Effect of Angiotensin 1-7 on Myocardial Calcium Pump in Salt-Sensitive Hypertensive Rats

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Abstract: Objective — To investigate the effects of angiotensin 1-7 (Ang1-7) on plasma membrane ATPase isoform 1 (PMCA1) in salt-sensitive hypertensive rats. Methods — Thirty newborn male Wistar rats were selected to establish the salt-sensitive hypertensive rat model with sensory nerve injury, which were then randomly divided into 5 groups (n=5), including model group, Telmisartan group, Ramipril group, Ang1-7 group, and A-779 group. Another normal control group was established (n=5). After 4 weeks of intervention, the tail blood pressure of rats in each group was measured, and then the apical tissue of left ventricle was cut. The contents of Ang Ⅱ and Ang1-7 in cardiomyocytes were detected by enzyme-linked immunosorbent assay. The expression of PMCA1 mRNA and protein in heart of salt-sensitive hypertensive rats were detected by RT-PCR and immunohistochemistry. Results — (1) Compared with the normal control group, the concentration of Ang Ⅱ in the myocardium of salt-sensitive hypertensive rats increased ($P < 0.05$), which decreased after the intervention of Telmisartan and Ramipril ($P < 0.05$), and no change occurred after the intervention of Ang1-7 in concentration ($P > 0.05$). (2) Compared with the normal control group, the concentration of myocardial Ang1-7 in salt-sensitive hypertensive rats decreased ($P < 0.05$), and increased after the intervention of telmisartan and ramipril ($P < 0.05$), and increased after the intervention of A-779 ($P < 0.05$). (3) The expression of PMCA1 mRNA and protein in salt-sensitive hypertensive rats was increased compared with the normal control group ($P < 0.05$), and the expression of Ang-(1-7), telmisartan and ramipril was decreased compared with the model group ($P < 0.05$). The expression of p38MAPK mRNA and p-p38MAPK protein in the myocardium of salt-sensitive hypertensive rats was increased compared with that in the normal control group ($P < 0.05$), and the expression of Ang-(1-7), Telmisartan and Ramipril was decreased compared with that in the model group ($P < 0.05$). Conclusion — Ang-(1-7) may be involved in the regulation of cardiac calcium pump, inhibiting its overcompensation and delaying the occurrence of calcium pump inhibition in the early stage of salt-sensitive hypertension. Ang-(1-7) can inhibit the activity of p38MAPK and protect the heart, and its regulation on PMCA1 may be mediated by the expression of p38MAPK pathway.

***Keywords*:** Ang1-7, salt-sensitive hypertension, PMCA1, p38MAPK

Hypertension is a chronic and complex disease caused by the interaction of multiple genes, multiple environmental factors and individuals' bad living habits.Studies have demonstrated that 50% of patients with hypertension and 25% of people with normal blood pressure displayed blood pressure salt sensitivity[1]. Long-term hypertension can lead to pathological changes such as myocardial fibrosis and myocardial remodeling. Ca^{2+} -ATPase, referred to as calcium pump, plays an important role in myocardial oxidative stress and ventricular remodeling. It can drive intracellular calcium ions to be pumped out of the cell or pumped into the endoplasmic reticulum for storage, so as to maintain a low concentration of free Ca^{2+} in the cell. Studies have shown that Ca^{2+} regulates hormones such as parathyroid hormone and vitamin D, which can regulate blood pressure by inhibiting the RAAS system [2]. Foreign studies by Hammad et al. found that under the intervention of Ang Ⅱ, PMCA1 heterozygous gene knockout mice had a more obvious degree of elevated blood pressure and myocardial hypertrophy than wild-type mice, indicating that PMCA1 plays an important role in the occurrence and development of hypertension[3]. However, Ang1-7 is hydrolyzed by Ang Ⅱ, and its physiological effects are mainly manifested as myocardial protective effects such as lowering blood pressure and anti-cardiac hypertrophy. Therefore, this study took salt-sensitive hypertensive rats with nerve injury as the research object to explore the effects of Ang1-7 on cardiac calcium pump activity and mRNA expression in salt-sensitive hypertensive rats.

1. Data and methods

1.1 Experimental animals

Thirty SPF newborn male Wistar rats were purchased from Beijing Vitong Lihua Experimental Animal Technology Co., Ltd. [Certificate No.: 11400700045542, Animal Laboratory License No.: SCXK(Beijing)2012-0001]. Subcutaneous injection of capsaicin 50mg/kg on day 1 and day 2 of birth was used to establish the salt-sensitive hypertensive rat model of sensory nerve injury. After 3 weeks of breast milk period, 4% NaCl high salt diet was fed for 4 weeks. Then the rats were randomly divided into 5 groups according to the intervention measures, with 5 rats in each group. Model group: continued to feed with 4% NaCl high salt diet; Telmisartan group: Telmisartan 10mg/kg·D was given by digestive tract every morning; Ramipril group: Ramipril 1mg/kg·D was given via upper digestive tract every morning; Ang1-7 group: Alzet microosmotic pump Ang1-7, 25μg×kg^{-1}×h^{-1}; Group A-779: Alzet microosmotic pump A-779, 25μg×kg^{-1}×h^{-1}; After that, each group was fed with 4% NaCl high salt diet. Another normal control group was fed with normal salt diet (normal feed containing 0.5%NaCl). They were fed for 4 weeks continuously.All animal experiment procedures were approved by the Animal Care and Use Committee of Zunyi Medical University, and all operations were performed under anesthesia.

1.2 Instruments and reagents

Capsaicin and Ang1-7 were purchased from Sigma Company in the United States, A-779 from Bachem Company in Switzerland, telmisartan from Boehringer Ingelheim Pharmaceutical Co., Ltd. (Shanghai), Ramipril from Sanofi-Avents Pharmaceutical Co., Ltd. (Beijing), Rat angiotensin Ⅱ ELISA kit and rat angiotensin 1-7 ELISA kit were purchased from Shanghai Huding Biotechnology Co., Ltd.; DEPC water, Trizol, reverse transcription kit, Real-time PCR kit and PMCA1 subunit monoclonal antibody were purchased from abcam Company.

1.3 Experimental methods

1.3.1 Blood pressure measurement of caudal artery of rats

The systolic blood pressure of rat caudal artery was measured by Softron BP-98A noninvasive manometer. The systolic blood pressure of the caudal artery was measured at a fixed time every week from the end of the lactation period (3 weeks of age), and was measured three times each time to take the average value.

1.3.2 Enzyme-linked immunoassay

After the intervention, the rats were sacrificed for sampling.An appropriate amount of left ventricle apical tissue was cut, blood was washed and removed, and the supernatant was homogenized, and the content of Ang1-7 and AngII in the supernatant was determined by enzyme-linked immunoassay.

1.3.3 RT-PCR assay

Total RNA was extracted from the apical tissue of the left ventricle of the rat, and cDNA was generated by reverse transcription kit (Dalian Baobio) and amplified.

1.3.4 Immunohistochemistry

The left ventricular apical tissue of rats was taken into paraffin sections, and the distribution of Ca^{2+}-ATPase PMCA1 in myocardial tissue was observed by immunohistochemical staining with GTVisionTM Ⅲ anti-mouse/rabbit universal immunohistochemical detection kit.Results Image data was analyzed by Image-Pro Plus 6.0 Image analysis software. The average optical density (MOD) of the positive color rendering part (brown) on each Image was calculated by the software. The MOD of the three visual field images on a slice was averaged as the MOD of the slice.

1.4 Statistical Methods

The experimental data was expressed as mean±standard deviation ($\bar{x}$±s), and the statistical software SPSS 20.0 was used to collate and analyze the data. Analysis of variance was used for comparison between groups, LSD method was used for homogeneity of variances, Tamhane's T2 method was used for heterogeneity of variances, and $P < 0.05$ was considered statistically significant.

2. Results

2.1 Effects of different interventions on blood pressure of SSH rats in each group

Compared with the normal control group, the blood pressure of the other high-salt feeding groups increased before intervention ($P < 0.05$). After 4 weeks of drug intervention, blood pressure in telmisartan group, ramipril group and Ang1-7

group decreased significantly compared with before intervention ($P < 0.05$), while blood pressure in model group and A-779 group increased ($P < 0.05$). Compared with model group, the blood pressure ratio of normal control group, Telmisartan group and Ramipril group was decreased ($P < 0.05$), but there was no significant difference between Ang1-7 group and model group ($P > 0.05$). See Table 1.

Table 1. Changes of systolic blood pressure in each group before and after intervention (mmHg, $\bar{x} \pm s$)

Groups	SBP/mmhg	
	Hypertension before intervention	Hypertension after 4 weeks of intervention
Normal control group	106.4±4.7	109.2±5.5 b
Model group	131.5±12.4 a	136.3±12.9 c
Telmisartan group	135.7±17.4 a	114.3±9.2 b c
Ramipril group	131.6±6.8 a	105.1±12.7 b c
Ang-(1-7) group	146.7±17.5 a	134.7±12.7 c
A-779 group	131.4±11.1 a	137.0±9.7 c

Note: a: Compared with normal control group, $P<0.05$; b: Compared with model group, $P<0.05$; c: Compared with pre-intervention blood pressure, $P<0.05$

2.2 Effects of different interventions on myocardial tissue ANG1-7 and ANGII in SSH rats

Enzyme-linked immunoassay assay showed that compared with the normal control group, the concentration of Ang1-7 in the cardiac tissue of the other salt-sensitive hypertension groups was increased ($P < 0.05$). Compared with model group, the concentration of Ang1-7 in myocardial tissue was increased after the intervention of Ang1-7 ($P < 0.05$), and the concentration of Ang1-7 in myocardial tissue was increased after the application of Telmisartan and Ramipril ($P < 0.05$). The content of Ang1-7 in myocardium of A-779 group was higher than that of model group ($P < 0.05$), but still significantly lower than that of Ang1-7 group ($P < 0.05$). Compared with model group, Ang Ⅱ was significantly decreased in normal control group ($P < 0.05$), and Ang Ⅱ was significantly decreased in Telmisartan group and Ramipril group after intervention ($P < 0.05$). There was no significant difference between Ang1-7 group and model group ($P > 0.05$). As is shown in Figure 1.

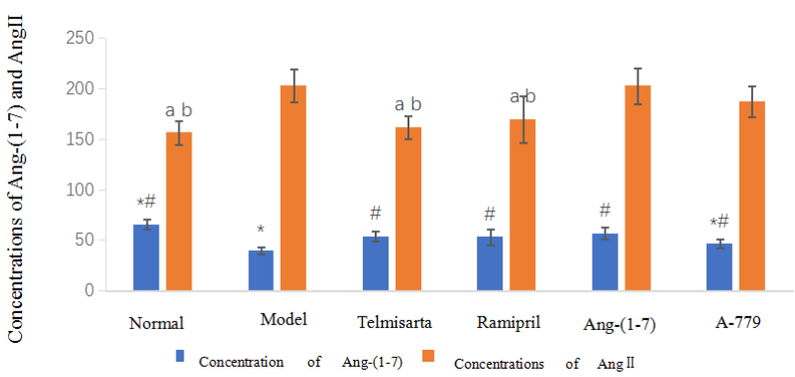

Figure 1. Changes in concentrations of Ang- (1-7) and Ang Ⅱ in myocardial tissue of rats in each group

Note: #: Compared with model group, $P<0.05$; *: Compared with Ang1-7 group, $P<0.05$; a: Compared with model group, $P<0.05$; b: Compared with Ang1-7 group, $P<0.05$.

2.3 Effects of different interventions on PMCA1 mRNA expression in myocardial tissue of rats in each group

RT-PCR indicated that compared with the normal control group, the expression of PMCA1 mRNA in the myocardium of rats in the model group was significantly increased ($P < 0.05$). Compared with the model group, the expression of PMCA1 mRNA in the myocardium of rats in the model group was decreased after the intervention of Telmisartan, Ramipril and Ang-(1-7) ($P < 0.05$). Compared with Ang-(1-7) group, the mRNA expression of PMCA1 in myocardial tissue was significantly increased after A-779 intervention ($P < 0.05$). As shown in Figure 2.

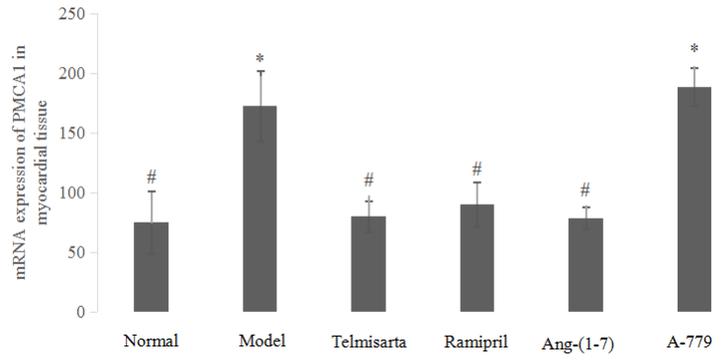

Figure 2. Comparison of relative mRNA expression levels of PMCA1 in myocardial tissue of rats in each

Note: # : Compared with model group, P<0.05;* : Compared with Ang-(1-7) group, P<0.05.

2.4 Effects of different interventions on AT1R mRNA expression in myocardium of SSH rats in each group

Compared with the normal control group, the mRNA expression of AT1R was increased in model group ($P < 0.05$), but decreased in Telmisartan group and Ramipril group ($P < 0.05$), and decreased in Ang-(1-7) group compared with the model group ($P < 0.05$). Compared with Ang-(1-7) group, the mRNA expression of AT1R in myocardial tissue was increased after A-779 intervention ($P < 0.05$). As is shown in Figure 3.

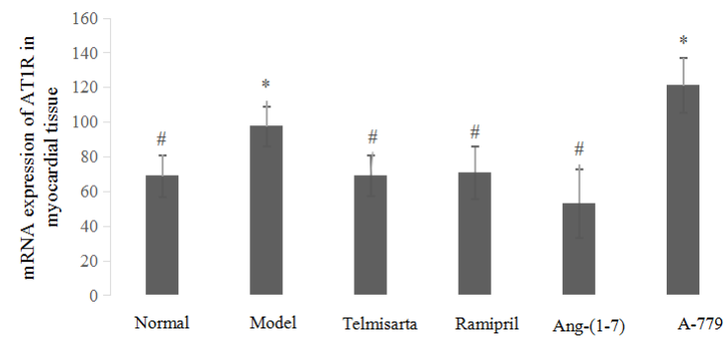

Figure 3. mRNA expression of AT1R in myocardium of rats in each group

Note: # : Compared with model group, P<0.05;* : Compared with Ang-(1-7) group, P<0.05.

2.5 Effects of different interventions on the expression of MAS mRNA in SHH rat myocardium

Compared with the normal control group, the expression of MAS mRNA in model group was decreased ($P < 0.05$), the expression of Telmisartan group was increased compared with the model group ($P < 0.05$), and the expression of Ang-(1-7) group was increased compared with model group ($P < 0.05$). Compared with Ang-(1-7) group, the mRNA expression of MAS in myocardium of rats in A-779 group was decreased ($P < 0.05$). See Figure 4.

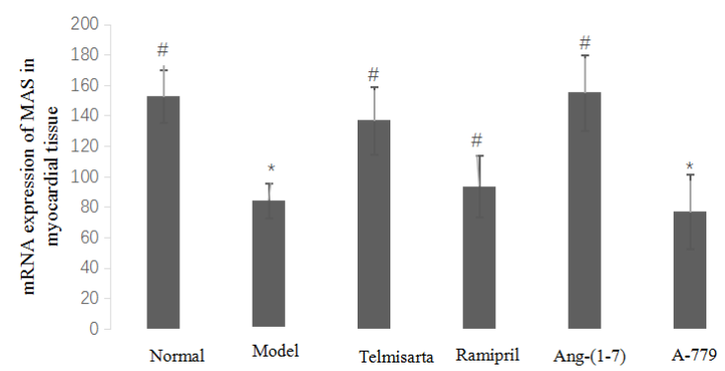

Figure 4. mRNA expression of MAS in myocardial tissue of rats in each group

Note: # : Compared with model group, P<0.05;* : Compared with Ang-(1-7) group, P<0.05.

2.6 Expression level of p38MAPK mRNA in rat myocardial tissue

Compared with the normal control group, the mRNA expression of p38MAPK in model group was increased ($P < 0.05$), the expression of telmisartan group and ramipril group was decreased compared with the model group ($P < 0.05$), and the expression of Ang-(1-7) group was decreased compared with the model group ($P < 0.05$). Compared with Ang-(1-7) group, the mRNA expression of p38MAPK in myocardium of rats in A-779 group was increased ($P < 0.05$). As shown in Figure 5.

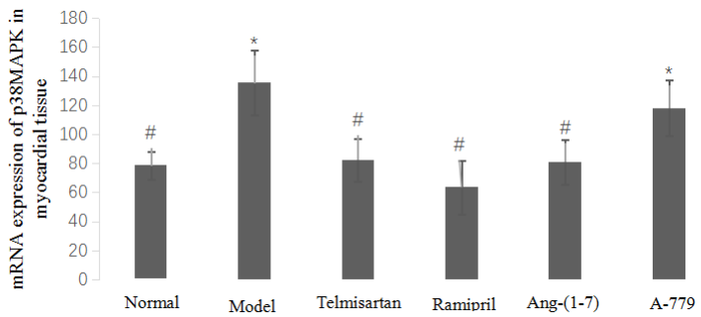

Figure 5. mRNA expression of p38MAPK in myocardial tissue of rats in each group

Note: # : Compared with model group, P<0.05;* : Compared with Ang-(1-7) group, P<0.05.

2.7 Influence of different interventions on PMCA1 protein expression in myocardial tissue of SSH rats

Immunohistochemical results showed that compared with the normal control group, the expression of PMCA1 protein in model group was increased ($P < 0.05$), compared with the model group, the expression of Telmisartan and Ramipril was decreased ($P < 0.05$), and the expression of Ang-(1-7) group was also decreased compared with the model group ($P < 0.05$). Compared with Ang-(1-7) group, the expression of PMCA1 protein in myocardium of rats in A-779 group was increased ($P < 0.05$). See Figure 6 and Figure 7.

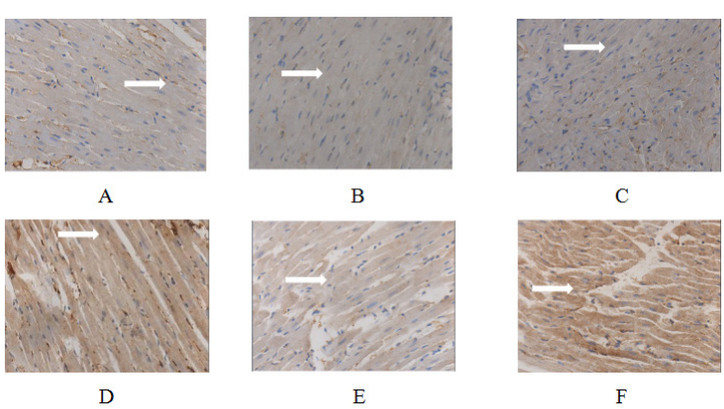

Figure 6. Immunohistochemical staining results of PMCA1 in myocardial tissue×400

Note: A) Normal control group; B) Telmisartan group; C) Ramipril group; D) Model group; E) Ang1-7 Group; F) Group A-779.

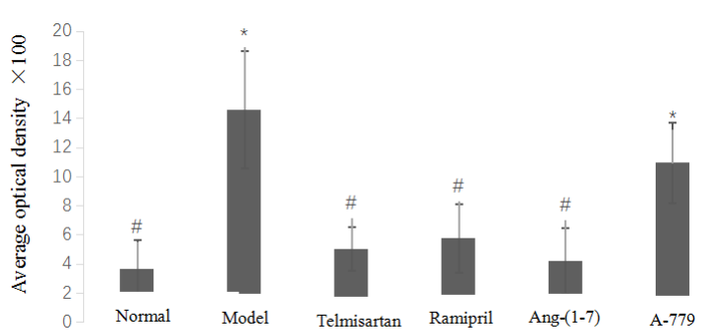

Figure 7. Immunohistochemical staining results of PMCA1 in myocardial tissue of rats in each group

Note: #: Compared with model group, P<0.05; *: Compared with ANG1-7 group, P<0.05.

3. Discussion

Ca^{2+} -ATPase, also known as the calcium pump,can be divided into plasma membrane Ca^{2+} -ATPase (PMCA) and endoplasmic reticulum (SARCO /endoplasmic reticulum SERCA) pumps. Although the density of PMCA was lower than that of SERCA, the effect of PMCA on Ca^{2+} effluent was still stronger than that of SERCA. Plasma membrane calcium pump can be divided into four subunits: PMCA1, PMCA2, PMCA3 and PMCA4, while PMCA1 and PMCA4 are mainly expressed in heart tissue[4]. PMCA in cardiomyocytes drives intracellular calcium ions to pump out of the cell or pump into the endoplasmic reticulum cavity for storage, thus maintaining the intracellular Ca^{2+} state of high external and low internal. Studies have found that Ca^{2+} -ATPase is closely related to hypertensive left ventricular hypertrophy, and the application of calcium channel blockers can reduce blood pressure and improve ventricular remodeling [5]. Cao Heng et al. [6] also observed in animal experiments that in SHR accompanied by significant LVH, Ca^{2+}-ATPase activity of myocardial cell membrane was decreased, while LVH of SHR was reversed after valsartan application, and Ca^{2+}-ATPase activity of myocardial cell membrane was restored. Foreign studies by Hammad et al. found that under the intervention of Ang Ⅱ, PMCA1 heterozygous gene knockout mice had a more obvious degree of elevated blood pressure and myocardial hypertrophy than wild-type mice, indicating that PMCA1 plays an important role in the occurrence and development of hypertension[7]. Other studies have shown that Ca^{2+} -ATPase expression is down-regulated in the sarcoplasmic reticulum of myocytes with mAs gene deletion, accompanied by transient decrease in Ca^{2+} concentration, suggesting that Ang-(1-7)/Mas may be involved in cardiac function regulation [8,9]. Ang-(1-7), as a cardiovascular protective peptide almost opposite to Ang Ⅱ, has not been fully determined whether Ang-(1-7) regulates the activity of cardiac calcium pump and mRNA expression by antagonizing the physiological effects of Ang-, thus affecting the occurrence and development of hypertension. In this study, the blood pressure of salt-sensitive hypertensive rats treated with capsaicin significantly decreased after the intervention of Ang1-7, which proved that Ang1-7 also had a hypotensive effect on the salt-sensitive hypertensive rats, which was similar to the hypotensive effect of Ang1-7 in other types of hypertensive rat models. However, the concentration of Ang Ⅱ in the myocardial tissue was not significantly reduced compared with that in the model group, which may be related to the effect of other pathways.

PMCA is an enzyme protein with low capacity and high affinity, which mainly regulates the intracellular Ca^{2+} concentration continuously and finely. Recent studies have shown that PMCA1 gene variation is closely associated with hypertension and other cardiovascular diseases. In the study of heterozygous PMCA1 gene deletion mice, it was found that the blood pressure of heterozygous mice was significantly higher than that of wild control group[10]. In this study, the expression of PMCA1 was increased in the myocardium of salt-sensitive hypertensive rats, but the mRNA and protein expression of PMCA1 were restored to the level of normal rats after the administration of Ang-(1-7), Telmisartan and Ramipril. This may be due to the fact that PMCA1 inhibits intracellular calcium overload in the form of compensatory increase in the early stage of hypertension, and has a certain protective effect on myocardium and vascular tissue cells, while ACEI drugs and ARB drugs can reduce this compensatory effect. Whether this indicates that the above drugs can inhibit the overexpression of PMCA1 through other pathways and delay the occurrence of its decompensation is still a question that needs further study. However, under the effect of Ang-(1-7), PMCA1 in the myocardium of salt-sensitive hypertensive rats was down-regulated and restored to the same degree as that in the normal group, suggesting that it had similar effects to Ramipril and Telmisartan.

Current studies have suggested that the activation of p38MAPK is closely related to vascular remodeling and cardiac hypertrophy in hypertension, and that tumor necrosis factor-α (TGF-α), interleukin-1β and transfer growth factor-β can induce cardiac hypertrophy, myocardial apoptosis and interstitial fibrosis through p38MAPK [11]. High salt can reduce the expression of vascular endothelial cells (eNOS), affect the release of nitric oxide and cause damage to vascular endothelial cells by activating p38MAPK, which is closely related to the ACE2-Ang-(1-7)-MAS axis. In this study, the expression of p38MAPK downstream of ACE2-Ang-(1-7)-Mas pathway was observed simultaneously. In salt-sensitive hypertension group, the expression of p38MAPK mRNA and phosphorylated protein were significantly increased, while in Ang-(1-7) intervention, MAS receptor was up-regulated and p-p38MAPK expression was significantly down-regulated, suggesting that Ang-(1-7) has the effect of inhibiting p38MAPK in the heart.

In conclusion, Ang-(1-7) can reduce blood pressure in salt-sensitive hypertensive rats. At the same time, the mRNA and protein expressions of PMCA1 in the cardiomyocytes of salt-sensitive hypertensive rats were increased, which could be reversed by Ang-(1-7), Telmisartana and Ramipril and it is related to the compensatory effect of calcium pump in the early and mild stages of salt-sensitive hypertensive rats. Whether Ang-(1-7) regulates the expression of PMCA1 through the p38MAPK pathway can be done with further and in-depth study.

Acknowledgments

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