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ACUPUNCTURE RESEARCH

Effect of Electroacupuncture Stimulation on mRNA Expression of Angiotensinogen, Angiotensin Ⅱ Type 1 Receptor, Endothelin-1, and Endothelin A Receptor in Spontaneously Hypertensive Rat Aorta

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ABSTRACT **Objective:** To observe the effect of electroacupuncture (EA) stimulation on the expressions of angiotensinogen (AGT), angiotensin Ⅱ type 1 receptor (AT1R), endothelin-1 (ET1), and endothelin A receptor (ETAR) mRNA in spontaneously hypertensive rat (SHR) aorta. **Methods:** Eighteen male SHRs were randomly divided into three groups, an SHR group, an SHR Baihui (DU 20) and Zusanli (ST 36) acupoint (SHR-AP) group, and an SHR non-acupoint (SHR-NAP) group, with 6 rats in each group. Six Wistar rats were used as a control. Rats in the SHR-AP group were stimulated by DU 20 and ST 36 acupoints, both of which were connected with EA. EA was handled one time every Monday, Wednesday and Friday, for total 24 times (8 weeks). SHR-NAP rats were acupointed at a 15° angle flat into 0.5 cm to two points, which were 1 and 2 cm from rail tip separately. EA parameters were the same as the SHR-AP rats. SHR control rats and Wistar rats were fixed without EA. Real-time quantitative polymerase chain reaction (PCR) was used to measure AGT, AT1R, ET1, and ETAR mRNA expression in rat aorta. **Results:** EA stimulation significantly reduced rat aorta vascular AGT, ET1, ETAR and AT1R mRNA expressions in the SHR-AP and SHR-NAP groups ($P<0.01$). Among these four genes, AT1R mRNA expression was significantly lower in the SHR-AP than in the SHR-NAP group ($P<0.01$). **Conclusion:** EA could reduce the AT1R mRNA expression in SHR-AP rat aorta, indicating a potential mechanism for the hypotensive effects of EA.

KEYWORDS electricacupunture, angiotensinogen, angiotensin Ⅱ type 1 receptor, endothelin-1, endothelin-1 A receptor

Hypertension can lead to damages to several organs, such as heart, brain and kidney. Therefore, an effective control of blood pressure exerts an important function in clinic research.[1] Up to date, evidence demonstrated that acupuncture and moxibustion application could benefit high blood pressure treatment.[2,3] Acupuncture can lower rat blood pressure by decreasing plasma noradrenaline and increase dopamine levels, thus modulating the excitability in peripheral and sympathetic nervous system.[4] Furthermore, acupuncture attributed to a reduction in blood pressure by modulating nitric oxide levels,[5] improving insulin resistance,[6] adjusting intra- and extracellular calcium concentrations,[7] and modulating immune function.[8]

Renin-angiotensin-aldosterone system (RAAS) plays an important role in the emergence and development of hypertension, and it is also important to the chronic regulation of hypertension.[9,10] By binding to its receptor (angiotensin Ⅱ type 1 receptor, ATIR), angiotensin Ⅱ (Ang Ⅱ) could cause blood vessels contraction, blood pressure elevation,[11] and endothelin 1 (ET1) levels up-regulation.[12] While interact with its receptor (endothelin A receptor, ETAR), ET1 could produce potent vasoconstrictor effects,[13] leading to high blood pressure.

Previous studies showed that electroacupuncture (EA) can reduce rats hypertension, and suggest that blood pressure reduction may be caused by decreased peripheral blood ET1 and Ang Ⅱ levels of hypertensive rats.[14,15] However, the effect of EA on local ET1 and

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Ang Ⅱ and their receptors remains unclear. To further clarify the antihypertensive effects of acupuncture, we used quantitative real-time polymerase chain reaction (RT-PCR) to determine the mRNA levels of angiotensinogen (AGT), AT1R, ET1 and ETAR in spontaneous hypertensive (SHR) rat aorta.

METHODS

Experimental Animals

Eighteen SHR rats (6 weeks, male, weight 125±5 g) were divided into an SHR group, an SHR Baihui (DU 20) and Zusanli (ST 36) acupoint (SHR-AP) group, and an SHR non-acupoint (SHR-NAP) group, with six rats in each group. Six Wistar rats (6 weeks, male, weight 125±5 g) were used as the control group. Animals were provided by the Experimental Animal Center of the Medical Department of Peking University (Beijing Animal License, SCXK-2006-0009). The animals were housed at 24±1 ℃ and relative humidity of 50%±1% with a 12 h light/dark cycle and given a standard laboratory diet and water. The animals were fasted for 12 h before experiments, but had free access to water. All animals were handled according to the guidelines of the Peking University Animal Research Committee. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments of Peking University Health Science Center (No. LA2015143).

Main Reagents and Equipment

The main reagents and equipment used were as follows: Qiagen RNeasy fibrous tissue kits (Qiagen, Germany); SuperScript™ Ⅲ One-Step real-time RT-PCR system with Platinum® TaqDNA polymerase (Invitrogen, USA); DNA Engine Opticon™ continuous fluorescence detection system (PTC-200 DNA Engine thermal cycler, CFD-3200 Opticon™ Tester, MJ Research, USA), blood pressure monitor (Type BP-98A, Softron Corporation,Toyko, Japan), HAN's EA instrument (HANS-202A, Shijiazhuang Forsyth Medical Company Ltd., Hebei, China), acupuncture needles (0.35 cm×2.0 cm, Global Brand, batch number 111120, Acupuncture Supplies Ltd., Suzhou, Jiangsu, China).

EA Treatment

Rats in the SHR-AP group were stimulated by DU 20 and ST 36 acupoints. Rats were given the acupuncture needles straight into the right side of conscious rats, with 0.35 cm×2.0 cm needle straightly inserting 0.5 cm into ST 36 and 1-inch needle go 30° angle obliquely backward 1 cm into DU 20. Acupoints were manipulated in accordance with national standard,[16] with ST 36 acupoint underneath the outside of hind knee, almost 5 mm below the head of fibula, and DU 20 in the midpoint of two-ear tip connection. Both of the two-acupoints were connected with EA, which were 202 A, 1 mA, 2 Hz and lasting 20 min. EA was handled one time every Monday, Wednesday and Friday, for total 24 times (8 weeks). SHR-NAP rats were acupointed 15° angle flat into 0.5 cm to two points, which were 1 cm and 2 cm from rail tip separately. EA parameters were the same as the SHR-AP rats. SHR control rats and Wistar rats were fixed without EA.

Blood Pressure Measurement

Blood pressure was measured in morning at the weekend of 2, 4, 6 and 8 weeks using a tail cuff method. Each conscious rat was measured mean blood pressure (MBP) 3 times continuously, with 60 s interval. MBP was calculated the average of 3 times.

Quantitative RT-PCR

On the second day of the 9th week after EA, rats were anesthetized by 20% uethane based on 1 mL/100 g weight. Aortic was taken, and aorta RNA was extracted by Qiagen RNeasy fibrous tissue kits. cDNA was synthesized by the SuperScript™ Ⅲ One-Step RT-PCR system with Platinum® TaqDNA polymerase. Quantitative detection of mRNA level was performed using the DNA Engine Opticon™ continuous fluorescence detection system, with reaction conditions: 50 ℃, 2 min; 95 ℃, 10 min; 95 ℃, 15 s; 60 ℃, 1 min, with a total of 40 cycles. Opticon Monitor 3.1 software was used to observe amplification curve and quantitatively analyze the product. Fold changes were calculated by $2^{-\triangle ct}$ method. Primers of AGT, AT1R, ET1, ETAR and housekeeping gene GAPDH were synthesized by TaKaRa Biotechnology (Dalian, Co., China, Table 1).

Statistical Analysis

SPSS 11.5 software was used for statistical analysis, and results were expressed as mean ± standard deviation ($\bar{x}\pm s$). Conformation to test conditions for parameter data sets was compared by independent sample *t*-test or single-factor analysis of variance, and differences between the groups were compared with the Bonferroni method. Data sets not eligible for parametric

Table 1. Primers Used for Quantitative RT-PCR Reactions

Product	Primer	Sequence
AGT	Forward	5' - GGCAAGATGGGTGACACCA-3'
	Reverse	5' CTGCTTGGAGTTCAAGGAGGAT-3'
AT1R	Forward	5' - AGTCCTGTTCCACCCGATCAC-3'
	Reverse	5' - GGTCTCAGACACTATTCGAAATCCA-3'
ET1	Forward	5' - ACCTGGACATCATCTGGGTCAAC-3'
	Reverse	5' - TTTGGTGAGCACACTGGCATC-3'
ETAR	Forward	5' - TCTCTGCGCTCTCAGTGTGGA-3'
	Reverse	5' - AGCCGATTGCTTCTGGGATG-3'
GAPDH	Forward	5' - GGCACAGTCAAGGCTGAGAATG-3'
	Reverse	5' - ATGGTGGTGAAGACGCCAGTA-3'

testing were compared by rank sum test. $P<0.05$ was considered a statistically significant difference.

RESULTS

Effects of EA on MBP in SHR Rats

MBP of rats did not change after a long-term EA stimulation, while the MBP significantly increased in the SHR rats. EA stimulation did not inhibit MBP in the SHR-NAP rats; however, the elevation was significantly lowered in the SHR-AP rats from 4 weeks ($P<0.05$, Figure 1).

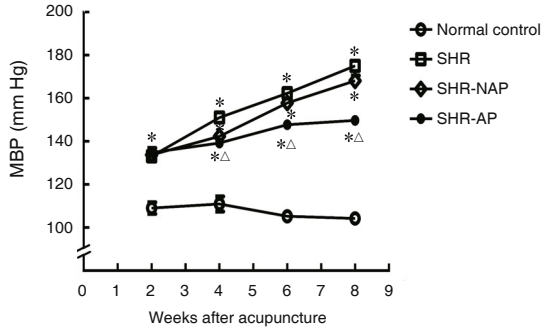

Figure 1. Effects of EA on MBP in SHR Rats

Notes: *$P<0.05$, compared with the normal control group; △$P<0.05$, compared with the SHR group

Effects of EA on mRNA Expressions of AGT and AT1R in SHR Rats

The mRNA expressions of AGT and AT1R were significantly elevated in the SHR group compared with control rats ($P<0.01$). EA reduced the expression of AGT (31%, 33%) and AT1R (29%, 53%) in SHR-NAP and SHR-AP groups (all $P<0.01$), respectively. Further statistical analysis showed that there was no significant difference in AGT mRNA expression between the two groups, while AT1R mRNA expression was significantly lower in the SHR-AP group than that in the SHR-NAP group ($P<0.01$, Figure 2).

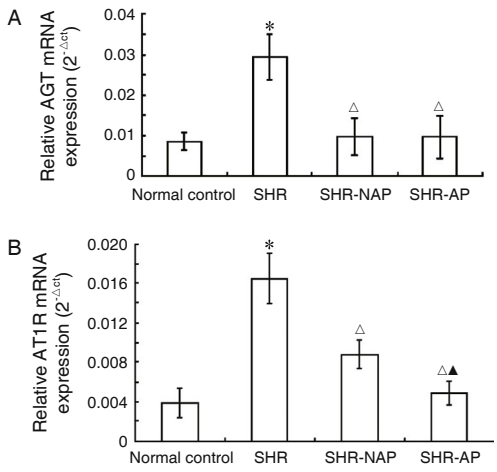

Figure 2. Effects of EA on Relative mRNA Expressions of AGT (A) and AT1R (B) in SHR Aorta

Notes: *$P<0.01$, compared with the normal control group; △$P<0.01$, compared with the SHR group; ▲$P<0.01$, compared with the SHR-NAP group

Effects of EA on mRNA Expression of ET1 and ETAR of SHR Rats

The mRNA expression of ET1 and ETAR was significantly elevated in SHR compared with control rats ($P<0.01$). EA reduced mRNA expression of ET1 and ETAR in the SHR-NAP and SHR-AP groups (all $P<0.01$). The mRNA expression of ET1 and ETAR was reduced by 22% ($P<0.01$) and 24% ($P<0.01$) respectively in the SHR-NAP group, and it was reduced by 29% ($P<0.01$) and 57% ($P<0.01$) respectively in the SHR-AP group (Figure 3).

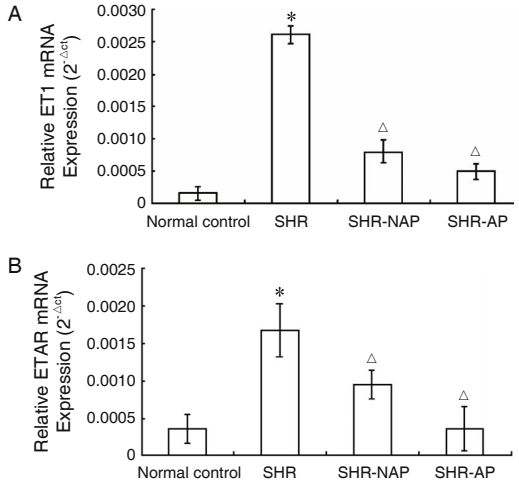

Figure 3. Effects of EA on Relative mRNA Expressions of ET1 (A) and ETAR (B) in SHR Aorta

Notes: *$P<0.01$, compared with the normal control group; △$P<0.01$, compared with the SHR group

DISCUSSION

Stomach (Wei) Meridian of Foot-Yangming (ST)

is rich in qi and blood. ST 36 acupoint, belongs to ST, can reduce the uplift of qi. It is frequently selected to use in the acupuncture treatment for hypertension.[17-19] DU 20 locates the vertex of human being. It is useful for balancing yang energy and low energy, as well as sedation. In clinical research, this is a main point for headache, dizziness, insomnia, cerebrovascular disease. Clinical practice had proven that functions on DU 20 can effectively reduce hypertension, as well as dizziness and headache because of hypertension.[20] The combination of DU 20 and ST 36 acupoints, scalp point and body point, can clearly reduce the hypertension.[21]

This study showed that EA at DU 20 and ST 36 acupoints can inhibit the elevation of blood pressure in SHR rats, and that stimulation of non-acupuncture points had a tendency to reduce elevated blood pressure (although no statistical significance was not shown), suggesting an ameliorating role for EA in the treatment of hypertension.

Following synthesis in the liver, AGT is hydrolyzed into its inactive form by renin angiotensin Ⅰ, under the influence of angiotensin-converting enzyme (ACE), to generate Ang Ⅱ which combines with its receptor (ATIR) to elevate blood pressure. Ang Ⅱ and AT1R thus play an important role in the development of hypertension.[22]

Previous studies have shown that EA may reduce plasma levels of Ang Ⅱ in SHR rats,[23] beside of systemic factors leading to the occurrence of high blood pressure, the role of the blood vessel itself is important as it can also produce renin and angiotensin. This study showed that EA reduced mRNA expression of AGT, the source of Ang Ⅱ, in the aorta of SHR rats. The reduction of plasma Ang Ⅱ results in the lowering of Ang Ⅱ and AT1R, thus inhibiting the elevation of blood pressure in SHR rats.

The study also showed that EA reduced mRNA expression of AT1R, further inhibiting the lowering of Ang Ⅱ and AT1R in combination and inhibiting the elevation of blood pressure in SHR rats. EA reduced the mRNA expressions of AGT and AT1R in aortas of SHR-NAP and SHR-AP rats. Furthermore, the changes in AT1R mRNA expression in SHR-AP were more significant than in SHR-NAP rats, indicating an important mechanism of the contribution of EA acupoint stimulation to the hypotensive effect.

In addition to the renin-angiotensin system, the role of endothelin is vital in the pathogenesis of hypertension. ET1 is a powerful vasoconstrictor secreted by endothelial cells, and exerts effects on the vascular endothelium through its receptor, ETAR, leading to vasoconstriction.[24,25] ET1 and ETAR combine to stimulate brain pressure, additional aldosterone secretion and release of catecholamines, growth factors and vascular smooth muscle contractility,[26,27] thus causing blood pressure to rise. Lower ET1 levels or a reduction in ETAR content can inhibit the development of hypertension.

This study showed that EA inhibited the elevation of vascular ET1 mRNA expression in SHR rats. This effect may be partly attributed to inhibition of the Ras-Raf-ERK pathway induced by AT1R, as AT1R mRNA expression was also reduced. The AT1R pathway induces the expression of AGT, thus reducing the combination of ET1 and ETAR. Furthermore, EA inhibited the elevation of ETAR mRNA expression to further reduce the combination of ET1 and ETAR, leading to a reduction in elevated blood pressure in SHR rats. Our data also showed that there was no difference in mRNA levels of ET1 and ETAR in SHR-NAP and SHR-AP rat vasculature, suggesting that EA at DU 20 and ST 36 acupoints had no significant effect on the ET system.

EA can inhibit elevated blood pressure, and the elevation of mRNA expression of AGT, AT1R, ET1 and ETAR in SHR rat aorta. Furthermore, EA at DU 20 and ST 36 acupoints was a more effective regulator of AT1R expression, a potential mechanism for lowering blood pressure.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Huo ZJ wrote the manuscript, Li D and Guo J participated the acupuncture experiments, Li S and Ding N helped wrote the manuscript and data analysis. Li ZX designed the experiments, performed most of the experiments, supervised the experiments and manuscript writing.

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