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ORIGINAL ARTICLE

Valsartan improves the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rats

Huang Zhi-Bin[1], Fang Chang[2], Lin Mao-Huan[2], Yuan Gui-Yi[2], Zhou Shu-Xian[2] and Wu Wei[3]

The objective was to investigate the effects of valsartan on the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rats (SHR). A total of 24 10-week-old male SHR were divided into two groups: valsartan and non-valsartan groups ($n=12$ in each). Twelve 10-week-old Wistar-Kyoto rats were served as the control group. Kv4.2 expression was measured in left ventricular myocardium using western blots. In addition, the systolic blood pressure, left ventricular mass index (LVMI), ventricular effective refractory period and ventricular fibrillation threshold (VFT) were also measured after eight weeks. I_{Na}, I_{CaL}, I_{to} and membrane capacitance were measured in left ventricular myocytes after 8 weeks by whole-cell patch clamp. Valsartan decreased LVMI compared with the non-Valsartan group (Valsartan *vs.* non-Valsartan: $3.2\pm0.03\,mg\,g^{-1}$ *vs.* $3.7\pm0.02\,mg\,g^{-1}$, $P<0.01$). Valsartan also enhanced the VFT compared with the non-Valsartan group (Valsartan *vs.* non-Valsartan: 18.6 ± 0.3 mA *vs.* 15.4 ± 0.4 mA, $P<0.01$). The expression of Kv4.2 was significantly lower in the non-Valsartan and Valsartan groups compared with the control group ($P<0.01$). The expression of Kv4.2 was significantly higher in the Valsartan group compared with the non-Valsartan group ($P<0.01$). Valsartan decreased the density of I_{CaL} compared with non-Valsartan group (Valsartan *vs.* non-Valsartan: -5.5 ± 0.6 pA/pF *vs.* -7.2 ± 0.9 pA/pF, $P<0.05$). Valsartan improved the density of I_{to} compared with non-Valsartan group(Valsartan *vs.* non-Valsartan: 13.93 ± 0.8 pA/pF *vs.* 11.22 ± 1.0 pA/pF, $P<0.05$). Valsartan improves the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rat.

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Keywords: electrophysiology; left ventricular hypertrophy; valsartan

INTRODUCTION

Studies have suggested that 60% of sufferers of high blood pressure also suffer from left ventricular hypertrophy (LVH).[1] Epidemiologic research suggests that LVH is an independent risk factor for sudden cardiac death (SCD).[2] The occurrence of SCD in patients with LVH is higher than in patients without LVH. The relationship between LVH caused by hypertension and SCD has been a major focus of research in recent years. The main pathophysiologic mechanism of SCD is the malignant ventricular arrhythmias, which occur in patients with LVH.[3] Angiotensin receptor blocker (ARB) has been widely used in clinical medicine in the treatment of hypertension and regression of LVH. ARB improves the prognosis of patients with LVH by decreasing the rates of SCD and heart failure.[4,5] Fluid excretion, reduction of catecholamines, diminution of peripheral vascular resistance and regression of LVH, which ameliorates abnormal electrophysiological characteristics, are commonly believed to be responsible for the antiarrhythmic action of ARB.[6]

Apart from these potential mechanisms, little is known about which membrane current components, ventricular effective refractory period (VERP) and ventricular fibrillation threshold (VFT), if any, may be influenced by ARB and whether these changes may contribute to the antiarrhythmic effect. Thus, in this study, we explored the effect of valsartan on the electrophysiological characteristics of left ventricular hypertrophied myocardium in spontaneously hypertensive 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. Spontaneously hypertensive rats and Wistar-Kyoto male rats aged 10 weeks (weight; ~200 g) were purchased from Vital River Experimental Animal Technology (Beijing, China). A total of 24 10-week-old male SHRs were randomly divided into the non-Valsartan

[1]Department of Cardiology, The First Affiliated Hospital, Sun Yat-sen University of Medical Sciences, Guangzhou, China; [2]Department of Cardiology, The Sun Yat-sen Memorial Hospital, Sun Yat-sen University of Medical Sciences, Guangzhou, China and [3]Department of Cardiology, The Fifth Affiliated Hospital, Sun Yat-sen University of Medical Sciences, Zhuhai, China
Correspondence: Professor W Wei, Department of Cardiology, The Fifth Affiliated Hospital, Sun Yat-sen University of Medical Sciences, Zhuhai 519000, China.
E-mail: wuwei2603@163.com
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Table 1 Comparison of VERP and VFT between the 3 groups

Group	n	*VERP (ms)*	*VFT (mA)*
Control	8	61.3 ± 0.8	24.3 ± 0.5
non-Valsartan	8	62.3 ± 0.6	15.4 ± 0.4[a]
Valsartan	8	61.5 ± 0.4	18.6 ± 0.3[a,b]

Abbreviations: VERP, ventricular effective refractory period; VFT, ventricular fibrillation threshold.
[a]Compared with the control group, $P<0.01$.
[b]Compared with the non-Valsartan group, $P<0.01$.

group and Valsartan group ($n=12$). A total of 12 10-week-old male Wistar Kyoto rats served as the control group. The Valsartan group received Valsartan (gift from Beijing Novis Pharmaceutical) 5 mg kg^{-1} per day orally for 8 weeks. The control and non-Valsartan groups received saline (0.9%) orally for 8 weeks. Rats were fed at the Sun Yat-sen University of Medical Sciences Animal Center.

Measurement of blood pressure

The tail artery systolic pressure was measured using a RBP-1 rat tail blood pressure meter (obtained from the China-Japan Friendship Hospital) during

Table 2 Comparison of the ionic channels in the left ventricular myocardium between the three groups

Group	n	C_m *(pF)*	I_{CaL} *(pA/pF)*	I_{Na} *(pA/pF)*	I_{to} *(pA/pF)*
Control	8	125.4 ± 3.3	−5.5 ± 0.6	−15.3 ± 2.0	16.12 ± 0.7
non-Valsartan	8	272.0 ± 8.2[a]	−7.2 ± 0.9[a]	−16.1 ± 1.1	11.22 ± 1.0[a]
Valsartan	8	201.8 ± 7.1[a,b]	−4.9 ± 0.3[b]	−16.7 ± 0.5	13.9 ± 30.8[a,b]

[a]Compared with the control group, $P<0.01$.
[b]Compared with the non-Valsartan group, $P<0.01$.

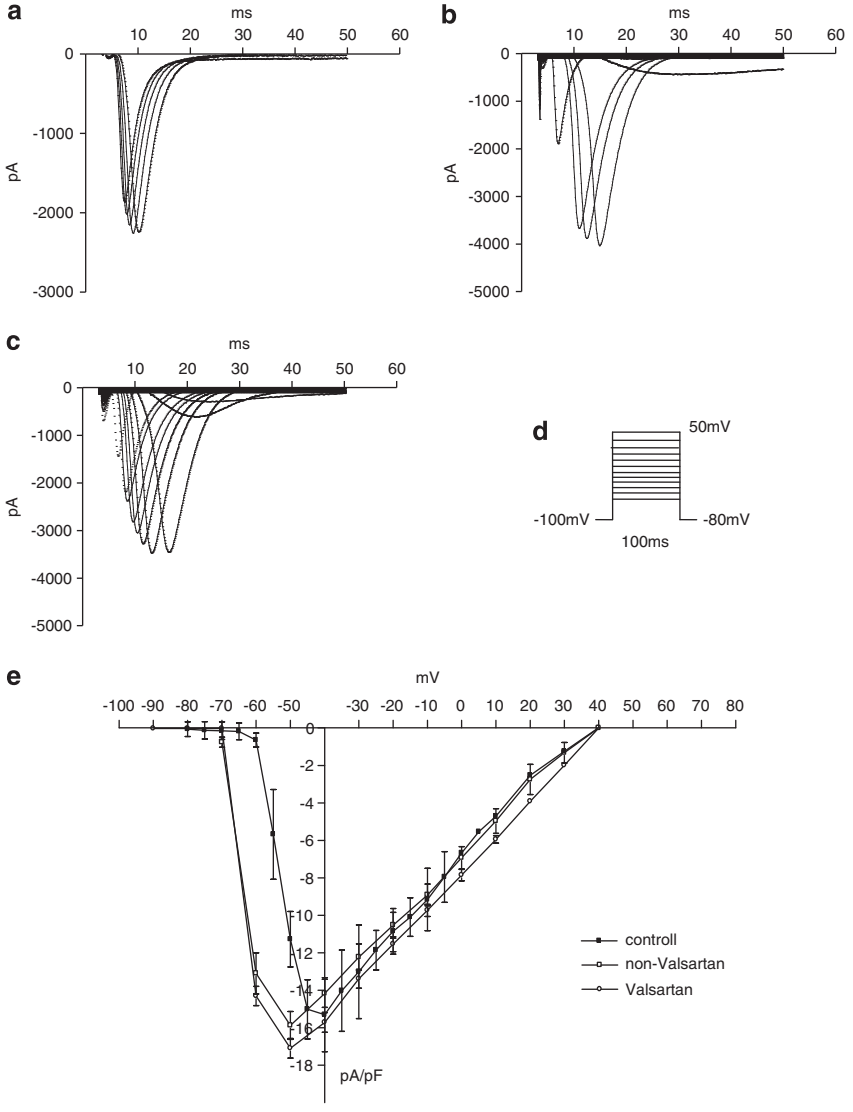

Figure 1 Effect of valsartan treatment on sodium current (I_{Na}). Typical recordings of I_{Na} in cells from control (**a**) non-valsartan (**b**) and valsartan (**c**). *x*-axis: time (ms: mini-second) ; *y*-axis: current volume (pA). (**e**): average I–V relationships of I_{Na} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), non-valsartan (□) and valsartan (○). The voltage clamp protocol is shown in (**d**).

awake and quiet conditions. Measurements were repeated three times, and the mean of three measurements was recorded.

Measurement of VERP and VFT

Rats were anesthetized with urethane (120 mg per 100 g body weight) via intraperitoneal injection. A tracheostomy was then performed, and the rat was placed on a servo-controlled heating table to maintain body temperature at 37 °C. The rat was connected to and ventilated by a small animal ventilator at a tidal volume of 1.7–2.5 ml, depending on body weight, and at a frequency of 60 breaths min^{-1}. Electrocardiogram signals were amplified and recorded on a multiple channel physiological recorder. After thoracotomy, two fishhook-like electrodes were placed in the apex of the left ventricle and connected to a program stimulator (type 5352, Medtronic Company, Louisville, CO, USA), isolation stimulator (type DSJ731-G-A) and a physiological stimulator (type DSJ731-2C-A).

The VERP was measured using extra stimuli delivered in 10 ms decrements (S_1S_2). The VERP was the longest S_1S_2 interval that failed to cause ventricular depolarization.

The heart was paced by a pacemaker at 500 b.p.m. Ventricular fibrillation was invoked by ultrarapid strand stimulation (10 stimuli, pulse width, 4 ms, 100 Hz; delay, 60 ms). The initial current intensity was 5 mA. The current was increased in 0.5 mA increments. The VFT was recorded as the lowest current intensity invoking ventricular fibrillation.

Measurement of left ventricular mass index

After VFT testing, the rats were killed and their hearts removed. Total heart mass and left ventricular mass were recorded. The ratio of left ventricular mass to body mass was used to calculate the left ventricular mass index ($mg\,g^{-1}$).

Isolation of ventricular myocytes

Isolated myocytes were obtained from the hearts of spontaneously hypertensive rats and Wistar-Kyoto rats by enzymatic dissociation. Briefly, hearts were quickly excised from anesthetized rats. The left ventricular apex was removed for protein studies and the ventriculotomy was closed. The heart was then mounted on a Langendorff apparatus and perfused with a Ca^{2+}-free solution composed of (in $mmol\,l^{-1}$) 136 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 1.0 $MgCl_2 \cdot 6H_2O$, 10.0 HEPES, 10 glucose, adjusted to pH 7.4 by NaOH, followed by Ca^{2+}-free solution with collagenase ($0.8\,mg\,ml^{-1}$, type II, Worthington Biochemical, Freehold, NJ, USA) and bovine serum albumin ($1\,mg\,ml^{-1}$). All perfusates were bubbled with 95% O_2 and 5% CO_2 and maintained at 37 °C and the flow rate was adjusted to maintain a perfusion pressure of 75 mm Hg. Cells were then filtered through a nylon mesh and stored at room temperature in the Krebs Buffer solution composed of (in $mmol\,l^{-1}$) 50 K-glutamate, 20 KOH, 40 KCl, 20 Taurine, 20 KH_2PO_4, 3.0 $MgCl_2$ $6H_2O$, 0.5 EGTA, 10.0 HEPES, 10 glucose, adjusted to pH 7.4 by KOH. Only rod-shaped cells with clear cross striations and without spontaneous contractions or granulations were selected for experiments.

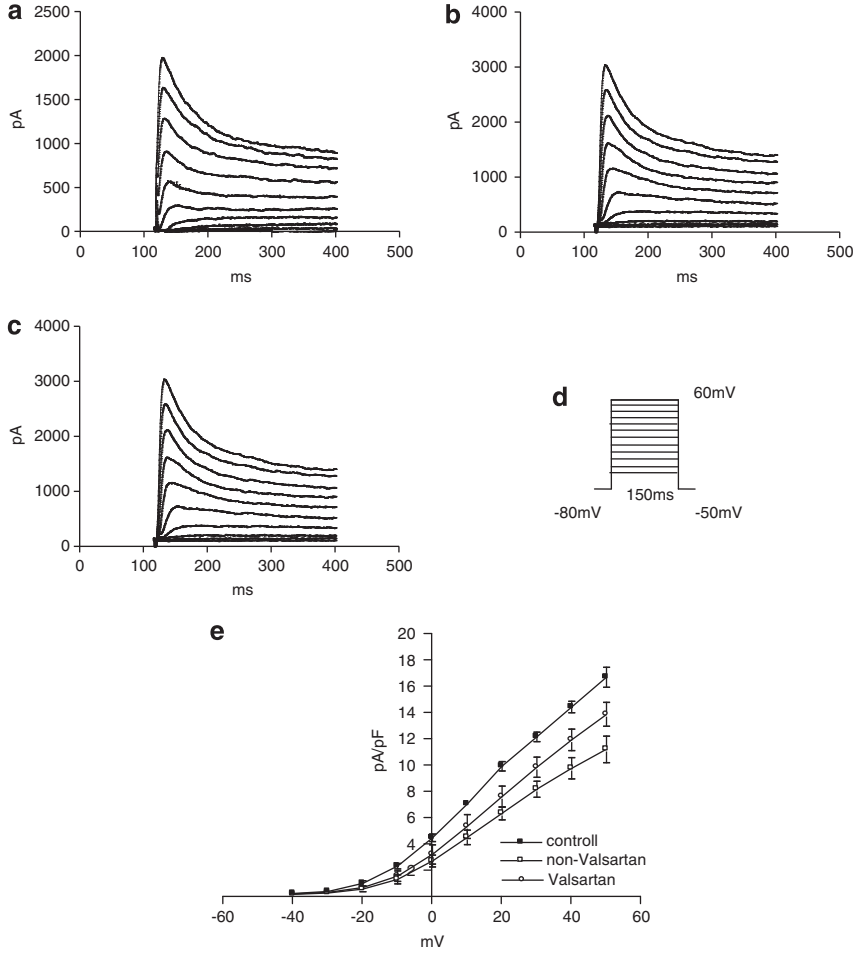

Figure 2 Effect of valsartan treatment on transient outward current. Typical recordings of I_{to} in cells from control (**a**) non-valsartan (**b**) and valsartan (**c**). The bath solution contained 0.3 mM Cd^{2+} to inhibit Ca^{2+} currents. *x*-axis: time (ms: mini-second) ; *y*-axis: current volume (pA). (**e**): average I–V relationships of I_{to} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), non-valsartan (□) and valsartan (○). The voltage clamp protocol is shown in (**d**).

Electrophysiological recordings

Whole-cell currents were recorded using an Axopatch 200 A amplifier (Axon Instruments, Foster City, CA, USA). Cell capacitance (C_m, pF) was calculated by integrating the area under an uncompensated capacity transiently 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 offline analysis. Data were analyzed using custom-written software.

I_{Na} was measured at 21 °C in an extracellular solution containing (in mmol l^{-1}): NaCl 5.0, Choline-Cl 130.0, CsC l5.4, HEPES 10.0, $MgCl_2$ $6H_2O$ 1.0, NaH_2PO_4 5.0, $CaCl_2$ 1.0, Glucose H_2O 10, Nicardipine 0.001, at pH 7.4. The intracellular solution contained (in mmol l^{-1}): CsC 120.0, CsF 110.0, NaCl 5.0, HEPES 5.0, EGTA 5.0, $MgCl_2$ $6H_2O$ 1.0, Na_2-ATP 5.0, at pH 7.2. I_{Na} was elicited from a holding potential of −100 mV by voltage steps of 100 ms from −80 mV to 50 mV in 10 mV increments at 0.5 Hz.

I_{CaL} was measured at 21 °C in an extracellular solution containing (in mmol l^{-1}): TEA-Cl 50.0, $MgCl_2$ $6H_2O$ 0.5, $CaCl_2$ 1.8, 4AP 3.0, HEPES 5.0, pH 7.4. The intracellular solution contained (in mmol l^{-1}): CsCl l00.0, TEA-Cl 20.0, Na_2-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 mV to 50 mV in 10 mV increments at 0.2 Hz.

I_{to} was measured at 21 °C in an extracellular solution containing (in mmol l^{-1}) NaCl 136, KCl 5.4, NaH_2PO_4 0.33, $MgCl_2$ $6H_2O$ 1.0, $CaCl_2$ 2, $BaCl_2$ 0.5, $CdCl_2$ 0.3, HEPES 10, and glucose 10, pH 7.4. The intracellular solution contained (in mmol l^{-1}) KCl 140, $MgCl_2$ 1, EGTA 5, HEPES 10 and Na_2-ATP 5, pH 7.2. I_{to} was elicited from a holding potential of −80 mV by voltage steps of 150 ms from −50 mV to 60 mV in 10 mV increments every 6 s. Standard pulse protocols were used to assay the biophysical properties of I_{to}.

Western blotting

Protein extracts were obtained by disrupting the tissue in lysis buffer. Supernatants containing cytoplasmic proteins were collected and the protein concentration determined by bicinchoninic acid assay method (Protein Assay Kit, Keygen Biotec, Nanjing, China). For each sample, 50 μg total protein was subjected to SDS-polyacrylamide gel electrophoresis on a 8% polyacylamide gel. Separated proteins were electrophoretically transferred onto polyvinylidene fluoride 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 enhanced chemiluminescence. The bands on the film were quantified

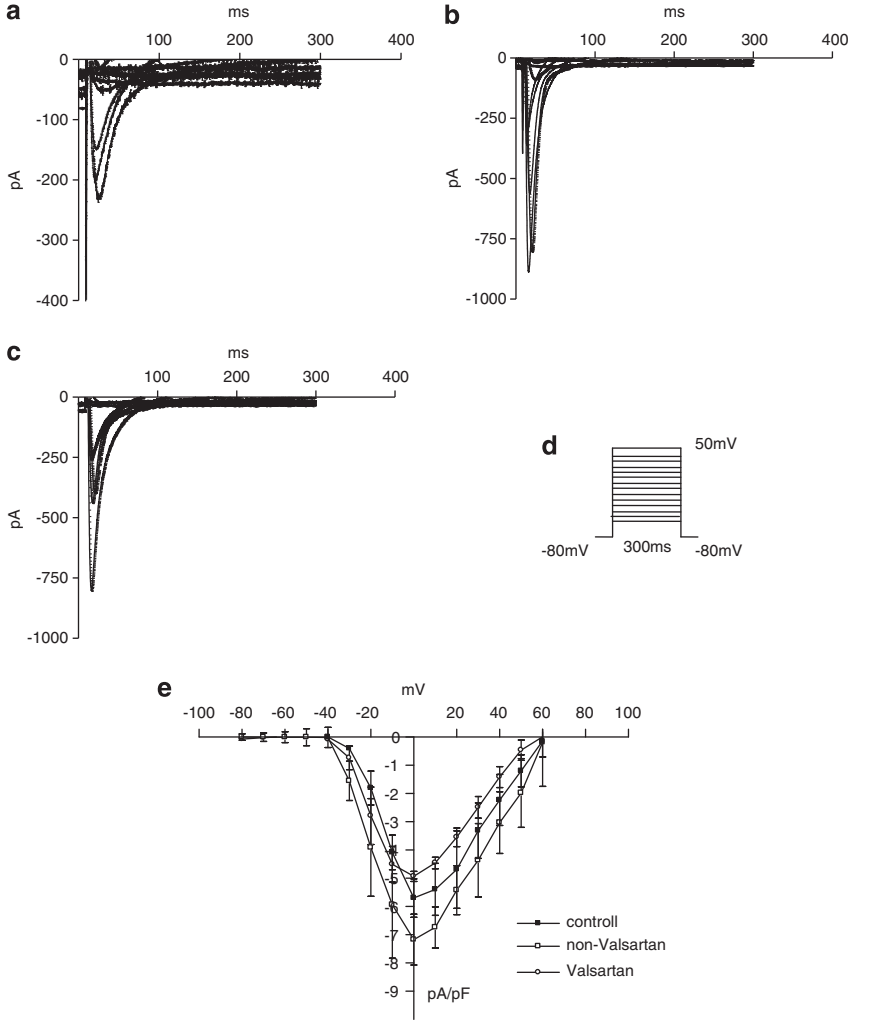

Figure 3 Effect of valsartan treatment on L-type calcium current (I_{CaL}). typical recordings of I_{CaL} in cells from control (**a**) non-valsartan (**b**) and valsartan (**c**). *x*-axis: time (ms: mini-second) ; *y*-axis: current volume (pA). (**e**): average I–V relationships of I_{CaL} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), non-valsartan (□) and valsartan (○). The voltage clamp protocol is shown in (**d**).

and normalized to α-tubulin. Antibody to Kv4.2 (07–491; rabbit) was purchased from Chemicon (Temecula, CA, USA).

Statistics

Results are expressed as mean ± s.d. Statistical analyses were performed using SPSS 10.0 (SPSS, Chicago, IL, USA). Differences between the mean values of multiple subgroups were evaluated by analysis of variance, and intergroup comparisons were performed using *t*-tests with analysis of variance (Bonferroni method). Statistical significance was accepted at $P<0.05$.

RESULTS

Comparison of systolic blood pressure and left ventricular mass index

The left ventricular mass index was significantly higher in the non-Valsartan and Valsartan groups compared with the control group ($3.7\pm0.02\,mg\,g^{-1}$ and $3.2\pm0.03\,mg\,g^{-1}$ *vs.* $2.5\pm0.03\,mg\,g^{-1}$, $P<0.01$). In addition, the left ventricular mass index was significantly higher in the non-Valsartan group compared with the Valsartan group ($3.7\pm0.02\,mg\,g^{-1}$ *vs.* $3.2\pm0.03\,mg\,g^{-1}$, $P<0.01$).

Comparison of VERP and VFT in rats

The VFT was significantly lower in the non-Valsartan and Valsartan groups compared with the control group (15.4 ± 0.4 mA and 18.6 ± 0.5 mA *vs.* 26.3 ± 0.5 mA, $P<0.01$). The VFT was significantly higher in the valsartan group compared with the non-Valsartan group (18.6 ± 0.5 mA *vs.* 15.4 ± 0.4 mA, $P<0.01$; Table 1).

Ionic channels in the left ventricular myocardium

The membrane capacitance of the non-Valsartan and Valsartan groups was significantly larger compared with the control group (273.0 ± 18.2 pF, 202.8 ± 7.1 pF *vs.* 124.4 ± 3.3 pF, $P<0.01$). In addition, the membrane capacitance of the Valsartan group was significantly lower compared with the non-Valsartan group (202.8 ± 7.1 pF *vs.* 273.0 ± 18.2 pF, $P<0.01$). The density of I_{CaL} in the non-Valsartan group was higher compared with the control and Valsartan groups (-7.1 ± 0.9 pA/pF *vs.* -5.6 ± 0.6 pA/pF, -4.8 ± 0.3 pA/pF, $P<0.05$). The density of I_{CaL} in the Valsartan group was similar to that in the control group (-5.6 ± 0.6 pA/pF *vs.* -4.8 ± 0.3 pA/pF, $P>0.05$). The density of I_{Na} was not significantly different among the three groups. Finally, the density of I_{to} in the non-Valsartan and Valsartan groups was significantly lower compared with the control group (11.23 ± 1.0 pA/pF, 13.91 ± 0.8 pA/pF *vs.* 16.1 ± 0.7 pA/pF, $P<0.05$). However, the density of I_{to} in the Valsartan group was significantly higher compared with the non-Valsartan group (13.91 ± 0.8 pA/pF *vs.* 11.23 ± 1.0 pA/pF, $P<0.05$; Table 2, Figures 1–3).

Comparison of Kv4.2 expression in the left ventricular myocardium

The Kv4.2 expression of the non-Valsartan and Valsartan groups was significantly lower compared with the control group ($P<0.01$). The Kv4.2 expression of the Valsartan group was significantly higher compared with the non-Valsartan group ($P<0.01$; Figure 4).

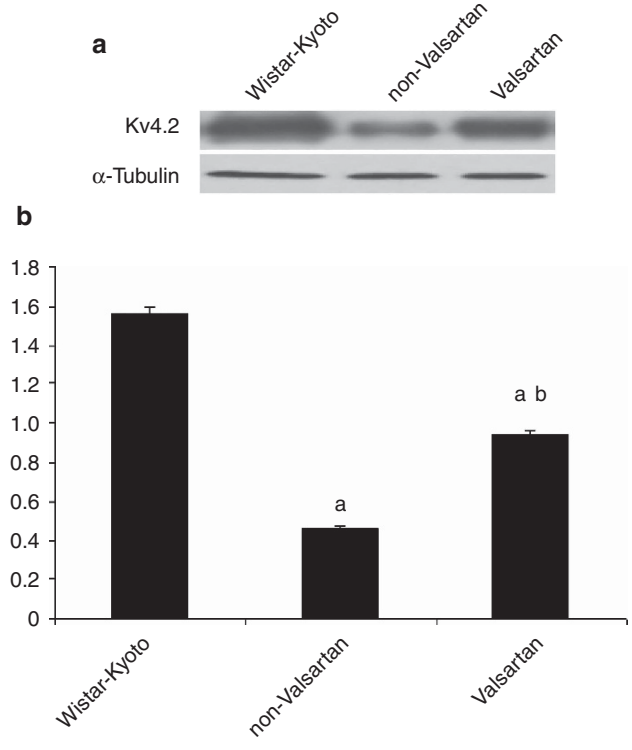

Figure 4 Kv4.2 expression in the three groups. (**a**) Compared with the control group, $P<0.01$. (**b**) Compared with the non-Valsartan group, $P<0.01$. There were eight samples per group.

DISCUSSION

LVH is a common cardiac complication in patients with hypertension.[1] Patients with LVH frequently have ventricular arrhythmia, especially malignant ventricular arrhythmia, which results in a high incidence of SCD. In the current study, valsartan improved hypertrophy of the left ventricle, similar to previous studies.[4] Previous data has shown that the electrophysiological stability of hypertrophic ventricular myocardium was improved by decreasing the hypertrophy of the left ventricle.[7,8] In addition, valsartan increased the VFT, suggesting that valsartan may decrease the incidence of malignant ventricular arrhythmias in hypertrophic left ventricles.

Angiotensin II[9,10] activates L-type Ca^{2+} channels and/or T-type Ca^{2+} channels, resulting in increases in $[Ca^{2+}]_i$ and calcineurin activity. Calcineurin is a Ca^{2+}-dependent protein phosphatase, and its activity is increased in pathological conditions, such as hypertrophy. NFAT3 is normally phosphorylated in the cytoplasm. When NFAT3 is dephosphorylated by calcineurin, it translocates into the nucleus. Kv4.2 has putative NFAT binding sites in promoter regions, and has been shown to be downregulated in an NFAT3-dependent mechanism.[11] In this study, Kv4.2 was downregulated in hypertrophic myocardium. Reduction of the transient outward potassium current (I_{to}) has been consistently observed in cardiac hypertrophy.[12–14] In hypertrophied myocytes, I_{to} was shown to be decreased secondary to reductions in the expression of Kv4.2 potassium channel genes.[15–17] The I_{to} was shown to be a major current contributing to early (phase 1) repolarization. By setting the membrane potential for the plateau phase of the cardiac action potential, I_{to} has an important influence on the electrical driving force for systolic Ca^{2+} entry into the cardiac myocyte and on the length of the action potential.[18] Impaired early repolarization is associated with desynchronized Ca^{2+} release from the sarcoplasmic reticulum,[19] action potential prolongation[20,21] and reduction of the transmural gradient of repolarization.[22–24] Downregulation of I_{to} density may therefore significantly contribute to the pathogenesis of excitation contraction abnormalities and cardiac arrhythmias. The expression of Kv4.2 is upregulated by valsartan in left hypertrophic ventricular myocardium, and we found in this study that the I_{to} density was increased in the Valsartan group. Speculatively, valsartan may have increased I_{to} density by causing over expression of Kv4.2. Studies

indicate that upregulation of I_{to} can improve the electrophysiological stability of hypertrophic ventricular myocardium.[17] The density of I_{CaL} was increased in hypertrophied myocytes, which resulted in increases in $[Ca^{2+}]_i$. Ca^{2+}-dependent signal pathways were likely activated, which led to downregulation of Kv4.2. Downregulation of I_{to} density has been shown before under such conditions.[25] The density of I_{CaL} was reduced by valsartan in hypertrophied myocytes, which may have led to decreases in $[Ca^{2+}]_i$ and weakening of the activity of Ca^{2+}-dependent signal pathways.

In summary, valsartan improved hypertrophy of the left ventricle, elevated the VFT, upregulated expression of Kv4.2, increased I_{to} density and downregulated I_{CaL} density in hypertrophied myocytes. Our findings indicate that valsartan improved the electrophysiological characteristics associated with hypertrophy of the left ventricle.

ACKNOWLEDGEMENTS

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