Original article

Involvement of connexin 43 in acupuncture analgesia

HUANG Guang-ying, ZHENG Cui-hong, YU Wei-chang, TIAN Dai-shi and WANG Wei

Keywords: *connexin 43; gene knockout; acupuncture analgesia; hot plate test; writhing response*

Background Connexin 43 (Cx43) is one of the major components of human keratinocyte gap junctions. To study whether gap junctional intercellular communication participates in the transfer of acupoint signals and acupuncture analgesia, the expression of Cx43 was studied in Zusanli (ST36) acupoints compared with control non-acupoint regions in rats after acupuncture. In addition, Cx43 heterozygous gene knockout mice were used to further explore the relationship between Cx43 and acupuncture analgesia.

Methods The expression of Cx43 was detected by immunohistochemistry, immunoblotting, and RT-PCR for the Cx43 protein and mRNA. The influence of the Cx43 gene knockout on acupuncture analgesia was measured by a hot plate and observing the writhing response on Cx43 heterozygous gene knockout mice.

Results Immunohistochemistry showed abundant Cx43 expression in some cells in the skin and subcutaneous tissue of rat ST36 acupoints. The mRNA and protein levels of Cx43 in acupoints were significantly higher than those in the control points in the non-acupuncture group, and even more so after acupuncture. The hot plate and writhing response experiments showed that partial knockout of the Cx43 gene decreased acupuncture analgesia.

Conclusion Cx43 expression and acupuncture analgesia showed a positive correlation.

Chin Med J 2009;122(1):54-60

Acupuncture and moxibustion, which belong to complementary and alternative medicine (CAM), have been used empirically in clinical practice in China for several millennia. Although a large number of previous clinical studies support the effectiveness of acupuncture and its existence is now generally accepted, little is known about the intercellular transfer pattern of the acupoint signal and the mechanism of the effect created by acupuncture.

Gap junctions, which provide low resistance pathways and a possibility for direct coupling of cells to be organized into functional units, are specialized plasma membrane domains composed of collections of the only channels that directly connect neighboring cells.[1] These pathways are responsible for the cell-to-cell transfer of ions and small hydrophilic molecules, including amino acids, nucleotides and second messengers (e.g. Ca^{2+}, cAMP, cGMP, IP3).[1,2] Structural studies have demonstrated that each gap junctional channel is composed of a connexon (hemichannel) in the plasma membrane of one cell and a connexon in the opposing cell membrane.[3] Each connexon, which provides a hydrophilic channel 2 nm in diameter, is an oligomer of 6 protein subunits, termed connexins because they belong to the connexins gene family.[4] Although there are more than 20 members in vertebrates, the major components of human keratinocyte gap junctions have been identified as connexin (Cx) 43 and Cx26.[5] Immunostaining for Cx43 has been observed in epidermal keratinocytes, sebaceous glands, eccrine sweat ducts, and hairs, but Cx26 has only been detectable in the two latter epidermal adnexae.[6]

To study whether gap junctional intercellular communication (GJIC) participates in the transfer of acupoint signals in acupuncture analgesia, the expression of Cx43 in Zusanli (ST36) acupoints and control points of rats in the condition of non-acupuncture or acupuncture were examined by a combination of immunohistochemistry, reverse transcriptase polymerase chain reaction (RT-PCR), and immunoblotting. Additionally, the influence of Cx43 gene knockout on acupuncture analgesia was observed by a hot plate and observing the writhing response on Cx43 heterozygous gene knockout mice.

METHODS

Animals and experimental design

Young adult female Sprague-Dawley (SD) rats (210—230 g, *n*=20) were obtained from the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology. Mating pairs of Cx43 heterozygous ($Cx43^{+/-}$) mice (C57BL/6J-$Gja1^{tm1kdr}$) were obtained from Jackson laboratories (Bar Harbor, ME,

DOI:10.3760/cma.j.issn.0366-6999.2009.01.011

Institute of Integrated Traditional and Western Medicine (Huang GY, Zheng CH and Yu WC), Department of Neurology (Tian DS and Wang W),Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China

HUANG Guang-ying and ZHENG Cui-hong equally contributed to this article.

Correspondence to: Dr. WANG Wei, Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China (Tel: 86-27-83662625. Fax: 86-27-83662871. Email: wwang_tjh@yahoo.com.cn)

This work was supported by the grants from the National Natural Science Foundation of China (No. 90209009) and the National Basic Research Program (No. 2006CB504502).

USA) and multiplied in our IVC-accredited animal facilities. The animals were housed in groups of 5 with continuous *ad libitum* access to food and water, and the room temperature was kept at (22±2)°C with a 12-hour light/dark cycle for at least 2 weeks before and throughout the experimental periods. All of the experiments were conducted in accordance with the Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain (IASP).

The rats were randomly divided into two groups (10 for each group): the non-acupuncture group and the acupuncture group. During manual acupuncture (MA) administration, the rats were kept in tailor-made cloth restraints, which were tacked to a board with the hind limbs exposed. Sterilized disposable stainless steel needles measuring 0.3 mm in diameter and 13 mm in length were perpendicularly inserted bilaterally to a depth of 5–7 mm into the ST36 regions, about 5 mm below the capitulum fibulae and lateral to the tibia. The control point was 5 mm away from the ST36, near to the fibula, at the same horizontal level as ST36, and received the same MA administration. The inserted needles were rotated clockwise and counter-clockwise at a frequency of about 2 Hz for 30 seconds in the 10 rats of the acupuncture group. We repeated the 2 Hz, 30-second needle stimulation every 10 minutes during a 30-minute total treatment period, once a day for 3 consecutive days. The rats in the non-acupuncture group were likewise fixed to boards but did not receive MA. After the last MA treatment on the 3rd day, the rats were anesthetized with an intraperitoneal (i.p.) injection of pentobarbital sodium (40 mg/kg), and then shaved before isolation. When the rats were killed, the skin and subcutaneous tissue were isolated together from bilateral ST36 and control points for each group. The experimenters were blinded as to the group condition, and tissues from the various groups were processed and run simultaneously in order for an appropriate comparison to be made. The right side fresh tissues were snap-frozen in liquid nitrogen and stored at –80°C until extraction for Western blotting and RT-PCR analysis, while the left side ones were fixed in 10% formaldehyde for at least 24 hours in preparation for Cx43 indirect immunohistochemistry.

Immunohistochemistry

Samples (n=10) fixed in formaldehyde were embedded in paraffin, cut into sections of 3-μm thickness, and collected on gelatinized slides for indirect immunohistochemistry. After deparaffinization and rehydration, the sections were blocked with 3% H_2O_2 for 40 minutes, and treated in 0.01 mol/L citric acid saline buffer at 95－98°C for about 10 minutes. The sections were placed in 7% non-fat milk for 30 minutes to block nonspecific background staining. The sections were then incubated overnight at 4°C with Cx43 antibody (1/1000, ADI, USA). After being washed, the sections were incubated for 1 hour with horseradish peroxidase (HRP)-conjugated secondary antibody. Diaminobenzidine (DAB) was employed to detect the immunocomplex and hematoxylin for nuclear counterstaining. To ascertain staining specificity, the sections were incubated with nonspecific rabbit IgG，which was used as the negative control. After being dehydrated through an ascending alcohol series and cleared with xylene, the sections were mounted and examined using a Nikon Eclipse E600 microscope equipped with a CFI60 Infinity Optical System.

RNA extraction and RT-PCR

The Cx43 mRNA level was detected in the samples (n=5 in each group) from ST36 and control points of each rat group by RT-PCR. The total RNA was extracted with TRIzol reagent (Takara, Japan), chloroform and isopropanol. The RT reagent kit and Taq DNA polymerase were obtained from Fermentas (Lithuania). The mix containing total RNA (2 μg), oligo (dT) 12－18 (50 μmol/L, 1 μl) and diethylpyrocarbonate (DEPC) -treated water (up to 15 μl) was incubated at 70°C for 5 minutes and then chilled on ice. With the addition of M-MuLV reverse transcriptase (200 U/μl, 1 μl), 5×reaction buffer (5 μl), dNTPs (10 mmol/L, 1.5 μl), RNase inhibitor (40 U/μl, 0.5 μl), and DEPC-treated water (13 μl), the mix was incubated at 42°C for 60 minutes and then reversely transcribed to DNA. The sense and anti-sense primers were purchased from Sangon (China). In the PCR protocol, 25 μl of the reaction mix was used, containing template DNA (4 μl), 10×PCR buffer (2.5 μl), $MgCl_2$ (25 mmol/L, 1.5 μl), dNTPs (10 mmol/L of each, 1 μl), Taq DNA polymerase (1 U/μl, 1 μl), and the sense and anti-sense primer (10 μmol/L, 1 μl of each) for rat Cx43 (sense: 5′-GAG CCC ATC AAA AGA CTG CG-3′ ; anti-sense: 5′-CTG GTT GTC GTC GGG GAA AT-3′)(265 bp product). The reversely transcribed products were amplified through 35 cycles of denaturation (90°C, 50 seconds), annealing (54°C, 50 seconds), and extension (72°C, 50 seconds). β-actin was used as the control (sense: 5′-CAT CCT GCG TCT GGA CCT-3′; antisense: 5′-TCA GGA GGA GCA ATG ATC TTG-3′) (480 bp product). The sizes of the PCR products were evaluated after electrophoresis in 1.5% agarose gels containing ethidium bromide.

Western blotting protocol

For Western blotting, the other 5 samples were prepared with lysis buffer containing 25 mmol/L Tris, 1% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol (ME), 10 mmol/L ethylenediamine tetraacetic acid (EDTA), 2 mmol/L phenylmethylsulfonyl fluoride (PMSF), 8 mol/L urea, and 10 mg/L of each aprotinin, leupeptin, antipain, and pepstatin, and then sonicated. The total protein concentration of each tissue was determined by Coomassie Brilliant Blue staining. The protein was separated by 12% SDS-PAGE, and then transferred to nitrocellulose (NC) membrane. After being blocked in 25 ml of 7% non-fat milk for 2 hours at room temperature,

the NC membrane was incubated with anti-Cx43, and anti-β-actin (1/2000, Sigma, USA), respectively, in TBS/Tween-20 containing 7% non-fat milk with gentle shaking overnight at 4°C. Then it was washed 4 times each for 15 minutes with TBS/Tween-20 and incubated with HRP-conjugated secondary antibody (1/5000 in TBS/Tween-20, Pierce, USA) with gentle shaking for 2 hours at room temperature. The blot was incubated with an enhanced chemiluminescence detection reagent (ECL, Pierce) for 5 minutes. Excess reagent was drained, and then the blot was covered with clear plastic wrap and exposed to X-ray film.

Genotyping the progeny of mice

Genotypes of offspring were determined from mice genomic tail DNA using a PCR assay. PCR reaction mix contained 0.25 μg genomic DNA, 2.5 μl 10×buffer, 2 μl 25 mmol/L $MgCl_2$, 0.5 μl 10 mmol/L dNTP, 1 μl Cx43 sense primer (5′-CCC CAC TCT CAC CTA TGT CTC C-3′), 1 μl Cx43 anti-sense primer (5′-ACT TTT GCC GCC TAG CTA TCC C-3′), 1 μl Neo sense primer (5′-CTT GGG TGG AGA GGC TAT TC-3′), 1 μl Neo anti-sense primer (5′-AGG TGA GAT GAC AGG AGA TC-3′), 0.5 μl 5 U/μl Taq DNA polymerase, in a final volume of 25 μl. All PCR reagents were from Fermentas. Thirty-seven cycles were performed on the samples as follows: denaturation at 94°C for 3 minutes, annealing at 58°C for 1 minute, extension at 72°C for 90 seconds, final extension cycle at 72°C for 7 minutes. Reaction products were analyzed on 1.2% agarose gels in which the following bands were expected: wildtype (+/+) allele: 519 bp; knockout (-/-) allele: 280 bp; heterotypic (+/-) alleles: both bands. The Cx43 null ($Cx43^{-/-}$, knockout, KO) offspring died at birth because pulmonary gas exchange did not occur, due to swelling and blockage of the right ventricular outflow tract from the heart, suggesting that Cx43 plays an essential role in heart development.[7] So only Cx43 HT ($Cx43^{+/-}$, heterozygous) and WT ($Cx43^{+/+}$, wildtype) mice could be used in our experiments.

Hot plate test

Female Cx43 mice, weighing 20–26 g and kept at a controlled temperature ((25±1)°C), were placed onto a stainless steel surface that was maintained at a temperature of (55.0±0.5)°C. Licking of the hind paws was taken as the endpoint. A pre-value of latency was measured twice on the day before the start of acupuncture, every other day. The two determinations were averaged as the basal reaction time. Mice with a pain threshold that was detected within 30 seconds were used in the experiments. All experimental animals were divided into the following 4 groups at random combined with the PCR results: WT control group, WT acupuncture group, HT control group, and HT acupuncture group (n=6 in each group). In the acupuncture groups, sterilized disposable stainless steel needles were perpendicularly inserted into bilateral Hegu (LI4), which was located on the dorsum of the front paw between the first and second metacarpal bones and Taichong (LR3), which was located on the top of the foot between the first and second toes. The methods of mice fixation and MA administration were the same as that used for the rats. The mice in the control group were restrained likewise but did not receive MA administration. Response time(s) was measured at 0minute (immediately post-acupuncture) and 15, 30, 45 and 60 minutes after acupuncture or blank administration. The cutoff latency of 60 seconds was adopted to prevent tissue damage. All the behavioral observations were made by co-workers who were blinded to the treatment protocol.

Writhing response

Mice of both sexes were used for the hot plate test and were randomly divided into the following 4 groups combined with the PCR results: WT control group, WT acupuncture group, HT control group, and HT acupuncture group (n=8 in each group). In the acupuncture group, sterilized disposable stainless steel needles were perpendicularly inserted into bilateral Zusanli (ST36) which was located in a position similar to the rat, and Zhongwan (CV12) which was located in the mid-abdominal area on a line extending between the xiphoid process and umbilicus. The method of mice fixation and MA administration were the same as for the hot plate test. The mice in the control group were treated the same, but without MA administration. After acupuncture, the mice were immediately placed individually in table-top observation rectangles. Acetic acid (0.1 ml/10 g of a solution at a concentration of 0.6%) was injected intraperitoneally to produce abdominal stretches, consisting of a contraction of the abdominal muscle together with a stretching of the hind limb. The latency period of the stretches was recorded, and the number of abdominal stretches per animal over the next 20 minutes was counted by two experienced investigators blinded to the experimental conditions. Antinociceptive activity was expressed as the reduction in the number of abdominal stretches, i.e. the difference between the control mice and mice receiving acupuncture.

Statistical analysis

Experimental data were expressed as the mean ± standard error (SE). We performed one-way analysis of variance (ANOVA), and the SNK test (except for paired t-test for the hot plate experiments data) using SPSS v.11.5. Statistical significance was set at the level of $P < 0.05$.

RESULTS

Immunohistochemistry for the expression of Cx43 in ST36 regions and non-acupoint control regions

The results of the immunohistochemical assay on sections of the skin and subcutaneous tissue from the Zusanli acupoint regions and control regions are shown in Figure 1. Brown spots are positive immunohistochemical staining of Cx43, and blue is nuclear hematoxylin counterstaining. As shown, the epidermis of adult rat skin mainly consists of stratum basale, stratum spinosum, stratum granulosum

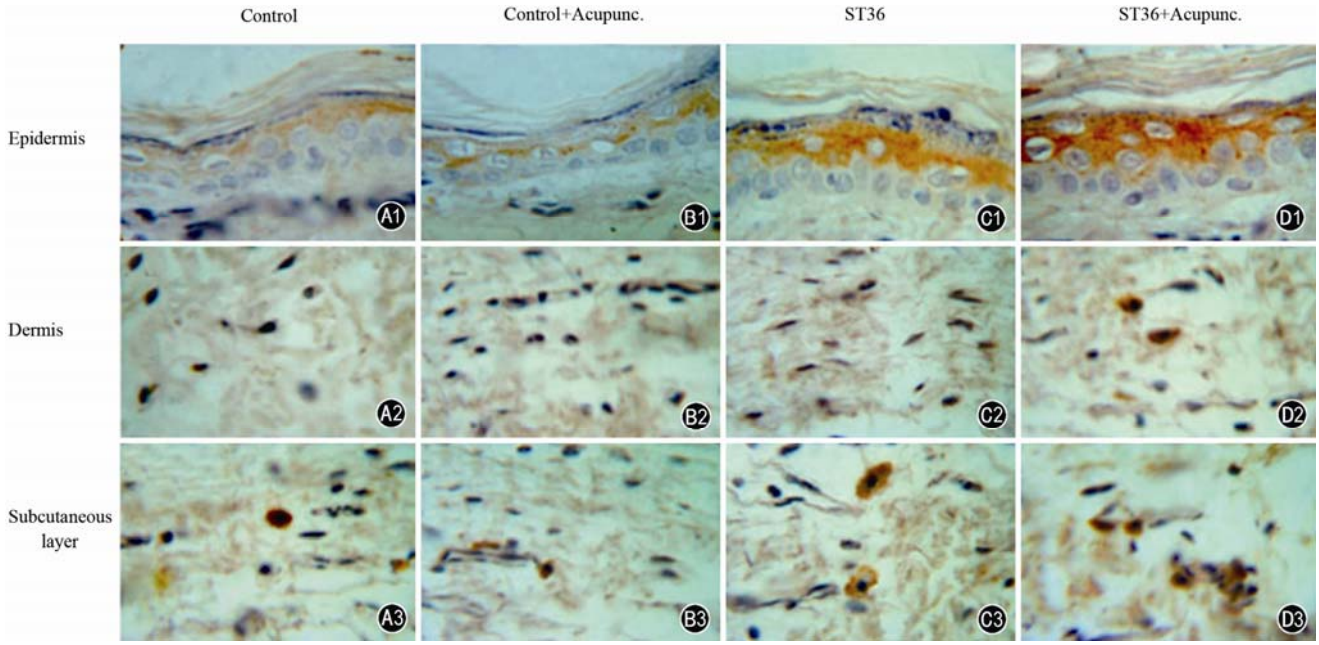

Figure 1. Immunolocalization of Cx43 in Zusanli (ST36) and control regions for different subgroups (Original magnification ×400). The expression of Cx43 was much higher in the spinous layer than elsewhere in the epidermis (**A1**–**D1**). In the dermis, there was positive but weak staining in fibroblasts (**A2**–**D2)**. Cx43 was found to be intensely expressed in some unknown cells in the subcutaneous layer (**A3**–**D3**) and in the dermis (**D2**). Staining was greater in ST36 (**C3**, **D3**) than in the control points (**A3**, **B3**), especially in the subcutaneous layer.

and stratum corneum. Cx43 was located mainly in the cell membrane and cytoplasm of keratinocytes in the epidermis, especially in the spinous layer (Figure 1 A1-D1). In the dermis, there was positive but weak staining in fibroblasts (Figure 1 A2-D2). But some large cells expressed Cx43 strongly in the dermis and the subcutaneous layer, which was greater in the acupoints (Figure 1 C3 and D3) than in the control (Figures 1 A3 and B3), especially in the subcutaneous layer.

RT-PCR and immunoblotting analysis for the expression of Cx43 in ST36 regions and non-acupoint control regions

Both RT-PCR (Figure 2) and immunoblotting (Figure 3) results revealed that in the non-acupuncture group Cx43 levels in ST36 acupoints were significantly higher than that in the control points (F=11.17, P <0.01; F=21.01, P <0.01). After acupuncture treatments in the acupoints, once a day for 3 days, the mRNA and protein levels of Cx43 in these acupoints of the acupuncture group increased significantly (P <0.05), but there was no statistical increase for the Cx43 levels in the control points where the same acupuncture treatments were performed (Figure 3).

Effect of acupuncture observed using the hot plate test

The effects of acupuncture in the hot plate test are shown in Figure 4. A comparison of all groups showed that there was no significant difference before acupuncture. The administration of acupuncture caused an increase in the latency of all mice in all analyzed periods (0, 15, 30, 45 and 60 minutes post-acupuncture). Mice that underwent acupuncture showed significant increases in the antinociceptive effect at both 0 (t=7.87, P <0.01) and 15 minutes (t=3.94, P <0.05) post-acupuncture, which was significantly different from the pre-acupuncture results in the WT group, but an effect was seen only at 0 minute (t =2.68, P <0.05) in the HT group. Increases of latency at 0 minute (F=3.24, P <0.05) and 15 minutes (F=2.45, P <0.05) post-acupuncture were markedly higher in the WT group, as compared with the HT group.

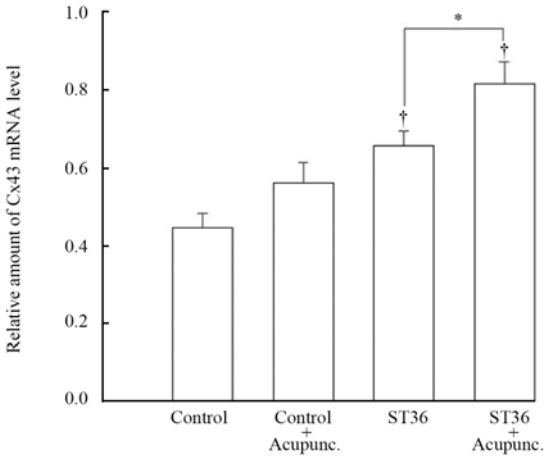

Figure 2. RT-PCR analysis for Cx43 mRNA in Zusanli (ST36) and control regions for different subgroups (n=5). $^{*}P$ <0.05, compared with the ST36; $^{\dagger}P$ <0.01, compared with the control (One–way ANOVA, SNK test).

Effect of acupuncture against acetic acid-induced visceral pain

The effects of acupuncture on the tolerance of acetic acid-induced visceral pain were also significant (Figure 5 A and B). There was no statistically significant difference between the WT and HT control groups before

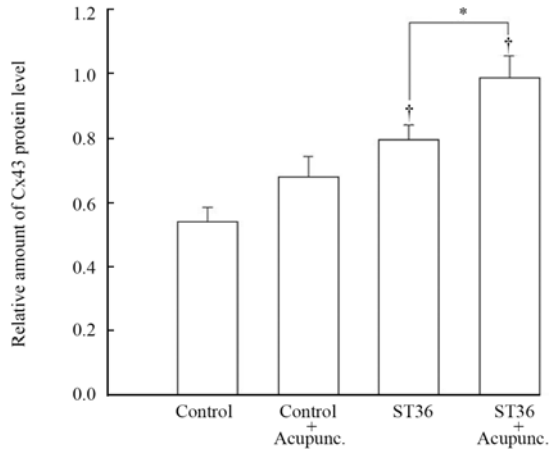

Figure 3. Immunoblotting assay for Cx43 protein levels detection in Zusanli (ST36) and control regions (n=5). *P < 0.05, compared with the ST36; †P < 0.01, compared with the control (One-way ANOVA, SNK test).

acupuncture. The administration of acupuncture to WT mice caused a marked inhibition of the acetic acid-induced visceral nociceptive response, which was expressed as a significant increase in the latency period of stretches (F =7.53, P <0.01) and reduction in the number of abdominal stretches (F =8.11, P <0.01). In the HT acupuncture group, the above tendency was also seen, but was not significantly different from the HT controls. Antinociceptive activity was 57% less in the WT and 23% less in the HT acupuncture groups than the non-acupunctured controls, respectively.

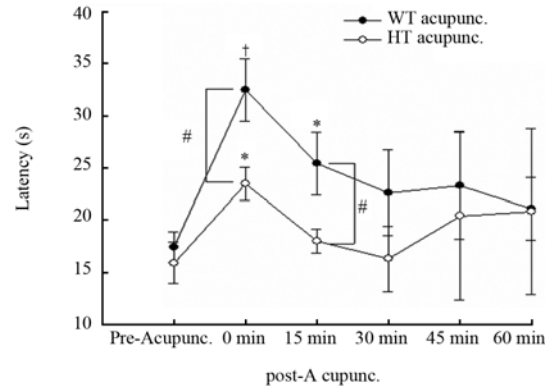

Figure 4. Effect of acupuncture on the hot-plate test in WT (●) and HT (○) mice. *P <0.05, †P <0.01 as compared with pre-acupuncture, ‡P <0.05 as compared with the HT acupuncture group.

DISCUSSION

GJIC plays an important role in embryonic development, cell differentiation, proliferation and growth control, etc., which is essential for proper development and health in animals and humans.[8,9] Multiple gap junction genes contribute to epidermal and follicular morphogenesis.[10] Transfers of electrical signal and mechanical stimulation have both been attributed to gap junctional communication. For example, both synchronous contraction of myocardial cells and electrical synaptic transmission between nerve cells depend on the integrity of gap junctions.[11] In other words，communication has to be present at gap junctions in order for a group of cells to simultaneously be activated. The data from the present study demonstrate that there is much more abundant Cx43 expression in the skin and subcutaneous tissue of rat ST36 acupoints than in the control, which provides the basic vehicle for GJIC in acupoints.

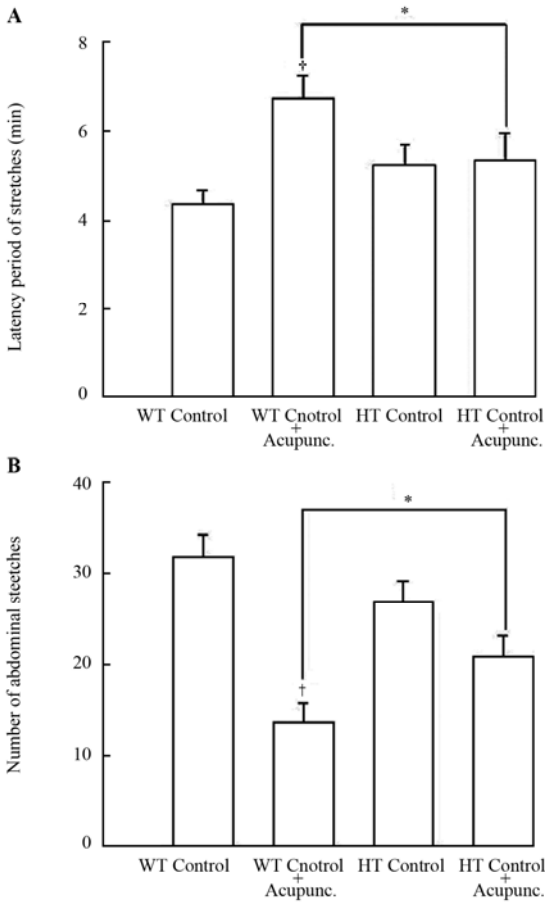

Figure 5. Effect of acupuncture against acetic acid-induced visceral pain in WT and HT mice. **A**: Effect of acupuncture on the latency period of stretches caused by acetic acid-induced nociception in WT and HT groups. **B**: Effect of acupuncture on the number of abdominal stretches resulting from acetic acid-induced nociception in WT and HT groups. *P <0.05, compared with the WT acupuncture group. †P <0.01, compared with the WT control group.

A mechanical signal is likely transferred to the tissue when needles are inserted and rotated in acupoints, which could be converted to bioelectrical or biochemical signals and induce a series of changes such as actin polymerization, extracellular matrix modification, signaling pathway activation, gene expression, and protein synthesis.[12-14] Changes in extracellular matrix composition in response to mechanical stimulation may also contribute to communication between different cell types.[15] Mechanical stimulation of a single cell induces an increase of the intracellular calcium concentration that propagates through adjacent cells via gap junctions, forming an intercellular Ca^{2+} wave.[16] Mechanically

induced intercellular Ca^{2+} waves can be inhibited by heparin, an inositol 1,4,5-triphosphate (IP3) receptor antagonist, demonstrating that the release of Ca^{2+} by IP3 is necessary for the propagation of intercellular Ca^{2+} waves. Possibly IP3 also moves through gap junctions to effect the intercellular transfer of Ca^{2+} waves.[17,18] Our data suggested that acupuncture might activate gap junctions and increase the synthesis of Cx43 mRNA and protein to increase the intercellular transfer of Ca^{2+} or IP3 via gap junctions in order to enhance intercellular communication.

Oligomerization of connexins results in hemichannels in single cells, or gap junctions with connexins from neighboring cells. It was thought that hemichannels could not open to the extracellular space and if there was an opening of hemichannels to the extracellular space, this would kill cells through loss of metabolites, collapse of ionic gradients and influx of Ca^{2+}.[19] However, a growing number of more recent findings indicate that the opening of non-junctional hemichannels does occur under both physiological and pathological conditions, and that such openings are functional or deleterious depending on the situation.[20-22] Hemichannels formed by Cx43 or Cx46 are mechanosensitive,[23-25] thus allowing the mechanical signal created by acupuncture to be transduced into a biochemical signal such as the release of signaling molecules, or a bioelectrical signal such as a change in the membrane potential and membrane current flow. As shown in Figure 1, Cx43 was expressed not only in the tight layer of epidermal keratinocytes, but also in some disseminated cells in the dermis and subcutaneous tissue, and these disseminated positive cells aggregated after acupuncture. The Cx43 mRNA and protein levels in ST36 acupoints of the acupuncture group were increased significantly after acupuncture stimulation, indicating that acupuncture might increase the synthesis of Cx43 in acupoints, and/or activate the non-junctional hemichannels composed of Cx43.

Spataro et al[26] found that intrathecal injection of carbenoxolone, a gap junction decoupler, reversed neuropathy-induced mirror image pain, suggesting that the spread of excitation via gap junctions might contribute importantly to inflammatory and traumatic neuropathic pain. In the present study, no significant difference in the basal reaction time was found between WT and HT mice, demonstrating that although gap junctions might play an important role in the processing of pain signals, Cx43 did not contribute to normal sensory or motor function. It was also found that sciatic nerve Cx37 mRNA increases were proportional to the extent of thermal hyperalgesia, [27] suggesting that other gap junction proteins might be responsible for the hyperexcitability following peripheral nerve injury. Quantitative studies showed an approximately two-fold difference in Cx43 mRNA and protein levels in wildtype and heterozygous mouse hearts [28] and in adult brains,[29] as expected based on gene dosage. The functional impact of the 50% reduction in Cx43 heterozygotes on impulse propagation might also be responsible for the above phenomenon. Acupuncture had a favorable modulation in both acute (inflammatory and neuropathic) and chronic pain, which has been confirmed by numerous clinical observations and experimental studies.[30]

In conclusion, the results of this study reconfirmed that acupuncture could produce significant antinociceptive action against classical chemical (acetic acid-induced visceral pain) and thermal (hot-plate test) models of nociception in mice. Acupuncture markedly improved the pain threshold in Cx43 WT mice, but in Cx43 HT mice this analgesic effect was not obvious. The current study provides evidence for involvement of Cx43 in acupuncture signal transmission, but the specific mechanisms and signaling pathways remain to be defined by further studies.

REFERENCES

1. Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Guldenagel M, et al. Structural and functional diversity of connexin genes in the mouse and human genome. Biol Chem 2002; 383: 725-737.
2. Richard G. Connexins: a connection with the skin. Exp Dermatol 2000; 9: 77-96.
3. Musil LS, Cunningham BA, Edelman GM, Goodenough DA. Differential phosphorylation of the gap junction protein connexin43 in junctional communication-competent and -deficient cell lines. J Cell Biol 1990; 111: 2077-2088.
4. Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. Annu Rev Biochem 1996; 65: 475-502.
5. Arita K, Akiyama M, Tsuji Y, McMillan JR, Eady RA, Shimizu H. Changes in gap junction distribution and connexin expression pattern during human fetal skin development. J Histochem Cytochem 2002; 50:1493-1500.
6. Salomon D, Masgrau E, Vischer S, Ullrich S, Dupont E, Sappino P, et al. Topography of mammalian connexins in human skin. J Invest Dermatol 1994; 103: 240-247.
7. Reaume AG, de Sousa PA, Kulkarni S, Langille BL, Zhu D, Davies TC, et al. Cardiac malformation in neonatal mice lacking connexin43. Science 1995; 267: 1831-1834.
8. Anand RJ, Hackm DJ. The role of gap junctions in health and disease. Crit Care Med 2005; 33: S535-S538.
9. Laird DW. Life cycle of connexins in health and disease. Biochem J 2006; 394: 527-543.
10. Risek B, Klier FG, Gilula NB. Multiple gap junction genes are utilized during rat skin and hair development. Development 1992; 116: 639-651.
11. Kumar NM, Gilula NB. The gap junction communication channel. Cell 1996; 84: 381-388.
12. Chiquet M. Regulation of extracellular matrix gene expression by mechanical stress. Matrix Biol 1999; 18: 417-426.
13. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999; 285:1028-1032.
14. Langevin HM, Yandow JA. Relationship of acupuncture points and meridians to connective tissue planes. Anat Rec

2002; 269: 257-265.
15. Swartz MA, Tschumperlin DJ, Kamm RD, Drazen JM. Mechanical stress is communicated between different cell types to elicit matrix remodeling. Proc Natl Acad Sci USA 2001; 98: 6180-6185.
16. Boitano S, Sanderson MJ, Dirksen ER. A role for Ca^{2+}-conducting ion channels in mechanically-induced signal transduction of airway epithelial cells. J Cell Sci 1994; 107: 3037-3044.
17. Boitano S, Dirksen ER, Sanderson MJ. Intercellular propagation of calcium waves mediated by inositol trisphosphate. Science 1992; 258: 292-295.
18. Sneyd J, Wilkins M, Strahonja A, Sanderson MJ. Calcium waves and oscillations driven by an intercellular gradient of inositol (1,4,5)-trisphosphate. Biophys Chem 1998; 72: 101-109.
19. Bennett MV, Contreras JE, Bukauskas FF, Saez JC. New roles for astrocytes: gap junction hemichannels have something to communicate. Trends Neurosc 2003; 26: 610-617.
20. Contreras JE, Sanchez HA, Eugenin EA, Speidel D, Theis M, Willecke K, et al. Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. Proc Natl Acad Sci USA 2002; 99: 495-500.
21. Evans WH, De Vuyst E, Leybaert L. The gap junction cellular internet: connexin hemichannels enter the signalling limelight. Biochem J 2006; 397: 1-14.
22. Saez JC, Contreras JE, Bukauskas FF, Retamal MA, Bennett MV. Gap junction hemichannels in astrocytes of the CNS. Acta Physiol Scand 2003; 179: 9-22.
23. Bao L, Sachs F, Dahl G. Connexins are mechanosensitive. Am J Physiol Cell Physiol 2004; 287: C1389-C1395.
24. Gluhak-Heinrich J, Gu S, Pavlin D, Jiang JX. Mechanical loading stimulates expression of connexin 43 in alveolar bone cells in the tooth movement model. Cell Commun Adhes 2006; 13:115-125.
25. Jiang JX, Cherian PP. Hemichannels formed by connexin 43 play an important role in the release of prostaglandin E(2) by osteocytes in response to mechanical strain. Cell Commun Adhes 2003; 10: 259-264.
26. Spataro LE, Sloane EM, Milligan ED, Wieseler-Frank J, Schoeniger D, Jekich BM, et al. Spinal gap junctions: potential involvement in pain facilitation. J Pain 2004; 5: 392-405.
27. Lin SH, Lu CY, Muhammad R, Chou WY, Lin FC, Wu PC, et al. Induction of connexin 37 expression in a rat model of neuropathic pain. Brain Res Mol Brain Res 2002; 99:134-140.
28. Guerrero PA, Schuessler RB, Davis LM, Beyer EC, Johnson CM, Yamada KA, et al. Slow ventricular conduction in mice heterozygous for a connexin43 null mutation. J Clin Invest 1997; 99:1991-1998.
29. Dermietzel R, Gao Y, Scemes E, Vieira D, Urban M, Kremer M, et al. Connexin43 null mice reveal that astrocytes express multiple connexins. Brain Res Rev 2000; 32: 45-56.
30. Ulett GA, Han S, Han JS. Electroacupuncture: mechanisms and clinical application. Biol Psychiatry 1998; 44: 129-138.

(Received July 8, 2008)
Edited by CHEN Li-min