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EXPERIMENTAL STUDY

Effect of electronic stimulation at Neiguan (PC 6) acupoint on gene expression of adenosine triphosphate-sensitive potassium channel and protein kinases in rats with myocardial ischemia

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Abstract

OBJECTIVE: To investigate the effects of electronic stimulation at acupoints Neiguan (PC 6) and Lieque (LU 7) on the gene expression of the adenosine triphosphate (ATP)-Sensitive potassium channel (KATP: Kir6.1, Kir6.2, SUR2A, and SUR2B) and protein kinases (PKA, PKG, and PKCβ2) in myocardial cells of rats with myocardial ischemia (MI) induced by isoproterenol (ISO).

METHODS: Rats were randomly divided into a control, model, Neiguan (PC 6), Lieque (LU 7), and non-acupoint groups. The MI model was established by injecting rats with ISO. Electro-acupuncture treatment was given to the acupuncture groups, once a day for 7 days. Gene expression was analyzed with real-time PCR.

RESULTS: The gene expression of KATP and protein kinases in the model group was higher than those in the control group ($P < 0.05$). After acupuncture treatment, the KATP and protein kinase expression levels were significantly lower in the Neiguan (PC 6) and Lieque (LU 7) groups compared with the model group ($P < 0.05$). The Neiguan (PC 6) group lowered these levels significantly more than that of the Lieque (LU 7) group ($P < 0.05$). No significant differences were observed between the model and non-acupoint groups ($P > 0.05$).

CONCLUSION: Our findings suggest that electronic needling of Neiguan (PC 6) can both reduce the gene expression of KATP and protein kinases in rats with ISO-induced MI.

Key words: Point PC6 (Neiguan); Acupuncture; Electric stimulation; KATP Channels; Protein Kinases; Myocardial ischemia

INTRODUCTION

According to Traditional Chinese Medicine (TCM) theory, the meridians are the passages in which energy flows around the human body. Acupoints are the sensitive points on the meridians. Acupoints, known as "the fifteen Luo-connecting points," link exterior-interior meridians. Neiguan (PC 6), one of "the fifteen Luo-connecting Points," is located in the hand and belongs to the Jueyin pericardium meridian. TCM theorizes that the pericardium is the first to be attacked when pathogenic factors invade the heart. Therefore, heart disease is mainly caused by pericardium disorders.[1]

Allies *et al* [2] injected two fluorescent dyes, true blue and diamidino yellow, into the pericardial sac, the medial brachial cutaneous nerve, or subcutaneously into the medial side of the brachium. Double-labeling was observed in the ipsilateral dorsal root ganglia neurons of spinal cord segments C8 (the eighth cervical vertebrae), T1 (the first thoracic vertebrae), and T2 (the second thoracic vertebrae), indicating that dichotomizing afferent fibers supply both the pericardium and the brachium.[2] This study provides a possible morphological explanation for referred cardiac pain. Lin Yang *et al* [3] and Qiang Liu *et al* [4] found that some nerve fibers in the heart and Neiguan (PC 6) originate from one same neuron in the spinal ganglions or the inferior ganglion of the vagus nerve by injecting peroxidase and cholergenoid β.[3,4]

Because of the relationship between Neiguan (PC 6) and the heart, stimulating Neiguan (PC 6) is essential to treating some heart diseases, such as MI.[5,6] Studies have shown that the mechanisms of the stimulation mainly involve blood rheology, bioactive substances, antioxidants, monoamines, intracellular signaling, myocardial enzymes, and energy metabolism.[7,8]

The adenosine triphosphate (ATP)-sensitive potassium channel (KATP) plays an important role in cardiovascular diseases, such as MI, ischemic preconditioning, arrhythmia, and hypertension.[9] As a new target of anti-MI drugs, KATP has received widespread attention.[10] In this study, Neiguan (PC 6) and Lieque (LU 7) were acupunctured in rat models of MI induced by injecting isoproterenol (ISO). RT-PCR was used to analyze changes in Kir6.1, Kir6.2, SUR2A, SUR2B, PKA, PKG, and PKCβ2 expression. This study aimed to reveal the relationship between acupuncturing Neiguan (PC 6) and gene expression of KATP.

MATERIALS AND METHODS

Animals

Healthy Sprague-dawley (SD) rats (specified pathogen free grade, 70 males) were obtained from Liaoning Chang Sheng Biotechnology (Benxi, China), production license No. SCXK [Liao] 2010-0001 and use license No. SYXK [Liao] 2010-0001. Rats received food and water ad libitum for 1 week before experiments [temperature at (24 ± 1) ℃ and humidity at 50% ± 5%]. The ethics committee from the Experimental Animal Center of Liaoning University of TCM (Traditional Chinese Medicine) approved the trial from National Essence Basic Research and Development 973 program. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals in Liaoning University of TCM.

Drugs and reagents

ISO was purchased from Sigma (St. Louis, MO, USA). TRIzol was obtained from Invitrogen (Carlsbad, CA, USA). Synthetic primers were provided by Beijing Genomics Institute. RT-PCR kit was purchased from Takara (Dalian, China).

Model establishment and grouping

Ten rats were selected randomly as the control group. Normal saline solution (85 mg/kg) was injected into the roots of the medial limbs of the control group, whereas ISO (isoproterenol, 85 mg/kg) was injected into the remaining groups.[11,12] The injection was performed on all the rats for a second time 24 h later, and then an ECG was recorded for each rat. The models were considered a success as previously described by Nandave *et al* [13] and Coval *et al.* [14] Briefly, the model was established when the ECG T-wave changed from positive to negative or biphasic, accompanied by ST-segment elevation, QRS wave widening, sinus tachycardia, contractions, or other arrhythmias.[13,14] There were no deaths in the control group after modeling. Forty rats survived the ISO injections. The remaining rats were randomly divided into model, Neiguan (PC 6), Lieque (LU 7), and non-acupoint groups, with 10 rats in each group.

Acupuncture methods

The electroacupuncture of Neiguan (PC 6) and Lieque (LU 7) was based on the rat acupuncture positioning method described in "Experimental Acupuncture Science." [15] The middle Tianshu (ST 25) and Shenque (RN 8) points were selected for the rats in the non-acupoint group. Needles (0.18 mm× 25 mm, Suzhou Hua Tuo Medical Instruments, Suzhou, China) were inserted into each corresponding point (bilateral). The needles were linked to a 6805-D EA (Shantou Medical Equipment Factory, Shantou, China) in sparse dense wave with the intensity of 2-20 Hz. The current intensity was considered adequate when a slight quiver of the forelimbs was observed, which is consistent with electroacupuncture in frequency. The same stimulation was applied to Neiguan (PC 6), Lieque (LU 7), and the non-acupoint groups. Acupuncture was maintained for 20 min per session, one time per day, for 7 days. The rats in the control and model groups did not receive needling.

Tissue sample preparation

Rats were sacrificed and their cervical vertebra and left ventricle tissues collected. The tissue was immediately placed into tubes after the addition of 1 ml TRIzol. Then, tissue samples were stored at − 80 ℃ until use.

Real-time PCR analysis

Total RNA was separated from approximately 100 mg of myocardial tissue, and subjected to one-step real-time PCR. RNA samples were deemed to be high quality if the sample A260/280 ratios ranged from 1.8 to 2.0. Total RNA (1 μL) was reverse transcribed into

cDNA with the designed gene primers according to the manufacturer's instructions. The primers were designed as follows: Kir6.1: 5′-CAACCTGGCTCACAAGAAC-3′ and 5′-CACCACATGATAGCGAAGA-3′ ; Kir6.2: 5′-TCCCGAAAGGGCATTAT-3′ and 5′-AAAGGAAGGCAGACGAAA-3′ ; SUR2A: 5′-GAGGGCGGTGACGAAT-3′ and 5′-GCCAAGTAGCGGAACG-3′ ; SUR2B: 5′-CGTTCCGCTACTTGGC-3′ and 5'-CGTGTTATTCTTCGGTTCA-3′ ; PKA: 5′-AAGACCCTTGGCACCG 3′ and 5′-GGCTCACTGAACCTCCC-3′ ; PKG: 5′-CTTCTTCGCCAACCTG-3′ and 5′-TGAAATCGGAATGAGCC-3′ ; PKCβ2: 5′ -TTGGAGTCCTGCTGTAT-3′ and 5′-CGTTTGCCTGGGTGT-3′ . GAPDH: 5′-CGTATCGGACGCCTGGTT-3′ and 5′-CGTGGGTAGAGTCATACTGGAAC-3′ . Following reverse transcription, cDNA quantity was determined and standardized to meet the required concentration for quantitative PCR. All genes were amplified with the following cycling parameters: 95 ℃ for 20 s and 40 cycles, 95 ℃ for 3 s, and 60 ℃ for 30 s. The amplicon was dissociated with the following cycling parameters: 95 ℃ for 15 s, 60 ℃ for 1 min, 95 ℃ for 15 s, and 60 ℃ for 15 s. The relative expression levels of the target gene were calculated according to the equation: ΔΔCt value = (target gene Ct-reference Ct) treated Groups-(target gene Ct-reference Ct) untreated Groups, and 2 [-Delta Delta C(t)] was analyzed.[16]

Statistical analysis

Data were analyzed by SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± standard deviation ($\bar{x}$ ± s). Differences between groups were evaluated with one-way analysis of variance. $P < 0.05$ was considered significant.

RESULTS

Changes in Kir6.1 and Kir6.2 expression

The expression level of Kir6.2 was approximately equal to that of Kir6.1 in the control group. Compared with the control group, the expression level of Kir6.1 and Kir6.2 was significantly higher in the model group ($P < 0.05$). After acupuncture treatment, the expression level of Kir6.1 and Kir6.2 was significantly lower in the Neiguan (PC 6) and Lieque (LU 7) groups compared with the model group ($P < 0.05$). The expression levels of Kir6.1 and Kir6.2 in the Neiguan (PC 6) group were significantly lower than those in the Lieque (LU 7) group ($P < 0.05$). No significant differences were observed between the model and non-acupoint groups ($P > 0.05$) (Figure 1A, 1B).

Expression of SUR2A and SUR2B

In the control group, the expression level of SUR2A was 7-fold greater than that of SUR2B. Compared with the control group, the expression levels of SUR2A and SUR2B were significantly higher than those in the model group ($P < 0.05$). After acupuncture treatment, the expression levels of SUR2A and SUR2B were significantly lower in the Neiguan (PC 6) and Lieque (LU 7) groups compared with the model group ($P < 0.05$). The expression levels of SUR2A and SUR2B in the Neiguan (PC 6) group were lower than those in the Lieque (LU 7) group ($P < 0.05$). No significant differences were observed between the model and non-acupoint groups ($P > 0.05$) (Figure 1C-1D).

Gene expression of PKA, PKG, and PKCβ2

The expression level of PKCβ2 was approximately 2-fold greater than those of PKA and PKG in the control group. Compared with the control group, the expression levels of PKA, PKG, and PKCβ2 were significantly higher than those in the model group ($P < 0.05$). After acupuncture treatment, the expression levels of PKA, PKG, and PKCβ2 were significantly lower in the Neiguan (PC 6) and Lieque (LU 7) groups compared with the model group ($P < 0.05$). The expression levels of PKA, PKG, and PKCβ2 in the Neiguan (PC 6) group were significantly lower than those in the Lieque (LU 7) group ($P < 0.05$). No significant differences were observed between the model and non-acupoint groups ($P > 0.05$) (Figure 1E-1G).

DISCUSSION

KATP is widely expressed in active metabolic tissues throughout the human body, and was originally discovered in cardiomyocytes by Noma.[17] The channel is composed of an inwardly rectifying potassium channel (Kir6.1 or Kir6.2) and an ATP-binding regulatory subunit (SUR2A or SUR2B) in a 1∶1 proportion.[18-20] KATP can regulate the plasma membrane's potential to match the demands of cellular metabolism.[21-23] As an endogenous protective mechanism in ischemic preconditioning, the activation of KATP can prevent MI by reducing elevated ST segments.[24,25] Mutations in KATP increases susceptibility to a range of life-threatening diseases.[26,27] Reduction in the cardiac action potential and increases in potentially deleterious calcium in the cytosol might serve as the mechanism of KATP regulating MI.[28]

We found that SUR2B is not highly expressed in normal cardiomyocytes, which is consistent with previous reports.[29] Kir6.1, Kir6.2, SUR2A, and SUR2B expression increased after MI and decreased after the electronic needling intervention. Our results indicate that acupuncturing Neiguan (PC 6) and Lieque (LU 7) could decrease the expression of Kir6.1, Kir6.2, SUR2A, and SUR2B during MI. However, there was a greater reduction during acupuncture at Neiguan (PC 6).

The activity of KATP is regulated by protein kinases (PKA, PKG, and PKCβ2). PKA and PKG can activate KATP in vascular smooth muscle, whereas PKCβ2 can

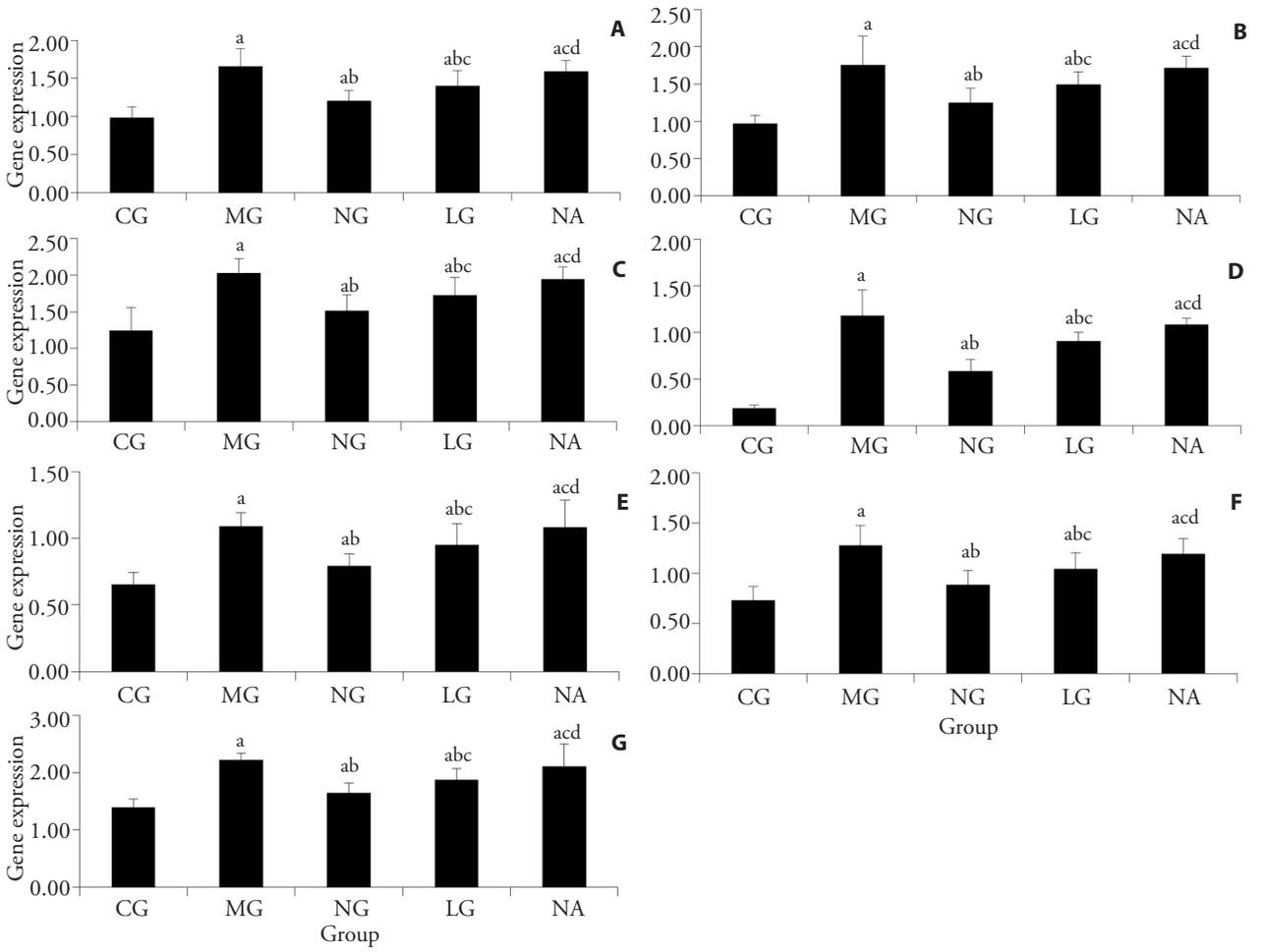

Figure 1 ATP-Sensitive potassium channels and protein kinase changes after electroacupuncture
A: Kir6.1; B: Kir6.2; C: SUR2A; D: SUR2B; E: PKA; F: PKG; G: PKCβ2. Kir6.1 and Kir6.2: ATP-Sensitive potassium channels; SUR2A and SUR2B: ATP-binding regulatory subunit; PKA, PKG, and PKCβ2: protein kinases. CG: control group; MG: model group; NG: Neiguan (PC 6) group; LG: Lieque (LU 7) group; and NA: non-acupoint group. Control and model groups were not treated with acupuncture. Neiguan (PC 6), Lieque (LU 7), and non-acupoint groups were acupunctured at their corresponding acupoints, once a day for 7 days. Comparison between control group and the other four groups, $^{a}P < 0.05$. After acupuncture, comparison between the model group and Neiguan (PC 6) or Lieque (LU 7) groups, $^{b}P < 0.05$. Comparison between Neiguan (PC 6), Lieque (LU 7), and non-acupoint groups, $^{c}P < 0.05$. Comparison between Lieque (LU 7) and non-acupoint groups, $^{d}P < 0.05$.

activate KATP in cardiomyocytes. The effect of PKCβ 2 on cardiomyocytes is related to the concentration of ATP. KATP is activated by PKCβ2 under high ATP concentrations, but it has the opposite reaction under low ATP concentrations.[19,30]

This study showed that the expression level of PKA, PKG, and PKCβ2 increased after MI, which is in agreement with previous reports.[31] PKA, PKG, and PKCβ2 expression level decreased after acupuncture at Neiguan (PC 6) and Lieque (LU 7). However, the change was more obvious after Neiguan (PC 6) acupuncture compared with Lieque (LU 7). This indicates that Neiguan (PC 6) is more closely connected to protein kinases than Lieque (LU 7).

Our results showed that both Neiguan (PC 6) from the pericardium meridian of hand-Jueyin and Lieque (LU 7) from the lung meridian of hand-Taiyin can change the expression of KATP and protein kinases in cardiomyocytes. This effect could be because both Neiguan (PC 6) and Lieque (LU 7) are controlled by the same spinal segment of C8-T1 because they are located close to each other on the medial forearm near the wrist joint.[32,33] Furthermore, previous study found that the heart and Neiguan (PC 6) in rats have the same nerve origin.[34] However, no direct link between Lieque (LU 7) and the heart has been confirmed. Therefore, the difference between the two points in this study probably indicates that Neiguan (PC 6) has an advantage over Lieque (LU 7) in regulating post-MI recovery.

Previous studies showed that Guanxinkang, a Chinese herbal medicine, can promote the expression of Kir6.1, Kir6.2, SUR2A, and SUR2B after MI.[35,36] This conclusion is discordant with ours, but no similar experiments have been reported to explain these differences. One possible explanation is that the herbal medicine experiment was performed *in vitro*. However, the actual process may not be reflected under the influence of many interference factors *in vivo*. In addition, as a preparation composed of several herbs, the effects of Guanxinkang on MI are difficult to predict exactly. Our experiment was performed *in vivo*, and it mainly reflected the regulating effects of acupuncture on MI. Never-

theless, our study scope was limited to KATP and protein kinases, so we cannot draw definite conclusions from the data available. The mechanisms of acupuncture on gene expression warrant further study.

Electroacupuncture at Neiguan (PC 6) could reduce both KATP and protein kinase expression in rats with isoproterenol-induced MI, which could be the mechanism underlying the treatment of MI-modeled rats by electroacupuncture.

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