Accepted Manuscript

Role of angiotensin II type 2 receptor during electrophysiological remodeling of left ventricular hypertrophic myocardium in spontaneously hypertensive rats

Xiao Ying, Long Wei-Qing, Guan Kai-Pan, Long Ming, Lu Gui-Hua, Huang Zhi-Bin

PII: S1933-1711(17)30343-1

DOI: 10.1016/j.jash.2017.09.014

Reference: JASH 1087

To appear in: *Journal of the American Society of Hypertension*

Received Date: 29 April 2017

Revised Date: 29 August 2017

Accepted Date: 26 September 2017

Please cite this article as: Ying X, Wei-Qing L, Kai-Pan G, Ming L, Gui-Hua L, Zhi-Bin H, Role of angiotensin II type 2 receptor during electrophysiological remodeling of left ventricular hypertrophic myocardium in spontaneously hypertensive rats, *Journal of the American Society of Hypertension* (2017), doi: 10.1016/j.jash.2017.09.014.

Role of angiotensin II type 2 receptor during electrophysiological remodeling of left ventricular hypertrophic myocardium in spontaneously hypertensive rats

Xiao Ying[a*], Long Wei-Qing[b*], Guan Kai-Pan[c], Long Ming[d], Lu Gui-Hua[d], Huang Zhi-Bin[d]

[a]Department of Anesthesiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[b]Department of Clinical Laboratory, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[c]Department of Respiratory Intensive Care Unit, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[d]Department of Cardiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

Corresponding author: Huang zhibin, Add: No. 58, Zhongshan II Road, Guangzhou, Tel: +8620 87755766 8180, Fax: +8620 87334143, E-mail: hzbin2603@163.com.

Running Title: angiotensin II type 2 receptor and electrophysiology

*These authors contributed equally to this study.

Abstract

The objective was to investigate the role of angiotensin II type 2 receptor during electrophysiological remodeling of left ventricular hypertrophic myocardium in spontaneously hypertensive rats (SHR). A total of 36 10-week-old male SHR were divided into 3 groups: control, valsartan and valsartan+PD123319 groups (n=12 in each). The systolic blood pressure, left ventricular mass index (LVMI), ventricular effective refractory period, and ventricular fibrillation threshold (VFT) were also measured after eight weeks. At the same time, I_{Na}, I_{CaL}, I_{to} and membrane capacitance were measured in left ventricular myocytes by whole-cell patch-clamp. The VFT of valsartan was higher than that of control (valsartan vs. control: 17.4±0.6mA vs. 15.8±0.5mA, P<0.05). The VFT of valsartan was higher than that of valsartan+PD123319 (valsartan vs. valsartan+PD123319: (17.4±0.6mA vs. 16.6±0.9mA, P<0.05). The density of I_{to} of valsartan was higher than that of control (valsartan vs. control: 14.7±0.42pA/pF vs. 11.2±0.15pA/pF, P<0.05). The density of I_{to} of valsartan was higher than that of valsartan+PD123319 (valsartan vs. valsartan+PD123319: 14.7±0.42pA/pF vs. 13.6±0.30pA/pF, P<0.05). The density of I_{CaL} of valsartan was lower than that of control (valsartan vs. control: -4.6±0.2pA/pF vs. -6.9±0.1pA/pF, P<0.05). The density of I_{CaL} of valsartan was lower than that of valsartan+PD123319 (valsartan vs. valsartan+PD123319: -4.6±0.2pA/pF vs. -5.4±0.1pA/pF, P<0.05).

These results demonstrated that the stimulation of angiotensin II type 2 receptor improved electrophysiological remodeling of left ventricular hypertrophic myocardium in SHR.

Keywords: angiotensin II type 2 receptor; electrophysiology; left ventricular hypertrophy; PD123319

Introduction

Cardiac hypertrophy was an adaptive process against increased work loads, however, hypertrophy also presented substrates for lethal ventricular arrhythmias, resulting in sudden arrhythmic deaths that accounted for about one third of deaths in cardiac hypertrophy.[1] Previous experimental data suggest that the alteration in activity of K^+ channels, particularly transient outward potassium current (I_{to}), associated with cardiac hypertrophy was a major cause of electrophysiological remodeling and arrhythmogeneity.[2~5] Angiotensin II was a key signal for myocyte hypertrophy,[6] at the same time, Angiotensin II exerted pro-arrhythmic effects by several mechanisms, including the modulation of voltage-dependent K^+ channels involved in human cardiac repolarization.[7] Angiotensin II binded to angiotensin II subtype-1 receptor (AT1R) and angiotensin II subtype-2 receptor (AT2R). Most of the known pathophysiologic effects of Angiotensin II were mediated by AT1R.[8] In studies, AT1R blockade prevented the development of myocyte hypertrophy and improved the electrophysiological remodeling of hypertrophied myocardium.[4, 9, 10] It had been well documented that AT2R activation counteracted most effects of AT1R.[11~13] However, the effect of AT2R on electrophysiological remodelling of hypertrophied myocardium was not fully elucidated.

To this aim, we studied cell capacitance, membrane currents (I_{Na},

I_{CaL} and I_{to}) and ventricular fibrillation threshold of hypertrophied myocardium from the heart of 10-week-old SHR after 8-week of treatment with valsartan or valsartan+PD123319.

Methods

Experimental animals

All animal experiments were performed in accordance with ethical principles of Declaration of Helsinki. Spontaneously hypertensive rats (SHR) aged 10-weeks (weight; ~200g) were purchased from Vital River Experimental Animal Technology (Beijing, China). A total of 36 10-week-old male SHRs were randomly divided into the control group (non-treatment), valsartan and valsartan+PD123319 group (n=12). The valsartan group received valsartan (gift from Beijing Novis Pharmaceutical Co., Ltd.) 20 mg/kg/d orally. The valsartan+PD123319 group received valsartan 20 mg/kg/d and PD123319 which was purchased from Selleck Chemicals (Houston, TX, USA) 30 mg/kg/d orally. Rats were fed at the Sun Yat-sen University of Medical Sciences Animal Center for 8 weeks.

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 awake and quiet conditions. Measurements were repeated three

times, and the mean of three measurements was recorded.

Measurement of ventricular effective refractory period (VERP) and ventricular fibrillation threshold (VFT)

Rats were anesthetized with urethane (120 mg/100g 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℃. The rat was connected to and ventilated by a small animal ventilator at a tidal volume of 1.7-2.5ml, depending on body weight, and at a frequency of 60 breaths/min. Electrocardiogram signals were amplified and recorded on a multiple channels 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, Colorado, 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 bpm. Ventricular fibrillation was invoked by ultra-rapid strand stimulation (10 stimuli, pulse width, 4 ms, 100Hz; 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 (LVMI) ($mg \cdot g^{-1}$).

Isolation of ventricular myocytes

Each heart was quickly excised and mounted on a Langendorff apparatus. Left ventricular myocytes were isolated according to the method described by Isenberg and Klöckner.[14] The aorta was retrogradely cannulated and perfused with nominally Ca^{2+}-free modified Tyrode's solution at 37 ℃ for 5 minutes. Perfusion pressure was 75 mmHg, and all solutions were equilibrated with 100% oxygen. Perfusion was continued for another 15 minutes 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 Ca^{2+} for another 5 minutes.

After perfusion, the left ventricular free wall was separated from the rest of the heart. As there are known differences in I_{to} magnitude between basal and apical regions of the left ventricle,[15] care was taken to isolate epicardial myocytes from the central portion of the left ventricular free wall. Epicardial tissue pieces were carefully dissected from the left

ventricular free wall with fine forceps, and the pieces were placed in cups. To further disaggregate the tissue pieces, they were gently shaken at 37 ℃, filtered through cotton mesh, and allowed to settle for 30 minutes. Cells were stored at room temperature in modified Tyrode's solution containing 100 μM Ca^{2+}. 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, CA, 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_{Na} was measured at 21℃ in an extracellular solution containing (in mmol/L): 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): 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℃ in an extracellular solution containing (in mmol/L): 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): 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℃ in an extracellular solution containing (in mmol/L) 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) KCl 140, $MgCl_2$ 1, EGTA 5, HEPES 10, and Na_2ATP 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 6s. Standard pulse protocols were used to assay the biophysical properties of I_{to}.

Statistics

Results are expressed as mean±SD. Statistical analyses were performed using SPSS 10.0 (SPSS, Chicago, IL, 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 systolic blood pressure and left ventricular mass index

The LVMI was significantly lower in the valsartan group compared with the control group (3.28±0.13mg/g vs. 3.61±0.17mg/g, P<0.05). In addition, the LVMI was lower in the valsartan group compared with the valsartan+PD123319 group (3.28±0.13mg/g vs. 3.47±0.11mg/g, P<0.05). (Table 1)

Comparison of VERP and VFT in rats

The VFT was significantly higher in the valsartan groups compared with the control group (17.4±0.6mA vs. 15.8±0.5mA, P<0.01). The VFT was higher in the valsartan group compared with the valsartan+PD123319 group (17.4±0.6±0.65mA vs. 16.6±0.9mA, P<0.05). (Table 2).

Ionic channels in the left ventricular myocardium

The membrane capacitance of the control group was significantly larger compared with the valsartan group (272.5±3.7pF vs. 198.5±5.6pF, P<0.05). In addition, the membrane capacitance of the valsartan+PD123319 group was larger compared with the valsartan group (224.3±4.9pF vs. 198.5±5.6pF, P<0.05). The density of I_{CaL} in the valsrtan group was lower compared with the control group (-4.6 ± 0.2pA/pF vs. -6.9±0.1pA/pF, P<0.05). The density of I_{CaL} in the valsartan

group was lower compared with the valsartan+PD123319 group (-4.6±0.2pA/pF vs. -5.4±0.1pA/pF, P<0.05). Finally, the density of I_{to} in the valsartan group was significantly higher compared with the control group (14.7±0.42pA/pF vs. 11.2±0.15pA/pF, P<0.05). The density of I_{to} in the valsartan group was higher compared with the valsrtan+PD123319 group (14.7±0.42pA/pF vs. 13.6±0.30pA/pF, P<0.05). (Table 3, Figures 1, 2, and 3).

Discussion

The main and novel finding of this study was that the stimulation of angiotensin II type-2 (AT2R) receptor not only improved electrophysiological remodeling of left ventricular hypertrophic myocardium in SHR, but also reserved the cardiac and cellular hypertrophy. To our knowledge, this was the first demonstration that the AT2R had effect on the electrophysiological characteristic in hypertrophied myocardium.

Angiotensin II binded two distinct renin–angiotensin system receptors, the angiotensin type-1 receptor (AT1R) and the angiotensin type-2 (AT2R) receptor, both AT1R and AT2R possessed similar affinity for angiotensin II.[16] The AT2R was highly expressed in foetal tissue, but levels declined rapidly after birth. In adults, AT2R was expressed in many tissues including heart, kidney, adrenal gland, brain, uterus and both

endothelial and vascular smooth muscle cells and AT2R expression was increased in cardiovascular relevant tissues in cardiovascular disease.[17] previous studies indicated that the AT2R expression was increased in hypertrophic myocardium in SHR.[18, 19] Angiotensin II plasma levels were increased with valsartan treatment (6-fold) in SHR.[19] Taken together, the simulation of AT2R can be achieved during valsartan treatment in SHR, which was served as the simulation of AT2R group. The combination group of valsartan and PD123319, one of AT2R antagonist, was served as the blocking of AT2R group.

Findings from the present study demonstrated that the mechanism associated with the regression of electrophysiological remodeling of hypertrophic myocardium during valsartan treatment can partially be accounted for by the actions of angiotensin II acting at the AT2R. In valsartan group, the ventricular fibrillation threshold (VFT) and transient outward potassium current (I_{to}) were significantly increased compared with control group, at the same time, the L type calcium current (I_{CaL}) was significantly decreased. In the treatment of combination of valsartan and PD123319, one of AT2R antagonist, the VFT and I_{to} were decreased compared with valsartan group, at the same time, the I_{CaL} was increased, however, the VFT and I_{to} were increased compared with control group, at the same time, the $I_{CaL}$was decreased. In conclusion, blocking of AT1R can improve the electrophysiological characteristic, which was same as

the previous studies, [4, 10] and activation of AT2R can further improve the electrophysiological characteristic, however, blocking of AT2R can worsen the electrophysiological characteristic. To our knowledge, this was the first study to examine the effect of AT2R on the electrophysiological remodelling of hypertrophied myocardium and reversal of abnormal ventricular electrophysiology both in vivo and at the cellular level. The present findings supported that the AT2R stimulation counteracted the AT1R-mediated action in electrophysiological remodeling of hypertrophied myocardium.

The left ventricular hypertrophy (LVH) was a strong independent predictor of cardiovascular morbidity and mortality. LVH increase the propensity to develop life threatening arrhythmias. The VFT was used as an index of vulnerability to ventricular fibrillation. A lower VFT indicated easier induction of ventricular fibrillation.[20] In this study, VFT was increased by the activation of AT2R. A low I_{to} was likely to be the ionic mechanism responsible for the prolongation of the action potential duration. As the decrease in I_{to} was dishomogeneous, it may cause dispersion of repolarization, which was in itself arrhythmogenic.[21] The lower I_{to} in ventricular myocytes in disease states associated with an increased propensity for ventricular arrhythmias.[20] In this study, the density of I_{to} was increased by the activation of AT2R. 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 decreasing of I_{to}.[22] In this study, the density of I_{CaL} was decreased by the activation of AT2R, which may led to decreases in $[Ca^{2+}]_i$ and weakening of the activity of Ca^{2+}-dependent signal pathways.

The systolic blood pressure had no change between valsartan group and valsartan+PD123319 group, which indicated that the AT2R had no effect on the systolic blood pressure. This was same as the previous studies.[12, 13, 17] However, the VFT and I_{to} were higher, at the same time, the LVMI, membrane capacitance, one of reliable parameters of cellular hypertrophy,[9, 21, 23, 24] and I_{CaL} were lower in the valsartan group compared with the valsartan+PD123319 group. Taken together, the results suggested that the AT2R had effect on improving electrophysiological remodeling and reserving the cardiac and cellular hypertrophy.

In summary, the activation of AT2R improved hypertrophy of the left ventricle, elevated the VFT, increased I_{to} density and decreased I_{CaL} density in hypertrophied myocytes. Our findings indicate that the activation of AT2R improved the electrophysiological characteristics associated with hypertrophy of the left ventricle.

Limitation

The ECG changes, systolic and/or diastolic function occurring in the hypertrophied heart may contribute to understanding the effect of the

activation of AT2R.

Acknowledgement

This study was supported by the Guangdong Province Science and Technology Project Plan and Social Development of China (No. 2010B031600060).

Conflict of interest: none.

References

1. Narayanan K, Reinier K, Teodorescu C, Uy-Evanado A, Chugh H, Gunson K, Jui J, Chugh SS. Electrocardiographic versus echocardiographic left ventricular hypertrophy and sudden cardiac arrest in the community. Heart Rhythm 2014;11:1040-1046.
2. Cutler MJ, Jeyaraj D, Rosenbaum DS. Cardiac electrical remodeling in health and disease. Trends Pharmacol Sci 2011;32:174-180.
3. Swynghedauw B. Molecular mechanisms of myocardial remodeling. Physiol Rev 1999;79:215-262.
4. Zhi-Bin H, Chang F, Mao-Huan L, Gui-Yi Y, Shu-Xian Z, Wei W. Valsartan improves the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rats. Hypertens Res 2014;37:824-829.

5. Furukawa T, Kurokawa J. Potassium channel remodeling in cardiac hypertrophy. J Mol Cell Cardiol 2006;41:753-761.
6. Ying X, Kai-Pan G, Wei-Qing L, Long-Yun P, De-Xi W, Zhi-Bin H. Long-term treatment of spontaneously hypertensive rats with PD123319 and electrophysiological remodeling of left ventricular myocardium. Naunyn Schmiedebergs Arch Pharmacol 2016;389:1333-1340.
7. Delpón E, Caballero R, Gómez R, Núñez L, Tamargo J. Angiotensin II, angiotensin II antagonists and spironolactone and their modulation of cardiac repolarization. Trends Pharmacol Sci 2005;26:155-161.
8. Yang BC, Phillips MI, Ambuehl PE, Shen LP, Mehta P, Mehta JL. Increase in angiotensin II type 1 receptor expression immediately after ischemia-reperfusion in isolated rat hearts. Circulation 1997;96:922-926.
9. Cerbai E, Crucitti A, Sartiani L, De Paoli P, Pino R, Rodriguez ML, Gensini G, Mugelli A. Long-term treatment of spontaneously hypertensive rats with losartan and electrophysiological remodeling of cardiac myocytes. Cardiovasc Res 2000;45:388-396.
10. Rials SJ, Xu X, Wu Y, Liu T, Marinchack RA, Kowey PR. Restoration of normal ventricular electrophysiology in renovascular hypertensive rabbits after treatment with losartan. J Cardiovasc Pharmacol 2001;37:317-323.

11. Matavelli LC, Siragy HM. AT2 receptor activities and pathophysiological implications. J Cardiovasc Pharmacol 2015;65:226-232.
12. Steckelings UM, Paulis L, Namsolleck P, Unger T. AT2 receptor agonists: hypertension and beyond. Curr Opin Nephrol Hypertens 2012;21:142-146.
13. Sumners C, de Kloet AD, Krause EG, Unger T, Steckelings UM. Angiotensin type 2 receptors: blood pressure regulation and end organ damage. Curr Opin Pharmacol 2015;21:115-121.
14. Isenberg G, Klöckner U. Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude. Pflugers Arch 1982395:30-41.
15. Gómez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. Science 1997;276:800-806.
16. Padia SH, Carey RM. AT2 receptors: beneficial counter-regulatory role in cardiovascular and renal function. Pflugers Arch 2013;465:99-110.
17. Widdop RE, Vinh A, Henrion D, Jones ES. Vascular angiotensin AT2 receptors in hypertension and ageing. Clin Exp Pharmacol Physiol. 2008;35:386-390.

18. Suzuki J, Matsubara H, Urakami M, Inada M. Rat angiotensin II (type 1A) receptor mRNA regulation and subtype expression in myocardial growth and hypertrophy. Circ Res 1993;73:439-447.
19. Der Sarkissian S, Tea BS, Touyz RM, deBlois D, Hale TM. Role of angiotensin II type 2 receptor during regression of cardiac hypertrophy in spontaneously hypertensive rats. J Am Soc Hypertens 2013;7:118-127.
20. Huang ZB, Deng CY, Lin MH, Yuan GY, Wu W. Fosinopril improves the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rats. Naunyn Schmiedebergs Arch Pharmacol 2014;387:1037-1044.
21. Marionneau C, Brunet S, Flagg TP, Pilgram TK, Demolombe S, Nerbonne JM. Distinct cellular and molecular mechanisms underlie functional remodeling of repolarizing K+ currents with left ventricular hypertrophy. Circ Res 2008;102:1406-1415.
22. Yang KC, Nerbonne JM. Mechanisms contributing to myocardial potassium channel diversity, regulation and remodeling. Trends Cardiovasc Med 2016;26:209-218.
23. Knollmann BC, Knollmann-Ritschel BE, Weissman NJ, Jones LR, Morad M. Remodelling of ionic currents in hypertrophied and failing hearts of transgenic mice overexpressing calsequestrin. J Physiol 2000;525 Pt 2:483-498.

24. Seidel T, Salameh A, Dhein S. A simulation study of cellular hypertrophy and connexin lateralization in cardiac tissue. Biophys J 2010;99:2821-2830.

Table 1 Comparison of SBP and LVMI between the 3 groups

Group	n	SBP(mmHg)	N	LVMI(mg/g)
Control	12	166±3.67	8	3.61±0.17
Valsartan	12	156±3.77[a]	8	3.28±0.13[a]
Valsartan+PD123319	12	158±4.79[a]	8	3.47±0.11[ab]

SBP: systolic blood pressure; LVMI: left ventricular mass index; n: number of annimals; N: number of heart.

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

Table 2 Comparison of VERP and VFT between the 3 groups

Group	N	VERP (ms)	VFT (mA)
Control	8	62.9±1.6	15.8±0.5
Valsartan	8	63.2±2.3	17.4±0.6[a]
Valsartan+PD123319	8	63.4±1.9	16.6±0.9[ab]

VERP: ventricular effective refractory period; VFT: ventricular fibrillation threshold;
N: number of heart.

[a]Compared with the control group, P<0.05. [b]Compared with the Valsartan group, P<0.05.

Table 3 Comparison of the ionic channels in the left ventricular myocardium between the 3 groups (n=8 / N=4).

Group	C_m (pF)	I_{CaL} (pA/pF)	I_{Na} (pA/pF)	I_{to} (pA/pF)
Control	272.5±3.7	-6.9±0.1	-16.7±0.2	11.2±0.15
Valsartan	198.5±5.6[a]	-4.6±0.2[a]	-16.9±0.5	14.7±0.42[a]
Valsartan+PD123319	224.3±4.9[ab]	-5.4±0.1[ab]	-16.8±0.4	13.6±0.30[ab]

C_m: cell capacitance; n: number of myocytes; N: number of heart.

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

Figure 1

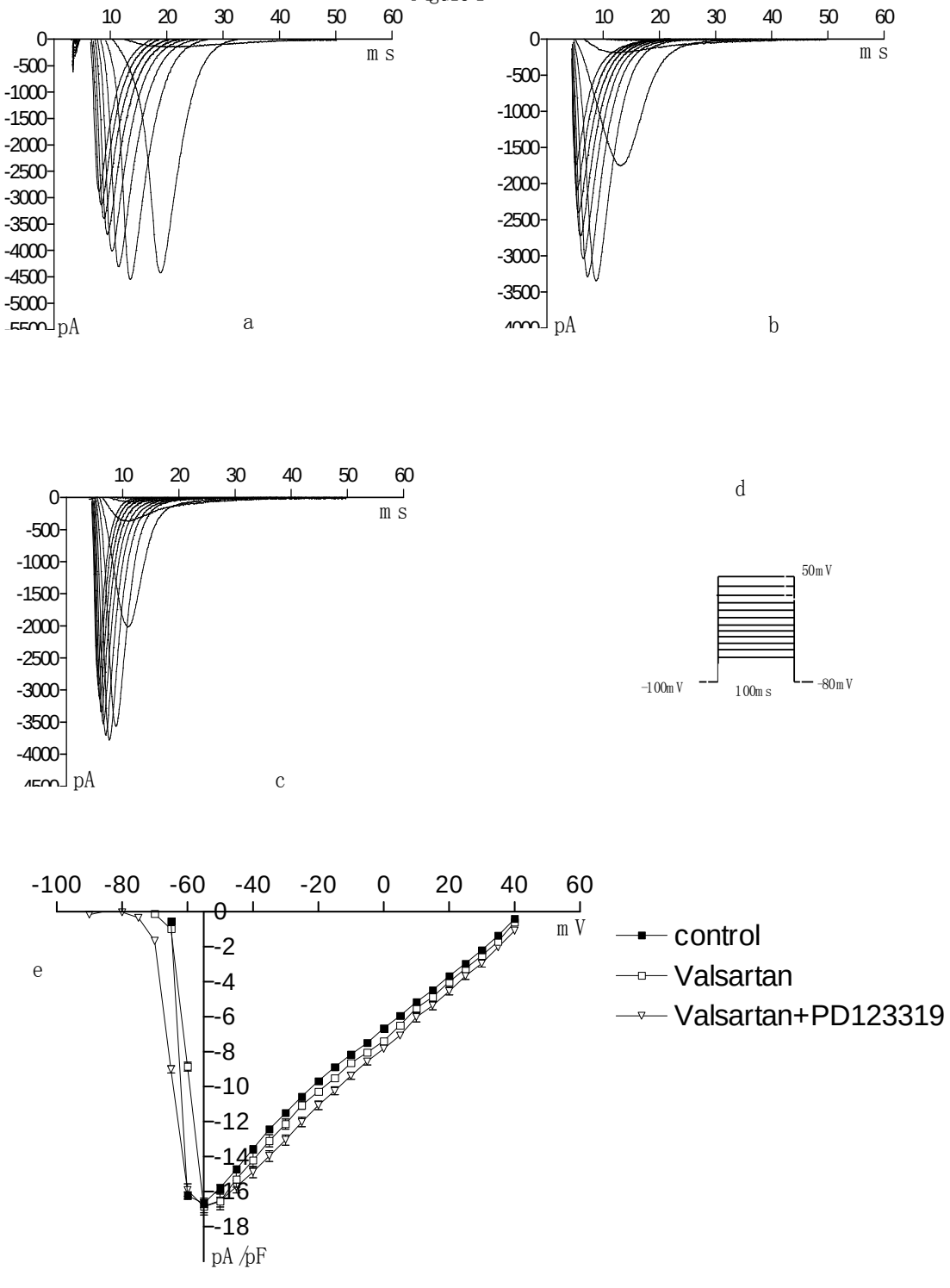

Figure 1. The change of sodium current (I_{Na}) in 3 group rats. Typical recordings of I_{Na} in cells from control (a) valsartan (b) and valsartan+PD 123319 (c). X axis: time (ms: mini-second); Y axis: current volume (pA). The voltage clamp protocol is shown in panel d. Panel e: average I-V relationships of I_{Na} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), valsartan (□) and valsartan+PD 123319 (▽).

Figure 2

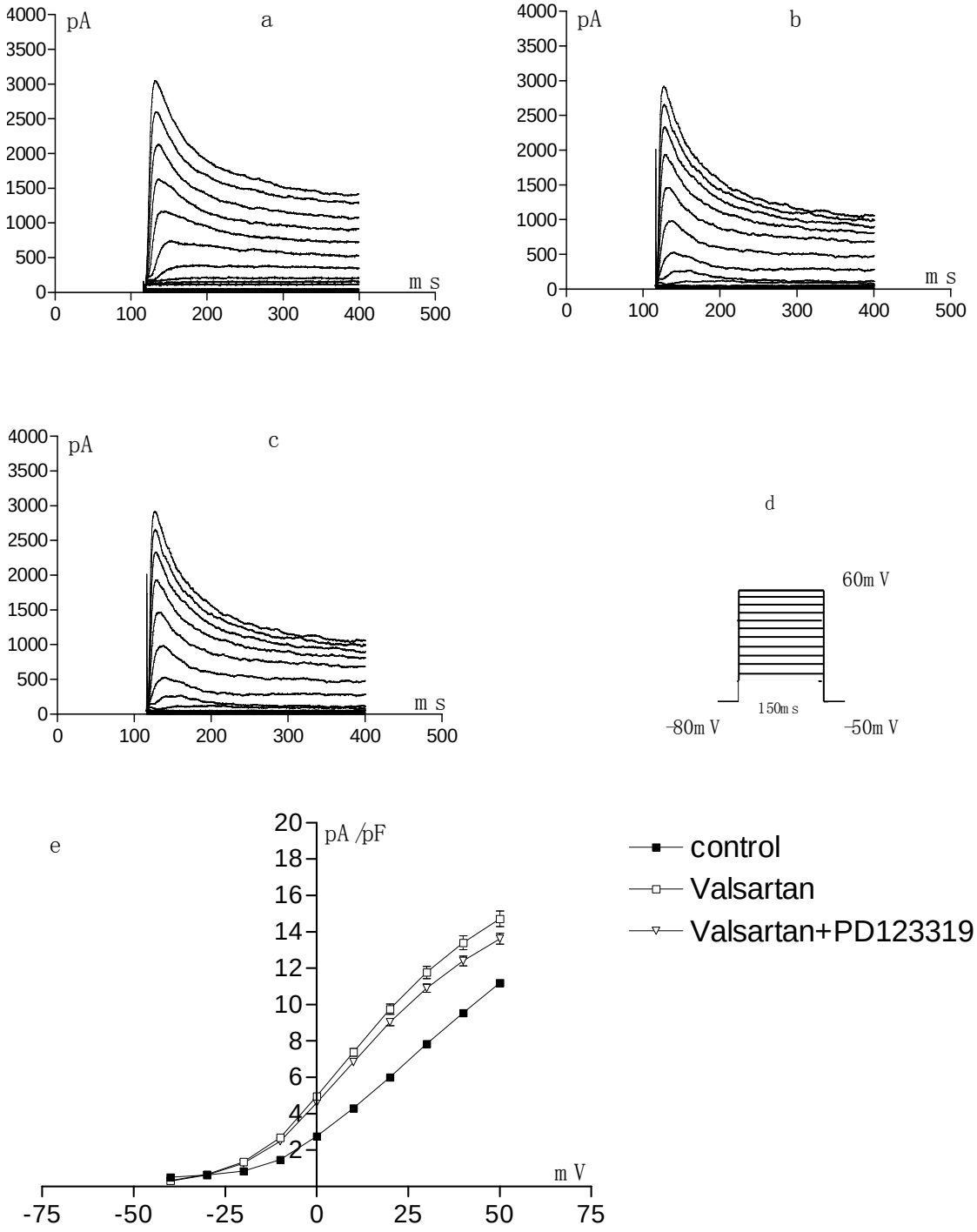

Figure 2. The change of transient outward current (I_{to}) in 3 group rats. Typical recordings of I_{to} in cells from control (a) valsartan (b) and valsartan+PD 123319 (c). X axis: time (ms: mini-second); Y axis: current volume (pA). The voltage clamp protocol is shown in panel d. Panel e: average I-V relationships of Ito density (in pA/pF) as a function of step potential (in mV), obtained in control (■), valsartan (□) and valsartan+PD 123319 (▽).

Figure 3

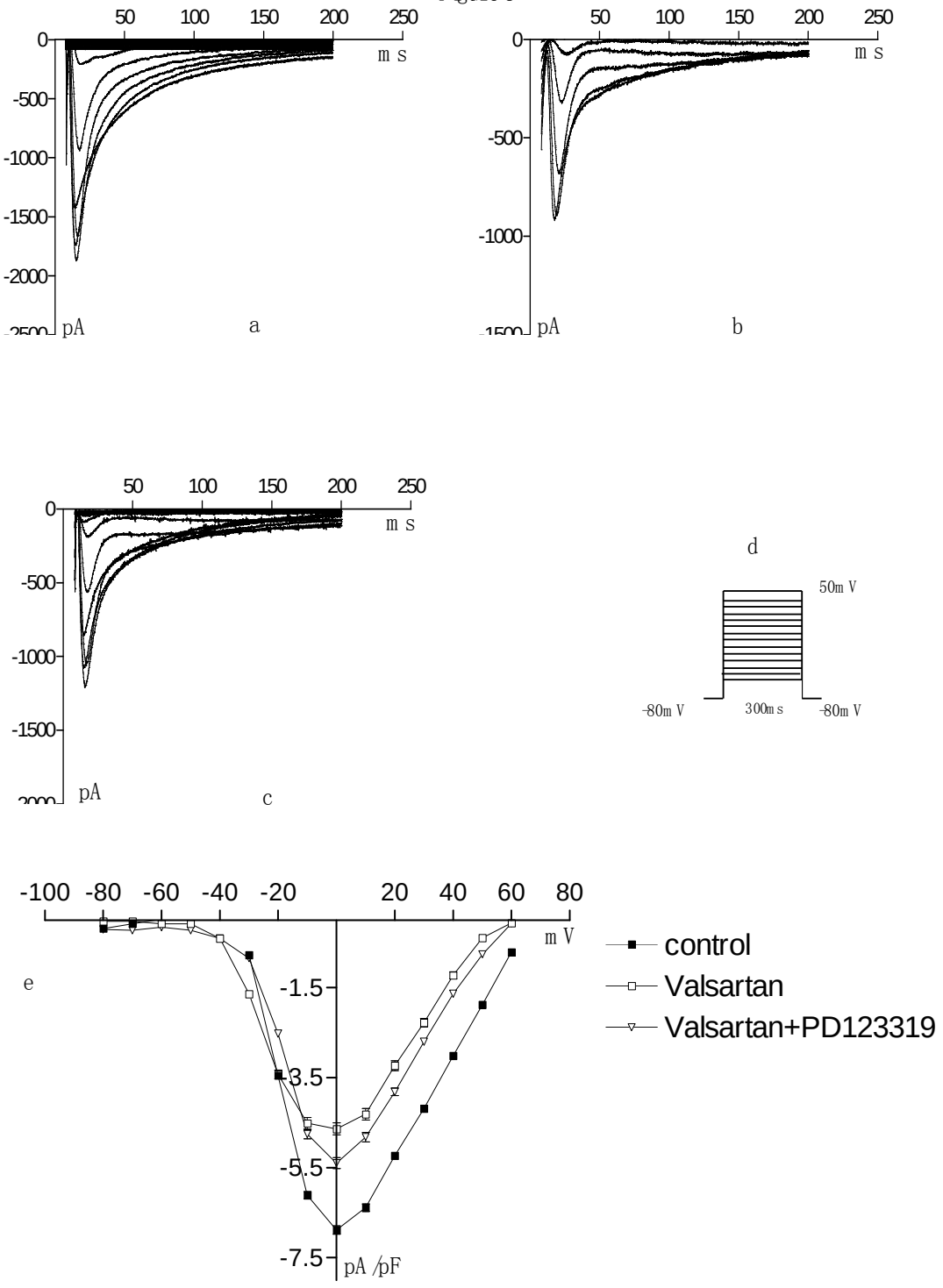

Figure 3. The change of L type calcium current (I_{CaL}) in 3 group rats. Typical recordings of I_{CaL} in cells from control (a) valsartan (b) and valsartan+PD123319 (c). X axis: time (ms: mini-second); Y axis: current volume (pA). The voltage clamp protocol is shown in panel d. Panel e: average I-V relationships of I_{CaL} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), valsartan (□) and valsartan+PD123319 (▽).

Figure 1

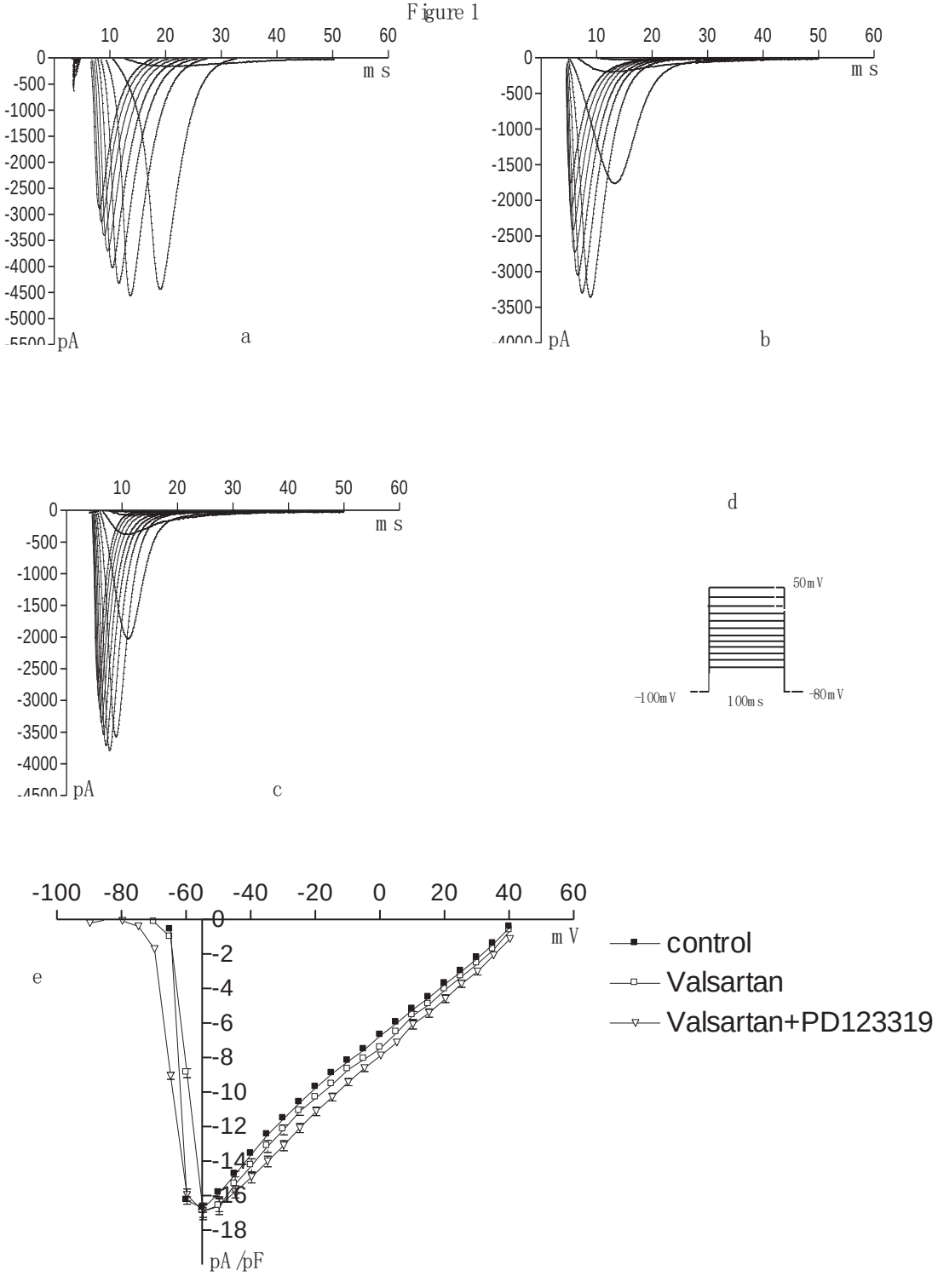

Figure 1. The change of sodium current (I_{Na}) in 3 group rats. Typical recordings of I_{Na} in cells from control (a) valsartan (b) and valsartan+PD 123319 (c). X axis: time (ms: mini-second); Y axis: current volume (pA). The voltage clamp protocol is shown in panel d. Panel e: average I-V relationships of I_{Na} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), valsartan (□) and valsartan+PD 123319 (▽).

Figure 2

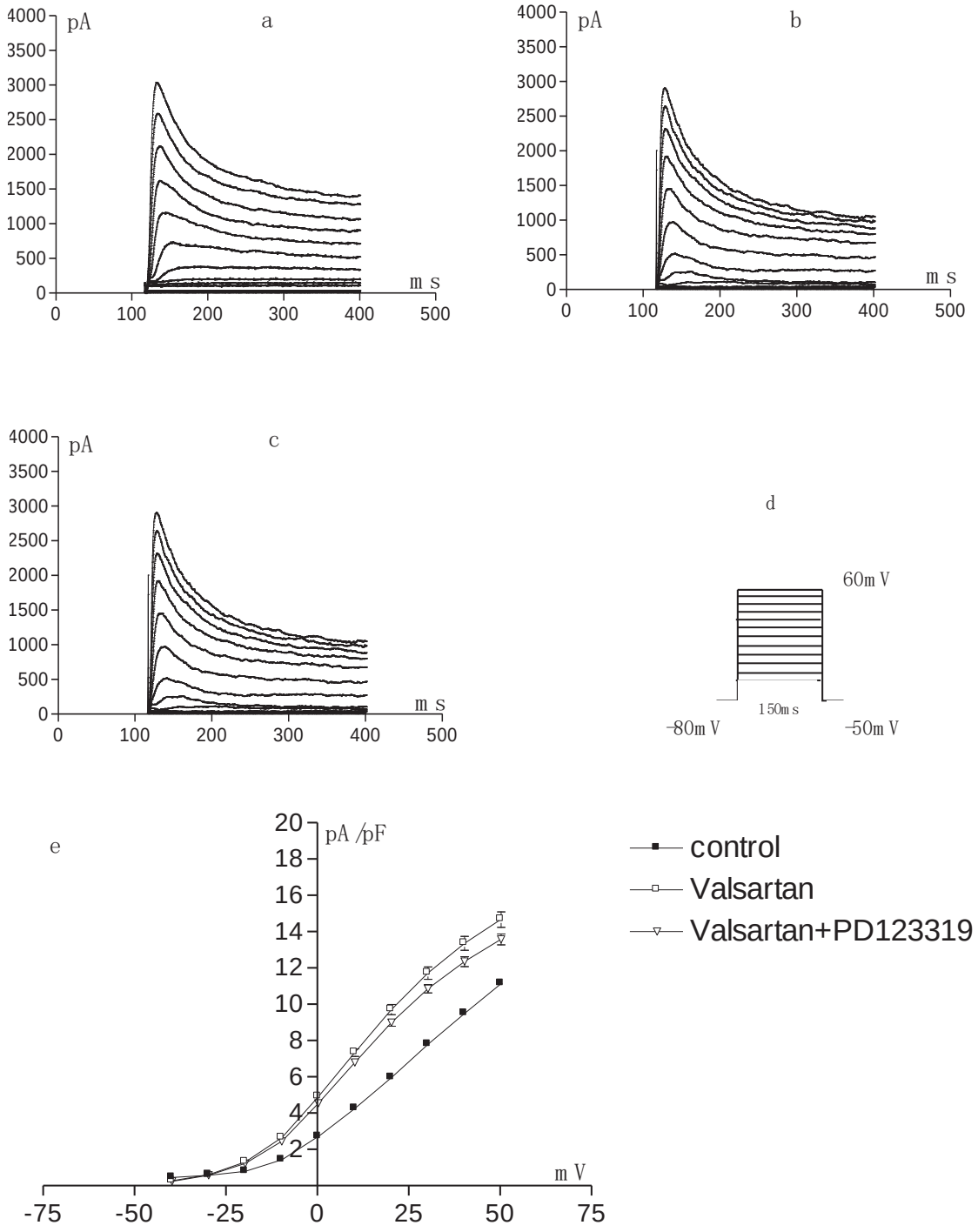

Figure 2. The change of transient outward current (I_{to}) in 3 group rats. Typical recordings of I_{to} in cells from control (a) valsartan (b) and valsartan+PD123319 (c). X axis: time (ms: mini-second); Y axis: current volume (pA). The voltage clamp protocol is shown in panel d. Panel e: average I-V relationships of Ito density (in pA/pF) as a function of step potential (in mV), obtained in control (■), valsartan (□) and valsartan+PD123319 (▽).

Figure 3

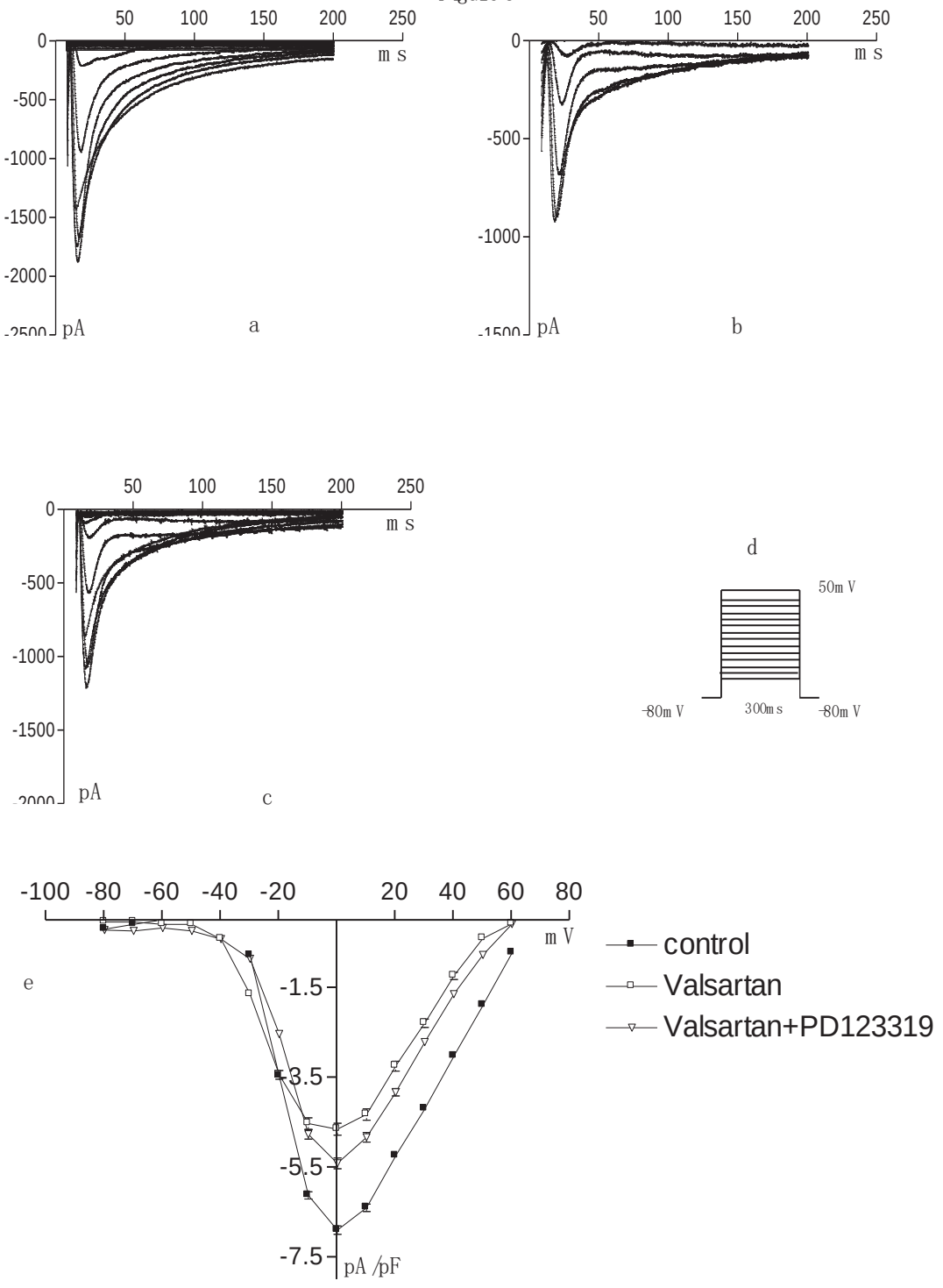

Figure 3. The change of L type calcium current (I_{CaL}) in 3 group rats. Typical recordings of I_{CaL} in cells from control (a) valsartan (b) and valsartan+PD123319 (c). X axis: time (ms: mini-second); Y axis: current volume (pA). The voltage clamp protocol is shown in panel d. Panel e: average I-V relationships of I_{CaL} density (in pA/pF) as a function of step potential (in mV), obtained in control (■), valsartan (□) and valsartan+PD123319 (▽).

Highlights

1. The activation of angiotensin II type-2 receptor (AT2R) improved the density of I_{to}.
2. The stimulation AT2R decreased the density of I_{CaL}.
3. The stimulation of AT2R reduced the left ventricular mass index.
4. The stimulation of AT2R decreased the membrane capacitance of cardiomyocyte.
5. The stimulation of AT2R improved the ventricular fibrillation threshold.