

Research Article

Effect of Valsartan on Sarcoplasmic Reticulum Ca^{2+} -ATPase Pump of the Left Ventricular Myocardium in Rats with Heart Failure with Preserved Ejection Fraction

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What Is It about?

Treatment of hypertension remains one of the most important factors in the management of heart failure with preserved ejection fraction (HFPEF). The Valsartan is one of the major treatments of hypertension. Various studies involving Valsartan use in HFPEF did not demonstrate significant benefits. Within the cardiomyocyte, Ca^{2+} is the main mediator of contraction and relaxation after electric activation. The sarcoplasmic reticulum Ca^{2+} -ATPase pump (SERCA) is the main mediator of Ca^{2+} . The activity and expression of SERCA decreases in HFPEF. We aim to determine the effect of Valsartan on SERCA of the left ventricular myocardium in HFPEF rats.

Key Words

Angiotensin type 1 receptor blocker · Sarcoplasmic reticulum Ca^{2+} -ATPase pump · L-type Ca^{2+} channel current · Heart failure with preserved ejection fraction

Abstract

Objectives: The aim was to investigate the effects of valsartan on the sarcoplasmic reticulum Ca^{2+} -ATPase pump (SERCA) and L-type Ca^{2+} channel current (I_{CaL}) of the left ventricular myocardium in rats with heart failure with preserved ejection fraction. **Methods:** The 30-week-old male spontaneously hypertensive rats (SHRs) are randomly divided into the non-Valsartan and Valsartan groups, and the 30-week-old male Wistar-Kyoto rats served as control rats. The expression of SERCA is measured by Western blot. The I_{CaL} is measured by whole-cell patch clamp. The left ventricular end-diastolic pressure and left ventricular relaxation time constant quantity are measured at the same time. **Results:** The left ventricular end-diastolic pressure

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is much higher in SHRs compared with that in control rats ($p < 0.01$). The left ventricular relaxation time constant quantity is markedly extended in SHRs compared with control rats ($p < 0.01$). Valsartan cannot increase the expression of SERCA nor decrease the density of I_{CaL} compared with the non-Valsartan group ($p > 0.05$). **Conclusions:** Valsartan has no effect on SERCA and I_{CaL} of the left ventricular myocardium in rats with heart failure with preserved ejection fraction.

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Introduction

The incidence of heart failure with preserved ejection fraction (HFPEF) had rapidly increased during the past decades and is becoming the dominant form of heart failure [1]. Chronic hypertension is the most common cause besides age, and it is suggested that up to 60% of patients with HFPEF are hypertensive [2, 3]. In HFPEF, left ventricular (LV) cavity dimensions are small, especially in the presence of LV hypertrophy [4]. Treatment of hypertension remains one of the most important factors in the management of diastolic dysfunction [5, 6]. The angiotensin type 1 receptor antagonist (ARB) is one of the major treatments of hypertension; at the same time, inhibition of the renin-angiotensin system with ARB induces regression of LV hypertrophy [7]. Studies of hypertensive subjects indicate that diastolic dysfunction improves with LV hypertrophy regression [8]. Although the association of renin-angiotensin system with many of the underlying processes behind HFPEF is well known, various studies involving ARB use in HFPEF did not demonstrate significant benefits [9]. What are the underlying mechanisms?

Within the cardiomyocyte, Ca²⁺ is the main mediator of contraction and relaxation after electric activation. Contraction is initiated by Ca²⁺ release from the sarcoplasmic reticulum (SR), triggered by Ca²⁺ influx through the sarcolemmal L-type Ca²⁺ channel. Simultaneous Ca²⁺-dependent activation of the myofilaments induces cell shortening. Ca²⁺ is then removed from the cytosol, mainly by the ATP-dependent SR Ca²⁺ pump (SERCA), to allow cell relaxation. Intracellular dyssynchrony of Ca²⁺ decay is increased in animal LV hypertrophy models and in human heart failure. Dyssynchronous diastolic Ca²⁺ decay within individual cardiomyocytes contributes to dyssynchronous intracellular sarcomere relengthening. It is conceivable that the subcellular synchrony of cytosolic Ca²⁺ decay and sarcomere relengthening determine the efficacy of cardiomyocyte relaxation [10]. The SERCA is the main mediator of Ca²⁺ decay. The activity and expression of SERCA decreases in HFPEF, which represents a potential new mechanism underlying diastolic dysfunction [11].

We have previously demonstrated that the angiotensin type 1 receptor antagonist valsartan decreases the density of L-type Ca²⁺ channel current (I_{CaL}) of the LV hypertrophy in adult spontaneously hypertensive rats (SHRs) that is mediated in part by regression of LV hypertrophy [12]. In the present study, we aim to determine the effect of Valsartan on the I_{CaL} and SERCA of LV myocardium in HFPEF rats.

Methods

Experimental Animals

All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and conformed to the Animal Regulations of Guangdong Province. All experiments were performed

with the approval of the President of Sun Yat-sen University of Medical Sciences. SHRs and Wistar-Kyoto male rats were purchased from Vital River Experimental Animal Technology (Beijing, China). The 30-week-old male SHRs were randomly divided into the non-Valsartan group and the Valsartan group. The 30-week-old male Wistar-Kyoto rats served as the control group. The Valsartan group received Valsartan (gift from Beijing Novis Pharmaceutical Co., Ltd.) 20 mg/kg/day orally for 4 weeks. The control and non-Valsartan groups received saline (0.9%) orally for 4 weeks. Rats were fed at the Sun Yat-sen University of Medical Sciences Animal Center.

Hemodynamic Measurements

Rats were anesthetized with urethane (120 mg/100 g body weight) via intraperitoneal injection. The 4- to 5-cm incision was made along the midline between the neck and the upper sternal border. The right common carotid artery was found by separating subcutaneous tissue. A Millar catheter was inserted in the right common carotid artery and connected to a 16-channel physiological recorder. LV systolic pressure, maximal rising rate of ventricular pressure (+dp/dt_{max}), LV end-diastolic pressure, maximal falling rate of ventricular pressure (-dp/dt_{max}), and LV relaxation time constant quantity (T) were measured.

Measurement of Blood Pressure

The tail artery systolic pressure was measured using an RBP-1 rat tail blood pressure meter (obtained from the China-Japan Friendship Hospital) during awake and quiet conditions. Measurements were repeated three times, and the mean of three measurements was recorded.

Measurement of LV Mass Index

The rats were killed and their hearts removed. Total heart mass and LV mass were recorded. The ratio of LV mass to body mass was used to calculate the LV mass index (LVMI; in mg · g⁻¹).

Isolation of Ventricular Myocytes

Each heart was quickly excised and mounted on a Langendorff apparatus; LV myocytes were isolated according to the method described by Isenberg and Klöckner [13]. The aorta was retrogradely cannulated and perfused with nominally Ca²⁺-free modified Tyrode's solution at 37°C for 5 min. Perfusion pressure was 75 mm Hg, and all solutions were equilibrated with 100% oxygen. Perfusion was continued for another 15 min with 20 ml of the same solution plus collagenase (type CLS II, 200 U/ml; Biochrom KG, Berlin, Germany) and protease (type XIV, 0.7 U/ml; Sigma, USA), and the solution was recirculated. Finally, the heart was perfused with modified Tyrode's solution containing 100 μM Ca²⁺ for another 5 min.

After perfusion, the LV free wall was separated from the rest of the heart. Epicardial tissue pieces were carefully dissected from the LV free wall with fine forceps, and the pieces were placed in cups. To further disaggregate the tissue pieces, they were gently shaken at 37°C, filtered through cotton mesh, and allowed to settle for 30 min. Cells were stored at room temperature in modified Tyrode's solution containing 100 μM Ca²⁺. Only single rod-shaped cells with clear cross-striations and no spontaneous contraction were used for experiments.

Electrophysiological Recordings

Whole-cell currents were recorded using an Axopatch 200A amplifier (Axon Instruments, Foster City, Calif., USA). Cell capacitance (C_m, pF) was calculated by integrating the area under an uncompensated capacity transient elicited by a 10-mV depolarizing pulse from a holding potential of 80 mV. Whole-cell currents were low-pass filtered at 1 kHz and digitized

at 5 kHz via a Digidata 1200 A/D converter (Axon Instruments) interface for off-line analysis. Data were analyzed using custom-written software.

I_{CaL} was measured at 21°C in an extracellular solution containing (in mmol/l): TEA-Cl 50.0, MgCl₂ 6, H₂O 0.5, CaCl₂ 1.8, 4AP 3.0, HEPES 5.0, pH 7.4. The intracellular solution contained (in mmol/l): CsCl 100.0, TEA-Cl 20.0, Na₂-ATP 5.0, HEPES 10.0, EGTA 10.0, pH 7.2. I_{CaL} was elicited from a holding potential of -80 mV by voltage steps of 300 ms from -80 to 50 in 10-mV increments at 0.2 Hz.

Western Blot

Protein extracts were obtained by disrupting the tissue in lysis buffer. Supernatants containing cytoplasmic proteins were collected and the protein concentration determined by the BCA method (Protein Assay Kit, Keygen Biotec). For each sample, 50 µg total protein was subjected to SDS-PAGE on an 8% polyacrylamide gel. Separated proteins were electrophoretically transferred onto polyvinylidene difluoride membranes for immunodetection. After a blocking step with 5% non-fat milk in 0.02 M Tris, 0.05% Tween 20, membranes were incubated with primary antibodies (diluted in blocking buffer) followed by horseradish peroxidase-conjugated appropriated secondary antibodies. Antigens were visualized by ECL. The bands on the film were quantified and normalized to α-tubulin. Antibody to SERCA was purchased from Beijing Solarbio Science & Technology Co., Ltd.

Statistics

Results are expressed as mean ± SD. Statistical analyses were performed using SPSS 10.0 (SPSS, Chicago, Ill., USA). Differences between the mean values of multiple subgroups were evaluated by ANOVA, and intergroup comparisons were performed using t tests with ANOVA (Bonferroni method). Statistical significance was accepted at p < 0.05.

Results

Comparison of LVMI and Hemodynamic Characteristics

The LV end-diastolic pressure, maximal falling rate of ventricular pressure and LV relaxation time constant quantity are significantly higher in SHRs than in control rats, which indicates that a model of HFPEF has been established. The systolic blood pressure is significantly higher in the non-Valsartan and Valsartan groups compared with the control group (p < 0.01). The systolic blood pressure is significantly lower in the Valsartan group compared with the non-Valsartan group (p < 0.05). The LVMI is significantly higher in the non-Valsartan and Valsartan groups compared with the control group (p < 0.01). The LV end-diastolic pressure, maximal falling rate of ventricular pressure and LV relaxation time constant quantity are significantly higher in the non-Valsartan and Valsartan groups compared with the control group (p < 0.01). The LVMI, LV end-diastolic pressure, maximal falling rate of ventricular pressure and LV relaxation time constant quantity of the Valsartan group are similar to those of the non-Valsartan group (p > 0.05) (table 1).

Ionic Channels in the LV Myocardium

The membrane capacitance of the non-Valsartan and Valsartan groups is significantly larger compared with that of the control group (p < 0.01). In addition, the membrane capacitance of the Valsartan group is close to that of the non-Valsartan group (p > 0.05). The density of I_{CaL} in the non-Valsartan and Valsartan groups is higher compared with that in the control group (p < 0.05). The density of I_{CaL} in the Valsartan group is similar to that in the control group (p > 0.05) (table 2; fig. 1).

Table 1. Comparison of LVMI and hemodynamic characteristics between groups (n = 5)

Parameters	30-week-old rats		34-week-old rats		
	control	HFPEF	control	HFPEF non-Valsartan	Valsartan
LVMI, mg/g	–	–	2.8±0.5	4.4±0.3*	4.3±0.5*
SBP, mm Hg	91±1.2	165±1.1*	101±1.8	170.4±1.1*	160±1.3*,#
+dp/dt _{max} , mm Hg/s	6,151±311.7	8,175±378.6*	6,178±289.2	8,375±367.6*	8,275±357.6*
LVSP, mm Hg	153.6±4.9	186.4±9.4*	157.2±3.2	197.4±7.4*	194.4±6.4*
–dp/dt _{max} , mm Hg/s	7,170±269.1	9,981±262.2*	7,870±239.3	10,815±298.1*	9,995±308.1*
LVEDP, mm Hg	3.2±0.5	9.9±1.0*	3.4±0.4	14.4±1.2*	10.1±1.5*
T, ms	626±31.1	895.8±33.2*	628±25.1	925.7±30.2*	919.8±28.4*

SBP = Systolic blood pressure; +dp/dt_{max} = maximal rising rate of ventricular pressure; LVSP = LV systolic pressure; –dp/dt_{max} = maximal falling rate of ventricular pressure; LVEDP = LV end-diastolic pressure; T = LV relaxation time constant quantity. * p < 0.01 compared with control rats, # p < 0.05 compared with non-Valsartan rats.

Table 2. Comparison of the I_{CaL} in the LV myocardium between the 3 groups (n^a = 4/n^b = 5)

Variables	Control rats	HFPEF rats	
		non-Valsartan	Valsartan
C _m , pF	207.67±25.86	335.70±24.23 ^c	331.50±23.60 ^c
I _{CaL} , pA/pF	–3.34±0.42	–5.30±0.39 ^c	–5.07±0.36 ^c

C_m = Cell capacitance.

^a Number of myocytes. ^b Number of animals. ^c Compared with the control group, p < 0.01.

Comparison of SERCA Expression in the LV Myocardium

The SERCA expression of the non-Valsartan and Valsartan groups is significantly lower compared with that of the control group (p < 0.01). The SERCA expression of the Valsartan group is close to that of the non-Valsartan group (p > 0.05) (fig. 2).

Discussion

The main findings of this study include the following: (1) valsartan cannot increase the expression of SERCA, which is significantly lower compared with that in the control group; (2) valsartan has no effect on the density of I_{CaL}, which is significantly higher compared with that in the control group, and (3) valsartan treatment has no effect on the regression of LV hypertrophy by decreasing systolic blood pressure.

Diastolic dysfunction is characterized by slow or incomplete relaxation of the ventricles during diastole, which is a key pathogenic feature of HFPEF [14]. Calcium mishandling is a hallmark of HFPEF [11]. During a cardiac action potential, Ca²⁺ is released from the SR, contributing approximately 70% to the cytosolic Ca²⁺ transient. The SR Ca²⁺ release is triggered by Ca²⁺ influx through the L-type Ca²⁺ channel, which contributes the remaining 10–30% to the Ca²⁺ transient. During diastole, Ca²⁺ is taken back up into the SR by the SR Ca²⁺

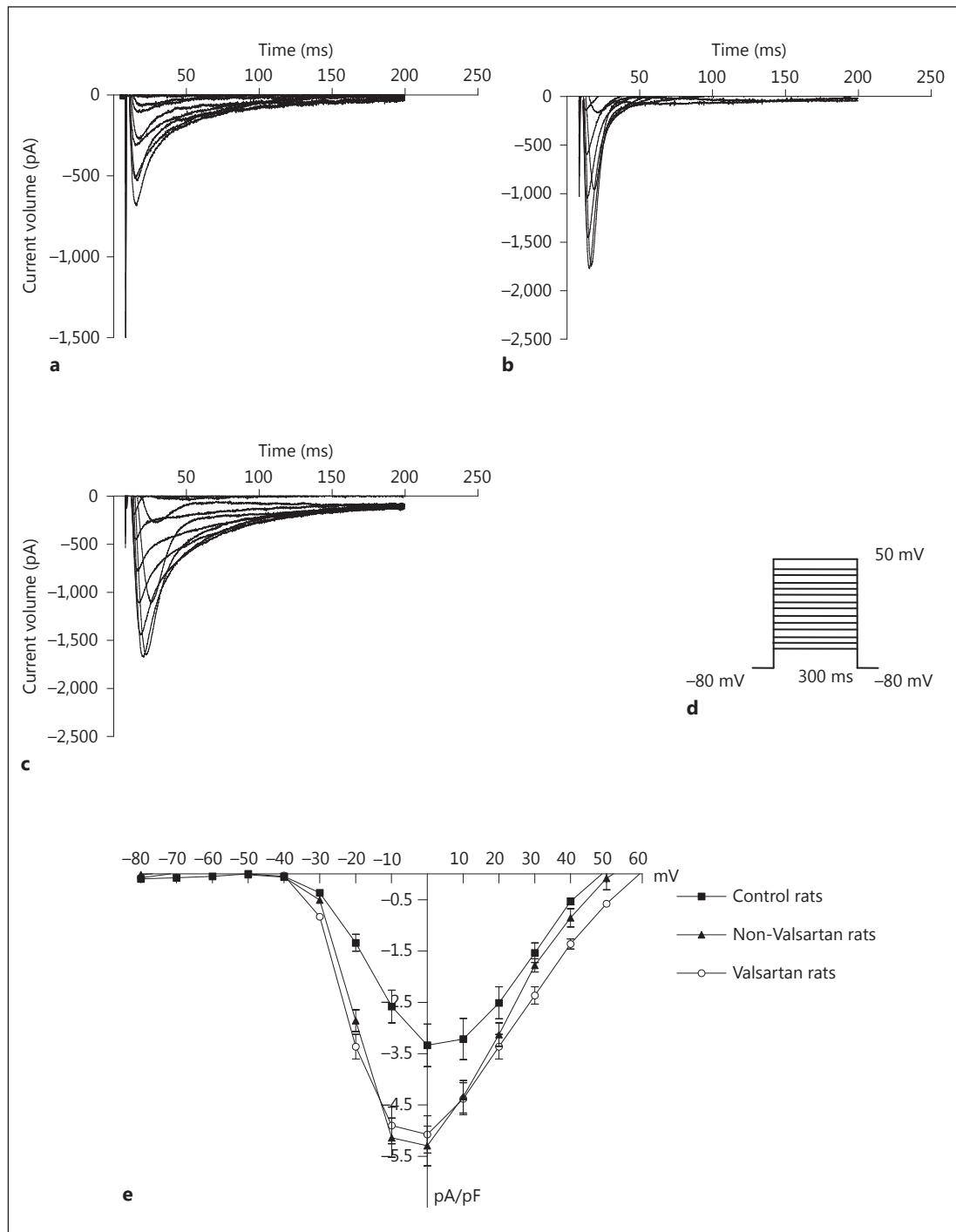


Fig. 1. Effect of valsartan treatment on I_{CaL} . Typical recordings of I_{CaL} in cells from the control (a), non-Valsartan (b) and Valsartan (c) groups. d The voltage clamp protocol. e Average I-V relationships of I_{CaL} density (in pA/pF) as a function of step potential (in mV), obtained in the control (■), non-Valsartan (▲) and Valsartan (○) groups.

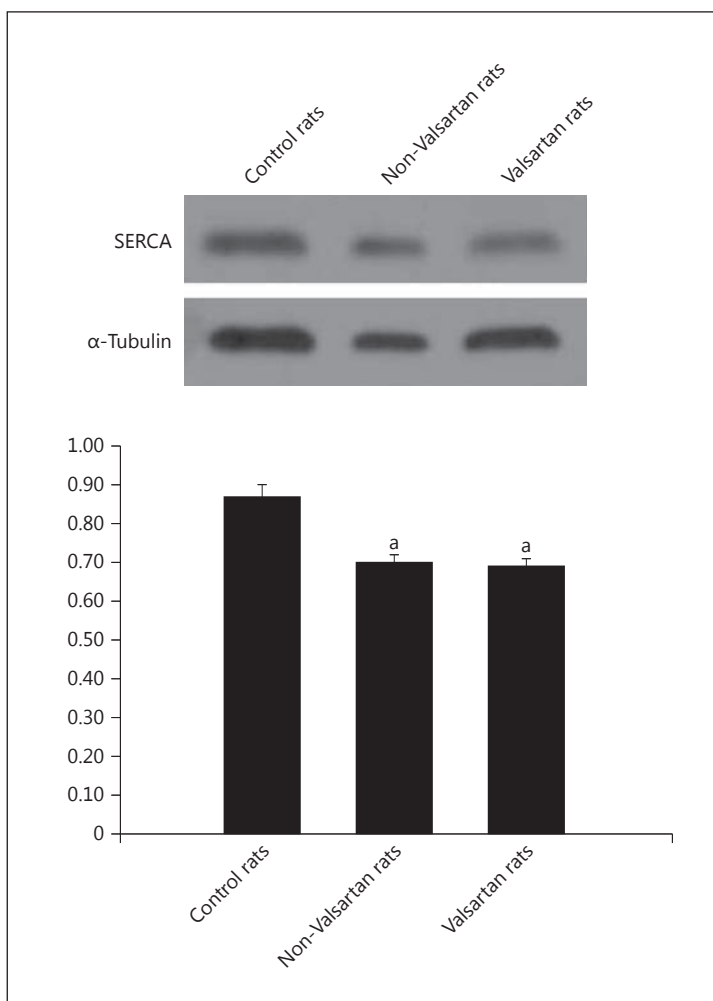


Fig. 2. Alternation of SERCA pumps in non-Valsartan, Valsartan and control rats. The top panel shows Western blot analysis of the SERCA expression in the non-Valsartan, Valsartan and control rats; the bottom panel shows quantification of SERCA from the blot. Each bar graph is a representative of 5 independent experiments. Values represent mean \pm SEM. ^a $p < 0.05$: compared with control rats.

ATPase to allow cell relaxation [15]. Defects in any of these mechanisms driving the Ca²⁺ flux can lead to profound cardiac dysfunction. In chronic heart failure, the underlying primary defect is decreased activity and expression of SR Ca²⁺ ATPase, which decrease the rate of cytosolic Ca²⁺ removal [10]. The cytosolic Ca²⁺ removal is the initiating step for relaxation, and the rate of cytosolic Ca²⁺ removal does influence relaxation kinetics in isolated cells [16], multicellular preparations [17, 18] as well as in vivo [19]. Reduced Ca²⁺ reuptake into the SR is a hallmark of the failing heart and an important contributor to Ca²⁺ mishandling and slow relaxation in diastolic dysfunction. Therefore, SERCA is an attractive therapeutic target [20]. In this regard, one approach could be restoring SR Ca²⁺ATPase expression. Our study indicates that the expression of SERCA significantly decreases in HFPEF rats, which is similar to the result of other researches [20, 21]. Four-week treatment with valsartan cannot increase the expression of SERCA, which may be one of the underlying mechanisms to understand the lack of efficacy of ARB in the treatment of HFPEF [22, 23].

In diastolic dysfunction, there may be an increase in sarcomere Ca²⁺ sensitivity and a decrease in the rate of Ca²⁺ reuptake via SERCA, all leading to a state of Ca²⁺ overload [24]. The Ca²⁺/calmodulin-dependent phosphatase calcineurin is initiated by Ca²⁺ overload and induces the hypertrophy of the myocardium [25]. Chronically, increased diastolic Ca²⁺ also activates cell death pathways and alters the metabolic profile, which leads to a decrease in

ATP production and an increase in the reactive oxygen species [26]. SERCA is sensitive to mitochondrial ATP production, and the organization of mitochondria and SR may directly influence the rate of SR Ca²⁺ reuptake, so the activity of SERCA decreases in a state of Ca²⁺ overload, which reduces the rate of cytosolic Ca²⁺ removal and deteriorates the state of Ca²⁺ overload [27, 28]. The reactive oxygen species promote the myocardial hypertrophy by the signal transduction pathway of Ca²⁺/calmodulin-dependent phosphatase calcineurin. Taken together, a vicious cycle is established among Ca²⁺ overload, insufficient energy and oxidative stress in heart failure. The hypertrophic growth of the myocardium is typically initiated by signal transduction pathways in response to increased mechanical load on the heart or through the action of neurohumoral mediators. Numerous signaling pathways coordinate the cardiac hypertrophic response. Among them, the Ca²⁺/calmodulin-dependent phosphatase calcineurin is considered as a central prohypertrophic signaling effector in the myocardium [25]. Valsartan has no effect on the expression of SERCA and the density of I_{CaL} in HFPEF rats, which indicates that valsartan has no effect on the state of Ca²⁺ overload; thus, it is easy to understand the lack of efficacy of valsartan in the regression of myocardial hypertrophy.

In conclusion, we do not find evidence that valsartan improves the expression of SERCA, the regression of myocardial hypertrophy and the density of I_{CaL} in HFPEF rats. This finding is consistent with the lack of efficacy of renin-angiotensin system inhibitors in the treatment of HFPEF [22, 23].

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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