**Original Article**

**Impacts of telmisartan and ramipril on Na\(^{+}\)-K\(^{+}\)-ATPase and Ca\(^{2+}\)-ATPase in thoracic aortic smooth muscle cells of salt-sensitive hypertensive rat**

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Received January 19, 2016; Accepted May 4, 2016; Epub June 15, 2016; Published June 30, 2016

**Abstract:** Objective: This study aimed to investigate the impacts and mechanisms of renin-angiotensin-aldosterone system (RAAS) inhibitors on Na\(^{+}\)-K\(^{+}\)-ATPase and Ca\(^{2+}\)-ATPase in the thoracic aortic smooth muscle cells of salt-sensitive hypertensive rat. Methods: The salt-sensitive hypertensive rat model was established and randomly divided into the telmisartan group (TEL), the ramipril group (RAM) and the model group, with 6 rats in each group. Another 6 healthy Wistar rats of the same age and weight were used as blank control group. The activities of Na\(^{+}\)-K\(^{+}\)-ATPase and Ca\(^{2+}\)-ATPase in the thoracic aortic smooth muscle cells, as well as the expressions of Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha\)-subunit, plasma membrane calcium ATPase isoform 1 (PMCA1) mRNA and protein were detected. Results: Telmisartan and ramipril could increase the activities of Na\(^{+}\)-K\(^{+}\)-ATPase and Ca\(^{2+}\)-ATPase and the expressions of Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha\)-subunit and PMCA1 mRNA \((P<0.05)\). Compared with the control group, the expression of \(\alpha\)-subunit protein of the model group was reduced, while the expression of PMCA1 protein was increased \((P<0.01)\). Compared with the model group, after the intervention of TEL and RAM, the expression of \(\alpha\)-subunit protein was increased, while that of PMCA1 protein was decreased \((P<0.01)\). Conclusions: Telmisartan and ramipril could reverse the activities of Na\(^{+}\)-K\(^{+}\)-ATPase and Ca\(^{2+}\)-ATPase, as well as the expressions of mRNA and protein, in the thoracic aortic smooth muscle cells of salt-sensitive hypertensive rat, which might be one of the mechanisms that RAAS inhibitors reduced the blood pressure of salt-sensitive hypertensive rat.

**Keywords:** Salt-sensitive, renin-angiotensin-aldosterone system (RAAS), hypertension, ion pump

**Introduction**

The salt-sensitive hypertension (SSH) referred to excessive salt intake-induced high blood pressure [1-4]. In China, SSH accounted for 50-60% of the adult hypertension population, therefore, the studies towards the pathogenesis and prevention of SSH might exhibit significant importance towards the health problems of Chinese population. Currently, several studies [5-10] revealed that the occurrence and development of SSH was closely related to the renin-angiotensin-aldosterone system and ion transportation, and the high-salt intake could lead to the defect of membrane ion transportation, abnormalities of Na\(^{+}\)/Ca\(^{2+}\) exchange, dysfunction of renal natriuresis and RAAS activation, while its cellular and molecular mechanisms had not been fully clarified. ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are the two widely used RAS antagonists, though in different ways. ACE inhibitors never block the RAS completely. Angiotensin receptor antagonists, on the other hand, feature a more specific mechanism since they selectively block the angiotensin receptor sub-type-1 (AT1), which is responsible for most of the deleterious effects of angiotensin II. In the present study, we established the sensory-nerve injured SSH rat model, then performed telmisartan (TEL) and ramipril (RAM) intervention, the activity changes of sodium pump and calcium pump in the thoracic aortic smooth muscle cells of SSH rat, as well as the changes of gene and protein expressions, were observed, aiming to explore the impact mechanisms of
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RAAS inhibitor-caused abnormal transportation of plasmalemmal Na\(^+\) and Ca\(^{2+}\) towards the blood pressure of SSH rat.

Materials and methods

Animals

The Wistar rats were provided by the Medical Laboratory Animal Center of Daping Hospital, Third Military Medical University, Chongqing [Certificate No: 0000651, animal laboratory license number: SCXK (Yu) 2012-0005]. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Chongqing Medical University. All experiments were conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (National Academy Press, revised 1996). All experiments were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Establishment of animal model and grouping

18 male Wistar rats were subcutaneously injected of 50 mg/kg capsaicin (Sigma, USA, dissolved in 10% ethanol + 10% Tween 80-containing saline) on the 1st and 2nd day of birth, after the lactation, 4% NaCl diet was giving to the rats for one month, and the rats were randomly divided into three groups according to the different intervention methods: the TEL group (Shanghai Boehringer Ingelheim Pharmaceutical Co., Ltd.), the RAM group (Beijing Sanofi-Aventis Pharma Co., Ltd) and the model group, with 6 rats in each group. Another 6 healthy Wistar rats of the same age and weight were used as blank control group. The TEL group (10 mg·kg\(^{-1}\)·d\(^{-1}\)) and the RAM group (1 mg·kg\(^{-1}\)·d\(^{-1}\)) were administered of the drugs through the digestive tract daily. The Softron BP-98A noninvasive manometry was used to detect and observe the changes of systolic blood pressure of rat tail.

Cell culture and identification

The animals were killed in the 9th week to collect the thoracic aortic smooth muscle cells for the primary and passage culture, the immunohistochemical method was performed to identify the α-SMActin cells, the 4th-generation cells were then collected for the experiments.

Activity determination of cellular Na\(^+\)-K\(^+\) pump

The ultrasonic pulverizer was used to disrupt the cells for the detection of sample protein concentration. The trace-amount ATP enzyme detection kit (Nanjing Jiancheng Bioengineering Institute) and the biochemical enzymatic method were used, the ATP enzyme could decompose ATP into ADP and inorganic phosphorus, the detection of inorganic phosphorus amount could determine the level of ATP activity, the operations were strictly in line with the instructions, the absorbance value of each tube was detected by 721 visible spectrophotometer at 660 nm wavelength, 1 cm and 0.5 cm diameter, which was then placed into the formula to calculate the ATP activity.

RT-PCR

The total cellular RNA was extracted, quantified and determined the purity. According to the reverse transcription kit (Takara, Dalian, China), the appropriate amount of reverse transcription working liquid was prepared for the RNA reverse transcription. The primer sequences were as follows (Takara, Dalian, China): the length of β-actin was 1296 bp, the upstream primer sequence was GGAGATTACTGCCCTGGGTCTCTA, the downstream primer sequence was GACTCATCGTACTCCTGCTTGCTG, the length of α\(_1\) subunit was 3636 bp, the upstream primer sequence was CTGATCAGCATGGCCTATGGAC, the downstream primer sequence was ACC-GTTCTCAGCGCCAGAATCAC, the length of PMCA1 was 4228 bp, the upstream primer sequence was GGAAGTTTCCGGATCTTCAGCA, the downstream primer sequence was CACTTTGGCCTACCATTAGCA.

Western-blot

The total cellular protein was extracted, and the protein concentrations were determined according to the BCA kit (Beyotime Institute of Biotechnology), the quantitation and expression were performed according to the sample protein concentrations.
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The experimental data were expressed as mean ± standard deviation (X±s), the processing and analysis of data was conducted by SPSS17.0 statistical software. The intergroup comparison used the analysis of variance, the LSD method was used towards the homogeneity of variance, otherwise the Tamhane’s T2 method was used, with P<0.05 considered as the statistical significance.

Results

Blood pressure changes

As shown in Figure 1, with the increasing of high-salt feeding time, the blood pressure of the model group was increased, significantly higher than that of the control group when fed with the high-salt diet for one month (P<0.05); on the fifth week, TEL and RAM were administrated for the intervention, and the blood pressures were gradually decreased (P<0.05).

Activities of Na⁺-K⁺-ATPase and Ca²⁺-ATPase

As shown in Table 1 and Figure 2, the activities of Na⁺-K⁺-ATPase and Ca²⁺-ATPase in the thoracic aortic smooth muscle cells of the model group were lower than the control group (P<0.05), after the intervention of TEL and RAM, the activities of Na⁺-K⁺-ATPase and Ca²⁺-ATPase were both increased (P<0.05), while there was no statistical difference in the activities of Na⁺-K⁺-ATPase and Ca²⁺-ATPase between the TEL and RAM group (P>0.05). The difference between the TEL group and the control group, as well as between the RAM group and the control group, was not statistically significant (P>0.05).

Expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 mRNA

The results were shown in Table 2 and Figure 3, compared with the control group, the expression of Na⁺-K⁺-ATPase α₁-subunit of the model group was reduced (P<0.05), compared with the model group, the expression levels of Na⁺-K⁺-ATPase α₁-subunit of the TEL group and the RAM group were increased (P<0.05), while there was no statistically significant difference between the TEL and RAM group.

Compared with the control group, the expression of PMCA1 in the thoracic aortic smooth muscle cells of the model group was reduced (P<0.05), compared with the model group, the expressions of PMCA1 of the TEL and RAM group were increased (P<0.05), among which the expression of PMCA1 of the RAM group was significantly higher than that of the TEL group (P<0.05).

Expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 proteins

As shown in Table 3 and Figure 4, compared with the control group, the expression of Na⁺-
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The plasmalemmal Na⁺-K⁺ pump is the important transportation substance to maintain the normal levels of Ca²⁺ and Na⁺, playing a very important role in the occurrence and development of hypertension [11-19]. Studies had shown [20, 21] that the salt-sensitive population had the phenomenon of intracellular calcium overload, indicating that the pressure boosting response of SSH was positively correlated with the intracellular Na⁺ and Ca²⁺ levels. Ushio-Yamana et al. [10] reported that the sense nerve injury-type SSH rat, established based on the capsicin-treat high-salt rat, exhibited the significantly increased intracellular Ca²⁺. Shang’s study about the thoracic aorta smooth muscle cells of hypertensive rats [22] showed that the activities of Na⁺-Ca²⁺ pump in the vascular smooth muscle cells of spontaneously hypertensive rat were decreased, and the application of partial antihypertensive drugs could make them increased. This study measured the activities of ion pumps in the vascular smooth muscle cells of SSH rat, and the results were consistent with the findings above, the activities of Na⁺ and Ca²⁺ pump in the thoracic aortic smooth muscle cells of the model group were significantly lower than the control group, while the TEL and RAM intervention could increase their activities. After the intervention of TEL and RAM, the activities of Na⁺ and Ca²⁺ pump were increased while the blood pressure was decreased, thus it could be speculated that the reduced activities of Na⁺ and Ca²⁺ pump might be one of the pathogeneses of SSH. The expressions of sodium pump mRNA and protein of the model group were decreased, while after the TEL and RAM intervention, the expressions were increased, so it could be speculated that the pathogenesis of SSH might be the reduced activity of sodium pump caused the abnormalities of Na⁺-Ca²⁺ exchange, leading to the vasoconstriction, which was followed by the increasing of blood pressure. This research found that the expression of Ca²⁺ pump mRNA of SSH rat was decreased, while those of the TEL and RAM group were increased, among which the increasing of the RAM group was significantly higher than the TEL group, which might be the significant difference caused by the relatively small sample numbers; the expression of Ca²⁺ pump protein in the thoracic aorta cells of the model group was increased, while reduced

**Figure 2.** The activity of Na⁺-K⁺-ATPase and Ca²⁺-ATPase in the rat’s smooth muscle cells of thoracic aorta on every group. *P<0.05, vs N; †P<0.05, vs M.

**Table 2.** The relative protein expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 mRNA in the rats’ smooth muscle cells of thoracic aorta of every group (X±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺-K⁺-ATPase α₁-subunit</th>
<th>PMCA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100±19.76b</td>
<td>100±10.93bd</td>
</tr>
<tr>
<td>Model</td>
<td>43.76±0.41ac,d</td>
<td>40.44±8.30ac,d</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>129.72±15.15b</td>
<td>164.51±24.51bd</td>
</tr>
<tr>
<td>Ramipril</td>
<td>150.16±13.18b</td>
<td>526.26±52.71abc</td>
</tr>
</tbody>
</table>

Note: *P<0.05, vs N; †P<0.05, vs M; ‡P<0.05, vs T; ‡‡P<0.05, vs R.

K⁺-ATPase α₁-subunit protein of the model group was significantly reduced (P<0.01), compared with the model group, after the TEL and RAM intervention, the expression of Na⁺-K⁺-ATPase α₁-subunit protein in the thoracic aortic smooth muscle cells was significantly increase (P<0.01), and the difference was statistically significant.

**Figure 4** showed that, compared with the control group, the expression of PMCA1 protein of the model group was increased (P<0.01). Compared with the model group, after the TEL and RAM intervention, the expression of PMCA1 protein was significantly reduced (P<0.01), and the difference was statistically significant.

**Discussion**

The plasmalemmal Na⁺-K⁺ pump is the important transportation substance to maintain the normal levels of Ca²⁺ and Na⁺, playing a very important role in the occurrence and development of hypertension [11-19]. Studies had shown [20, 21] that the salt-sensitive population had the phenomenon of intracellular calcium overload, indicating that the pressure boosting response of SSH was positively correlated with the intracellular Na⁺ and Ca²⁺ levels. Ushio-Yamana et al. [10] reported that the sense nerve injury-type SSH rat, established based on the capsicin-treat high-salt rat, exhibited the significantly increased intracellular Ca²⁺. Shang’s study about the thoracic aorta smooth muscle cells of hypertensive rats [22] showed that the activities of Na⁺-Ca²⁺ pump in the vascular smooth muscle cells of spontaneously hypertensive rat were decreased, and the application of partial antihypertensive drugs could make them increased. This study measured the activities of ion pumps in the vascular smooth muscle cells of SSH rat, and the results were consistent with the findings above, the activities of Na⁺ and Ca²⁺ pump in the thoracic aortic smooth muscle cells of the model group were significantly lower than the control group, while the TEL and RAM intervention could increase their activities. After the intervention of TEL and RAM, the activities of Na⁺ and Ca²⁺ pump were increased while the blood pressure was decreased, thus it could be speculated that the reduced activities of Na⁺ and Ca²⁺ pump might be one of the pathogeneses of SSH. The expressions of sodium pump mRNA and protein of the model group were decreased, while after the TEL and RAM intervention, the expressions were increased, so it could be speculated that the pathogenesis of SSH might be the reduced activity of sodium pump caused the abnormalities of Na⁺-Ca²⁺ exchange, leading to the vasoconstriction, which was followed by the increasing of blood pressure. This research found that the expression of Ca²⁺ pump mRNA of SSH rat was decreased, while those of the TEL and RAM group were increased, among which the increasing of the RAM group was significantly higher than the TEL group, which might be the significant difference caused by the relatively small sample numbers; the expression of Ca²⁺ pump protein in the thoracic aorta cells of the model group was increased, while reduced
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after the intervention of TEL and RAM, contrast to the previous similar studies, the inconsistency of increased calcium pump protein expression and decreased mRNA expression of the model group might because the activity of plasmalemmal Na⁺ pump was reduced, thus the intracellular Na⁺ load was increased, the resulted abnormality of Na⁺-Ca²⁺ exchange made the intracellular calcium overloaded, thus reactively caused the compensatory increasing of Ca²⁺ pump protein, while the decreased gene expression might because the high salt feed induced the expression of Ca²⁺ pump mRNA prior to the protein.

Thus, it was suspected that the reduced mRNA expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 in the thoracic aortic smooth muscle cells of SSH rat might because the activities of Na⁺-K⁺-ATPase and Ca²⁺-ATPase were decreased, leading to the abnormalities of plasmalemmal ion transportation, thereby the vascular remodeling was caused. TEL and RAM intervention could reverse this phenomenon and decrease the blood pressure. While how could TEL and RAM regulate the gene and protein expressions of Na⁺-K⁺-ATPase and Ca²⁺-ATPase, thus achieved the reversal of this phenomenon, how was the intracellular free Ca²⁺ concentration in the thoracic aortic smooth muscle cells of SSH rat and what were the changes of intracellular free Ca²⁺ concentration after the TEL and RAM intervention, what kind of relationship existed among the activities of Na⁺ and Ca²⁺ pump and intracellular free Ca²⁺ concentration of SSH rat, such a series of issues still needed the further researches and explorations, so that the pathogenesis of abnormal plasmalemmal ion transportation-caused hypertension could be further explained.

In summary, TEL and RAM could decrease the activities of Na⁺ and Ca²⁺ pump in the thoracic aortic smooth muscle cells of SSH rat, and down-regulate their mRNA expressions, it could be speculated that the reduced activities of Na⁺ and Ca²⁺ pump might be one of the pathogeneses of SSH. While the difference of Ca²⁺ pump protein expression might because of the compensatory increasing of calcium pump protein caused by the abnormal intracellular Na⁺-Ca²⁺ exchange.

Acknowledgements

This study was supported by Science and technology Foundation of Guizhou Provincial Science Department (Qiankehe (2009) 08).

Table 3. The protein expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 mRNA in the rats' smooth muscle cells of thoracic aorta of every group (x±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺-K⁺-ATPase α₁-subunit</th>
<th>PMCA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.62±0.07⁰</td>
<td>0.67±0.04⁰</td>
</tr>
<tr>
<td>Model</td>
<td>0.59±0.36ª,ª</td>
<td>1.39±0.31ª,ª</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>1.55±0.23ª</td>
<td>0.74±0.27ª</td>
</tr>
<tr>
<td>Ramipril</td>
<td>1.44±0.16⁰</td>
<td>0.37±0.23⁰</td>
</tr>
</tbody>
</table>

Note: ¹P<0.01, vs N; ²P<0.01, vs M; ³P<0.01, vs T; ⁴P<0.01, vs R.

Figure 3. The mRNA expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 in the rats' smooth muscle cells of thoracic aorta of every group. *P<0.05, vs N.
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Figure 4. The protein expressions of Na⁺-K⁺-ATPase α₁-subunit and PMCA1 in the rats' smooth muscle cells of thoracic aorta of every group. **P<0.05, vs N; ***P<0.05, vs M.

Disclosure of conflict of interest

None.

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