peak appeared after NA and E, which was not confirmed (Fig. 9). In contrast without acupuncture, no NA or E could be observed in the liquid of the incision of the rats. Because of the low concentration of NA, the actual amount was not be analyzed.

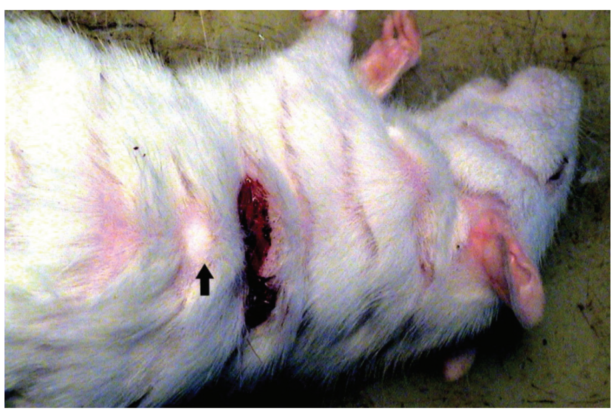

Fig. 6. Incision-blocked pilomotor line in a rat. When the skin of the rat was incised, the pilomotor line induced by injecting phenylephrine (arrow) did not cross the incision.

Discussion

The Pilomotor Line for Transmission of Acupuncture Signals

The SSLs and HLLs have a predominantly longitudinal distribution and form a linear system throughout the skin, corresponding with concentrated sympathetic clusters in the AP muscles of the hair follicle (Fig. 10). Why does the SSL exist? Why do the innervations of sympathetic endings in hair follicles occur in the form of lines? One explanation for the distribution may be that there has been a fundamental difference between the hair follicles online and offline. When certain AP muscles and hair folli-

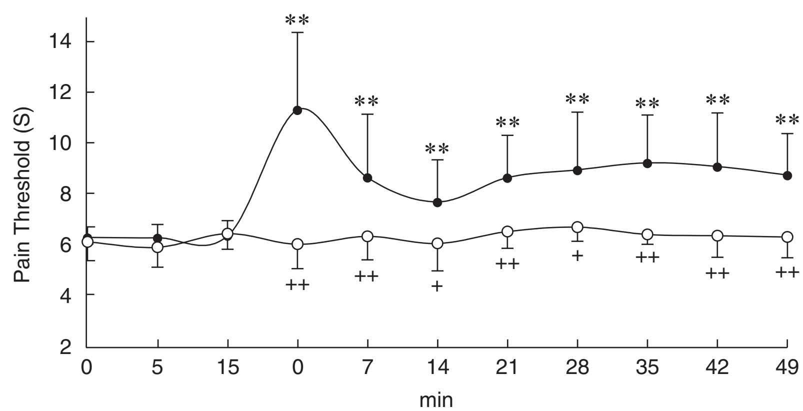

Fig. 7. Variations of pain threshold (Mean ± SD) after acupuncture when the rat's skin was incised on the hind leg along the Meridian line (○) or away from the Meridian line (●) (n = 12). In the X-axis, 0-15 is basic pain threshold, 0-49 is pain threshold after acupuncture. Comparing PT to BPT in the Control group, $P < 0.01$ (**). Comparing the Control group to Test group, $P < 0.05$ (+) or $P < 0.01$ (++).

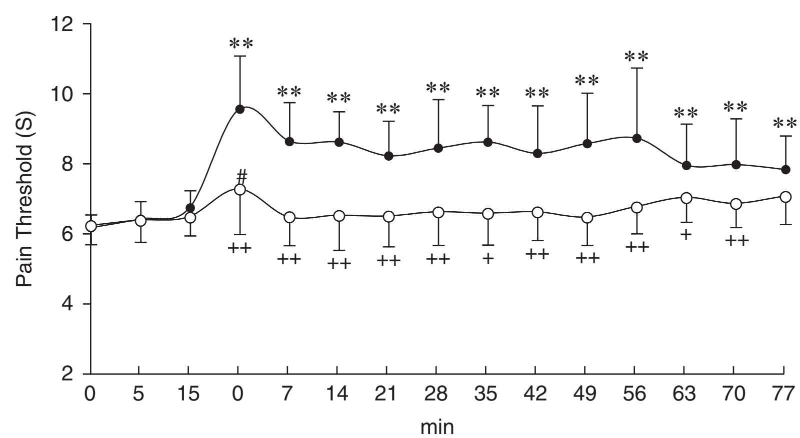

Fig. 8. Variations of pain threshold (Mean ± SD) after acupuncture when the rat's skin was incised along the SSL (○) at the back or away from the SSL at the thorax (●) (n = 12). In the X-axis, 0-15 is the basic pain thresholds; 0-77 is the pain threshold after acupuncture. Comparing PT to BPT in the Control group, $P < 0.01$(**). Comparing PT to BPT in the Test group, $P < 0.05$ (#). Comparing the Control group to Test group, $P < 0.05$ (+) or $P < 0.01$ (++).

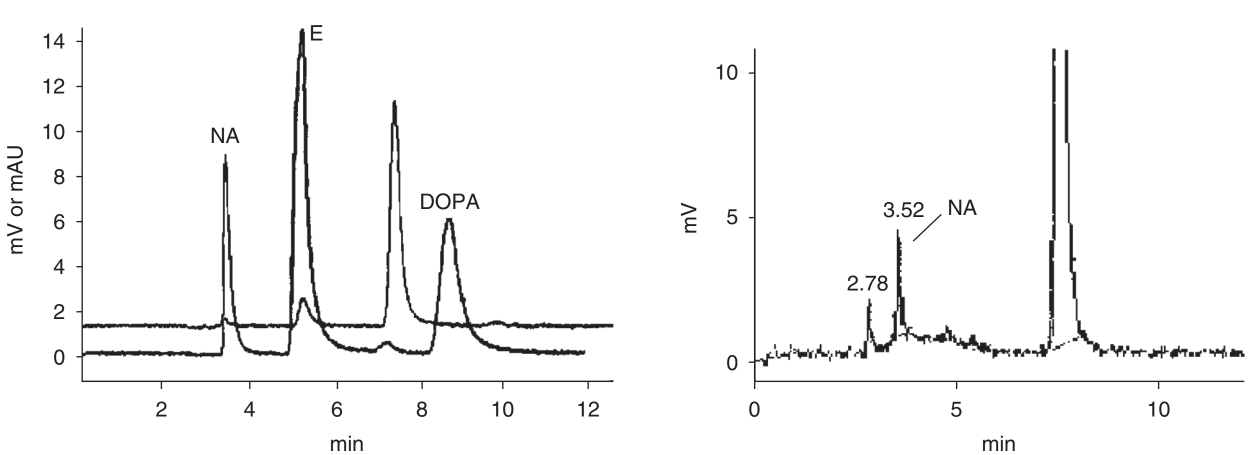

Fig. 9. The separation of the three components of catecholamines (NA, E and Dopa) by HPLC. Left: Large peaks, standard substances; small peaks, homogenate of skin. Right: NA was checked out from the liquid of the skin incision of a rat after acupuncture.

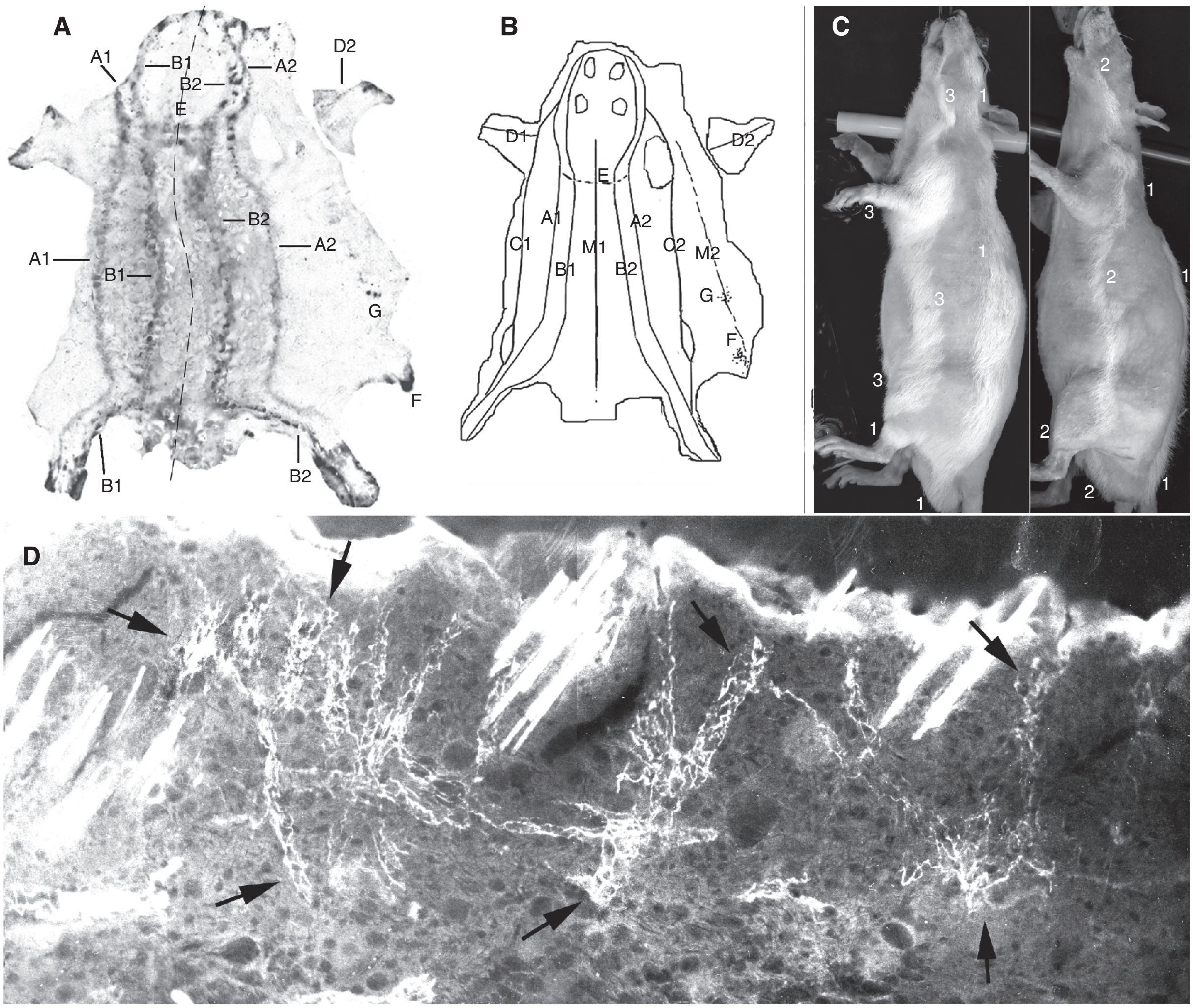

Fig. 10. SSL and HLL in rat's skin. A. whole rat's skin showimg labeled two pairs of sympathetic substance-lines (SSL, A1-A2 and B1-B2). B. Schematic representation of all SSLs in the rat's skin (A-D). C. Re-growing hair-loop-lines occurred after shaving the rat's hairs (number 1-3 label three lines respectively). D. A micrograph of sympathetic fibers with catecholamine-containing fluorescence in the AP muscles of rat's skin (Arrows).

cles are arranged as the SSL, they may provide some integrated function, for instance, for transmitting signals along the line. If so, every hair follicle along the line should be linked with adjacent hair follicles and served as intermediate bits. The contraction of AP muscles may provide a mechanical force for the transmission of signals. The courses of SSLs are quite similar to the courses of Meridian lines. If the SSL is the Meridian line, the signals of acupuncture are probably transmitted along the SSL, and the transmission should involve the activity of sympathetic and the contraction of AP muscles. Here, such speculations are proved by our experiments.

When acupuncture was performed at the acupoints of the rats, or when phenylephrine was injected into the dermis of the rats to excite the α receptor in the AP muscles, local hairs stood up quickly and a pilomotor line subsequently appeared along the SSL. Such a pilomotor line is a type of signal's transmission. It is the first time that we witness the transmission of signals produced by acupuncture by naked eyes. Therefore, one could build a simple model for observing the transmission of the acupuncture signals. In the normal rats, because the hairs overlap together, even if some hairs stand up, the transmission cannot be observed. When the hairs were shaved into zones, the pilomotor line became visible.

Associated with each hair follicle is the AP muscle, innervated by sympathetic with α receptor. Generally, the contraction of AP muscles pulls the hair follicles into an upright position and dimples the skin surface to produce goose bumps in response to cold or fear. Phenylephrine is a specific agonist for the α receptor. It is certain that injecting a α receptor agonist can stimulate the local piloerection. Acupuncture can stretch the dense fibers of local sympathetic in the

SSL, forming the local pilomotor. The question then is why and how the pilomotor transmits along a line.

Blocking of the Pilomotor Line and Mechanical Transmission of Signals

When the rat's skin was incised, the pilomotor line did not cross the incision. This indicates that the piloerection was directly transmitted from one part of the skin to the adjacent part of the skin, but did not depend on the reflex through the central nervous system (CNS). That is to say, the piloerection was transmitted through the skin independently without intermediary of the CNS. When regitin was injected to block the α receptor along the SSL, the phenylephrine-induced pilomotor line did not cross the injected point. This indicates that the transmission of the piloerection depends on the contraction of AP muscles of hair follicles along the line. When the contraction of the intermediate AP muscles was blocked, the next AP muscles did not contract again. Thus, the pilomotor line is most likely produced as a chain reaction along the skin, relaying from one hair follicle to adjacent hair follicles along the line. It is possible that the contraction of AP muscles of one group of hair follicles stretches the sympathetic fibers innervated the adjacent hair follicles, resulting in new contraction of AP muscles and a new group of piloerection. Thus then, a kind of mechanical transmission of signals occurs. Because the pilomotor line coincides with the SSL and the AP muscles of hair follicles along the SSL are innervated with a great number of sympathetic endings, the piloerection is reasonably transmitted along a line to form the pilomotor line.

Furthermore, whether the pilomotor line is only an incidental phenomenon or is the primary pathway of the transmission of acupuncture signals for producing AE or AA. In order to observe whether AE or AA is relative to the SSL and the pilomotor line, three functional experiments were processed.

AE and Acupuncture Analgesia

In order to directly display the dynamic regularity of the AE or AA, the animals were stimulated with traditional needles, and the effects were observed after acupuncture. I also analyzed the every measured point respectively. But in many related researches, electrical acupuncture was used and AE or AA was measured during the electrical acupuncture. I am always suspect such results while the animal was stimulated by electrical pulses, accompanying intense contraction of the body muscles and tremble of the limbs (4). The electrical acupuncture should be different to the traditional acupuncture in many respects.

Both the rats and rabbits showed steady BPT before acupuncture and significantly increased PT after acupuncture. This indicates that the acupuncture produced obvious analgesia. The cheek skin of the rabbits was often burned by the heat radiation when the PT was over two times that of the BPT (*i.e.* 12 S). The PT showed a large variety in different animals and wave obviously in individuals, so the standard deviations, as the SD shown in the figures, are always large. It is also noticed that the PT undulated regularly in a period about 28 min.

Signal Pathways of Acupuncture along Skin

In the group of Leg-incision, when the skin incisions of rats were made along the SSL or Meridian line, the AA was wholly blocked. But when the same incisions were made 5 mm away from the line, the PT increased significantly. This indicates that the AA depends on the transmission of acupuncture signals along a confirmed pathway in the skin, and that the transmission depends on a relaying role between the adjacent areas of the skin. According to modern physiology, acupuncture firstly excites the local sensory receptors and then the pulses are transmitted from those receptors to the CNS through afferent nerves to produce effects (29). However, according to CTM description, acupuncture signals must be transmitted firstly along certain Meridian lines and then arrive at the focal place of the body to produce therapeutic effects. This process is also called focal arrival of acu-esthesia. In my experiments, acupuncture signals must be transmitted firstly along a skin line, the SSL or the pilomotor line, then producing effects, just as described in CTM. Because the innervated nerve around the acupoint (St36) descends along the medial skin of the hind limb, the incision did not affect the afferent nerves. During the experiments, the rats with blocked analgesia always struggled and fidgeted during acupuncture, which also indicates that the afferent signals of pain were increased. One rabbit with exceptionally good effects even went to sleep after acupuncture.

According to CTM, the Meridian line that passes through St36 ascends along the clavicular median line toward the head in the human beings. In the rats, however, the SSL passes through the St36 ascends along the scapular median line toward the head. In the group of Back-incision, when the incision was made in the back along the SSL, the AA was significantly blocked. But when the incision was made in the thorax along the clavicular median line, the AA appeared obviously. This indicates that the acupuncture signals in the rats are transmitted along the SSL. The different pathways between the rats and humans may vary among species.

The AP Muscles and the Transmission of Acupuncture Signals

When trying to observe the influence of a drug on AA at a local skin area along a Meridian line (not the whole body), the drug only can be injected into the dermis with small doses over a longer duration. The initial dose of first injection is always bigger than the maintenance dose of flowing injection. Thus, the rabbit was selected because it is docile and large enough for performing injection in awake condition. By the contrary, the rat's leg is always in movement. The injected drug was in low concentration and in small volumes, and the injected speed was very slow, which gave little physical and chemical stimulation on the local tissue of skin and little pharmacological action on the whole body, but continuously pharmacological acting on the local skin. The skin of rabbit loosely connects with deep tissue, making it almost impossible for the drug to diffuse into deeper tissue. According to our primitive experiments, the results from the rats were coincidental with that of the rabbits.

When regitin was injected away from the Meridian line or physiological saline was injected along the Meridian line in the rabbits, the AA increased significantly. But when regitin was injected along the Meridian line, the AA was remarkably blocked, producing notable influence from the drug. These indicate that: [1] The action of regitin was limited to the injected point, not the entire body; [2] The AA depends on the transmission of acupuncture signals along a confirmed skin pathway—the Meridian line and the SSL/HLL, similar to the experiments of skin incision in the rats; [3] Because regitin is a specific antagonist for α receptors, the α receptors in the skin are closely related to the transmission of acupuncture signals. Therefore, the transmission of acupuncture signals is mediated by the α receptor, a known receptor in the autonomic nervous system. The hair follicles, with dense noradrenergic sympathetic fibers in the AP muscles under the SSL, should be the target organs in the skin. Otherwise, we have observed that when the hair follicles above and below an acupoint along the Meridian line were pulled, the AA was markedly blocked (15).

It should be noted that when the injection of regitin ceased, the PT in the Test group tended to increase further. This can be understood as when the drug's action was cancelled, the residual acupuncture signals passed through the injected point again and produced the AA.

In a few trial tests, regitin was only injected above acupoint St36 along the Meridian line, with more than ten times the dosage, but the blocked effect was not obvious, which indicates bidirectional transmission of the acupuncture signals, similar to that of the pilomotor line. This is the reason why I needed to incise or inject at two sites simultaneously, one above and one below the acupoint.

By HPLC determination, NA was observed to be released from the skin along the Meridian line, which indicates that the sympathetic along Meridian line became active while acupuncture was performed at the acupoint. Still I was surprised to see that injecting phenylephrine into the skin at the acupoint produced analgesia significantly and steadily. Even when the injection was terminated, the PT increased again and maintained for a longer time. Such an intense effect was beyond initial anticipation. When phenylephrine was injected at the non-acupoint, there was no visible effect, which indicates that the action of the drug depends on the α receptors at the acupoint along the Meridian line, but not at any random site or the entire body.

As a summary, the injection of an agonist for the α receptor not only stimulated the pilomotor line but also simulated AA simultaneously, while the injection of the antagonist for α receptor blocked both the pilomotor line and AA. These identical actions strongly suggest that the pilomotor line is just the transmission of acupuncture signals and the transmission depends on the α receptor in the AP muscles, specifically the contraction of the AP muscles. The acupuncture signals for producing AE definitely transmit along certain skin pathways that are the SSLs, the HLLs, the pilomotor lines, or the Meridian lines. This is another type of signal transmission, which are horizontal pathways, in contrast to lengthways pathways of signal transmission from the acupoint through afferent nerves to the CNS in modern physiology. The Meridian in CTM should really exist, most likely as the SSL and HLL and shown as the pilomotor line.

Many other evidences also support this claim. The resistance value of skin during ophthalmic surgery under AA exhibits a marked decrease in many Meridians, indicating an increase of sympathetic activity along the Meridian (19). In healthy volunteers, almost all functional indicators of autonomic nervous system, such as the peripheral blood circulation in fingertips and nasal mucous membrane, the skin temperature at various sites, the skin conductance, the heart frequency and respiration, show identical, transient, and quick reflex responses of the sympathetic nervous system after acupuncture (25). In the presence of a combined electrical stimulation analgesia through two needles attached to each ear, eight of 11 patients undergoing open heart surgery showed an up to 20-fold increase in the level of adrenaline in plasma. In five patients with abdominal surgery, NA was elevated with the increase of adrenaline (9), indicating an increase in the activity of the sympathetic nervous system, or a massive acti-

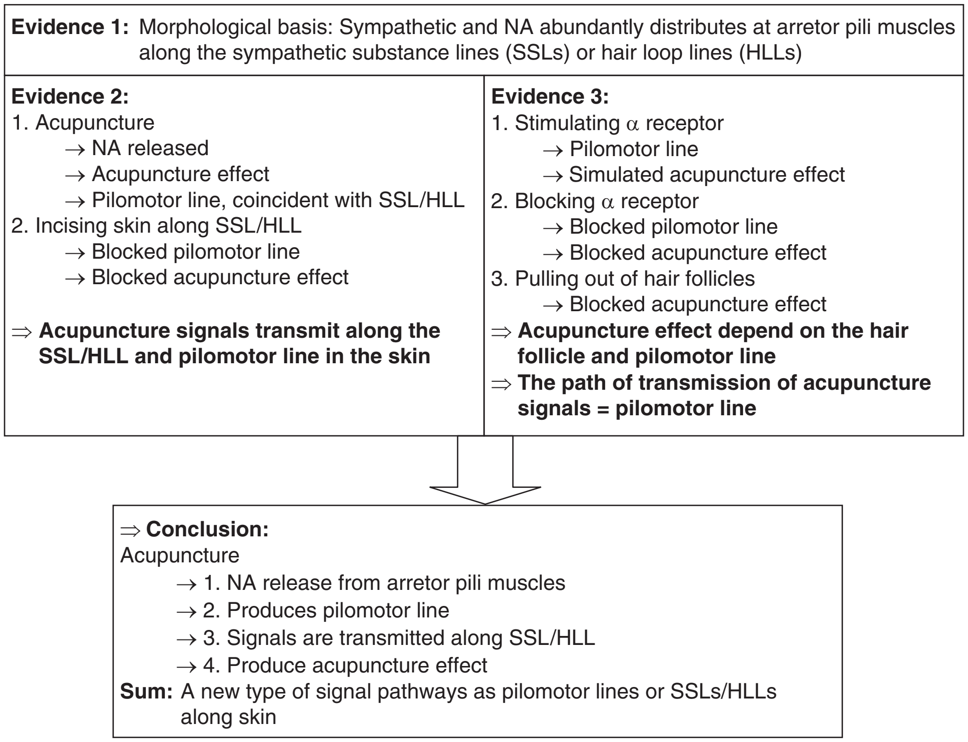

Fig. 11. A summary of evidences on the signal pathway along skin for transmitting acupuncture signals to produce acupuncture effect.

vation of the adrenal medulla. All these can be regarded as: [1] the stimulation of acupuncture caused reflex responses (*i.e.* pain reflex) of the central sympathetic nervous system of the whole body, and [2] large amount of NA released from the pilomotor line when acupuncture. According to my macro-autoradiography study, the SSL is the largest storeroom of sympathetic substances, which must play a very important role in body function. It should be noted that the activity of the sympathetic by acupuncture is not limited to one region, but spreads throughout the linear system of whole body skin, which must be tremendous physiological action. All these evidences can be summed in Fig. 11.

The Peripheral Mechanism of Acupuncture Analgesia

Most of previous and present studies of AA concentrated on the CNS, which states that many brain nuclei composing a complicated network involved in processing AA, and that diverse signal molecules contribute to mediate AA. Among these, the opioid peptides after acupuncture play a pivotal role (6, 27-29). Many reports claimed that endorphin in the brain and spine was released after acupuncture and that AA could be reversed by naloxone (17, 21, 24). Other researchers, however, reported that naloxone failed to reverse AA and that endorphins could be released in response to a stressor (20, 22). I must point out here that the AA/AE was estimated during electrical acupuncture in most of these experiments, and the results measured after acupuncture were referred to as after-effects. The so called after-effects are always small and weak, and are very obviously smaller than the effects after acupuncture in our experiments. I seriously suspect that their effects mainly represent the pain produced from acupuncture counteracted by the pain produced from the stimulation of measurement. The AA in my experiments, however, was measured after acupuncture while the animals remained uninfluenced by anything other than the non-contacted heat radiation, representing pure AA. Thus, it is reasonably believed the results in my experiments: there is an important mechanism of AA in the peripheral skin tissue based on the Meridian lines and SSLs/HLLs along skin.

Hair follicles have plenty of sensory nerve endings and slow adaptive receptors (7, 8, 18). Bending the hair stimulates these nerve endings and receptors. The afferent nerve fibers from hair follicles branch to form large ramifications into different territories in the dorsal horn of the spinal cord (23). When the AP muscles along the SSL contract, they produce large amounts of afferent signals to CNS. The role of signals from such hair follicles in the CNS has not been carefully researched by modern physiology. They may serve to adjust body function with some unknown mechanism.

It is interesting that there has been such a large linear system hidden under our skin, yet unknown up to now. I am sure that the new morphological characteristics of the SSL/HLL and the phenomenon of pilomotor line will lead us to open new ways for exploring AA/AE. These should be a new morphological system and a new physiological type of single transmission, and also a new mechanism as horizontal adjustment of whole body function for balance and coordination.

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