

Long-term treatment of spontaneously hypertensive rats with PD123319 and electrophysiological remodeling of left ventricular myocardium

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Abstract To investigate the effects of PD123319, an antagonist of angiotensin II subtype-2 receptor (AT2R), on the electrophysiological characteristics of the left ventricular hypertrophic myocardium in spontaneously hypertensive rats (SHR). A total of twenty-four 10-week-old male SHR were divided into two groups: PD123319 and non-PD123319 groups ($n = 12$ in each). Twelve 10-week-old Wistar-Kyoto rats served as the control group. Systolic blood pressure, left ventricular mass index (LVMI), ventricular effective refractory period, and ventricular fibrillation threshold were also measured after 8 weeks. I_{Na} , I_{CaL} , I_{to} , and membrane capacitance were measured in the left ventricular myocytes after 8 weeks by whole-cell patch clamp. PD123319 increased LVMI compared with the non-PD123319 group (PD123319 vs. non-PD123319, 3.83 ± 0.11 vs. 3.60 ± 0.19 mg/g; $P < 0.01$). PD123319 also decreased the ventricular fibrillation threshold compared with the non-PD123319 group (PD123319 vs. non-PD123319, 14.75 ± 0.65 vs. 16.0 ± 0.86 mA; $P < 0.01$). PD123319 enhanced membrane capacitance compared with the non-PD123319 group (PD123319 vs. non-PD123319, 283.63 ± 5.80 vs. 276.50 ± 4.28 pF; $P < 0.05$). PD123319 increased the density

of I_{CaL} compared with the non-PD123319 group (PD123319 vs. non-PD123319, -6.76 ± 0.48 vs. -6.13 ± 0.30 pA/pF; $P < 0.05$). PD123319 decreased the density of I_{to} compared with the non-PD123319 group (PD123319 vs. non-PD123319, 11.49 ± 0.50 vs. 12.23 ± 0.36 pA/pF; $P < 0.05$). Long-term treatment with PD123319 worsened the development of myocyte hypertrophy and associated electrophysiological alterations in spontaneously hypertensive rat.

Keywords PD123319 · Electrophysiology · Hypertrophy · Hypertension

Introduction

Sudden death due to ventricular arrhythmias was a major risk factor in patients with myocardial hypertrophy (Levy et al. 1987; Messerli et al. 1984). Angiotensin II was a key signal for myocyte hypertrophy (Patel and Mehta 2012). Angiotensin II binded to angiotensin II subtype-1 receptor (AT1R) and angiotensin II subtype-2 receptor (AT2R). Most of the known pathophysiological effects of Ang II were mediated by AT1R, including vasoconstriction and increased blood pressure, promotion of tissue inflammation and fibrosis, increased oxidative stress, and aldosterone production (Yang et al. 1997). It had been well documented that AT2R activation counteracted most effects of AT1R by inhibiting cell proliferation and differentiation, promoting vasodilation, and reducing inflammation and oxidative stress (Matavelli and Siragy 2015; Steckelings et al. 2012; Sumners et al. 2015). In studies, AT1R blockade prevented the development of myocyte hypertrophy and improved the electrophysiological remodeling of hypertrophic myocardium (Cerbai et al. 2000a; Rials et al. 2001; Zhi-Bin et al. 2014). However, the effect of AT2R

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blockade on electrophysiological remodeling of hypertrophic myocardium was not fully elucidated.

For all these reasons, we thought it would be interesting to assess the effect of long-term treatment with PD123319, a non-peptidic antagonist of AT2R, on the electrophysiological alterations occurring in SHR during the development of cardiac hypertrophy. To this aim, we studied cell capacitance, membrane currents (I_{Na} , I_{CaL} , and I_{to}) and ventricular fibrillation threshold of hypertrophic myocardium from the heart of a 10-week-old SHR after 8 weeks of treatment with saline or PD123319.

Materials and methods

Experimental animals

All animal experiments were performed in accordance with the ethical principles of the Declaration of Helsinki. Spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) male rats aged 10 weeks (weight, ~200 g) were purchased from Vital River Experimental Animal Technology (Beijing, China). A total of twenty-four 10-week-old male SHR were randomly divided into the non-PD123319 group and PD123319 group ($n = 12$). A total of twelve 10-week-old male Wistar-Kyoto rats served as the control group. The PD123319 group received PD123319, which was purchased from Selleck Chemicals (Houston, TX, USA) at $30 \text{ mg kg}^{-1} \text{ day}^{-1}$ orally for 8 weeks. The control and non-PD123319 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 awake and quiet conditions. Measurements were repeated three times, and the mean of three measurements was recorded.

Measurement of ventricular effective refractory period and ventricular fibrillation threshold

Rats were anesthetized with urethane (120 mg/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. 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, Colorado, USA), isolation stimulator (type DSJ731-G-A), and a physiological stimulator (type DSJ731-2C-A).

The ventricular effective refractory period (VERP) was measured using extra-stimuli delivered in 10 ms decrements (S_1S_2) by a program stimulator (type 5352, Medtronic Company, Colorado, USA). The VERP was the longest S_1S_2 interval that failed to cause ventricular depolarization.

The heart was paced by a physiological stimulator (type DSJ731-2C-A) at 500 bpm. Ventricular fibrillation was invoked by ultra-rapid strand stimulation (10 stimuli, pulse width, 4 ms, 100 Hz; delay, 60 ms) released from isolation stimulator (type DSJ731-G-A). The initial current intensity was 5 mA. The current was increased in 0.5 mA increments. The ventricular fibrillation threshold (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 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 (1982). The aorta was retrogradely cannulated and perfused with nominally Ca^{2+} -free-modified Tyrode's solution at 37°C for 5 min. Perfusion pressure was 75 mmHg, 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 $100 \mu\text{M Ca}^{2+}$ for another 5 min.

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 (Gómez et al. 1997), 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°C , filtered through cotton mesh, and allowed to settle for 30 min. Cells were stored at room temperature in modified Tyrode's solution containing $100 \mu\text{M Ca}^{2+}$. 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 1200A/D converter (Axon Instruments) interface for off-line analysis. Data were analyzed using custom-written software.

I_{Na} was measured at 21 °C in an extracellular solution containing (mmol/l) 5.0 NaCl, 130.0 choline-Cl, 15.4 CsCl, 10.0 HEPES, 1.0 $MgCl_2 \cdot 6H_2O$, 5.0 NaH_2PO_4 , 1.0 $CaCl_2$, 10 glucose H_2O , and 0.001 nicardipine, at pH 7.4. The intracellular solution contained (mmol/l) 120.0 CsCl, 110.0 CsF, 5.0 NaCl, 5.0 HEPES, 5.0 EGTA, 1.0 $MgCl_2 \cdot 6H_2O$, and 5.0 Na_2-ATP , at pH 7.2. I_{Na} was elicited from a holding potential of -100 mV by voltage steps of 100 ms from -80 to 50 mV in 10-mV increments at 0.5 Hz.

I_{CaL} was measured at 21 °C in an extracellular solution containing (mmol/l) 50.0 TEA-Cl, 0.5 $MgCl_2 \cdot 6H_2O$, 1.8 $CaCl_2$, 3.0 4AP, and 5.0 HEPES, pH 7.4. The intracellular solution contained (mmol/l) 100.0 CsCl, 20.0 TEA-Cl, 5.0 Na_2-ATP , 10.0 HEPES, and 10.0 EGTA, pH 7.2. I_{CaL} was elicited from a holding potential of -80 mV by voltage steps of 300 ms from -80 to 50 mV in 10-mV increments at 0.2 Hz.

I_{to} was measured at 21 °C in an extracellular solution containing (mmol/l) 136 NaCl, 5.4 KCl, 0.33 NaH_2PO_4 , 1.0 $MgCl_2 \cdot 6H_2O$, 2 $CaCl_2$, 0.5 $BaCl_2$, 0.3 $CdCl_2$, 10 HEPES, and 10 glucose, at pH 7.4. The intracellular solution contained (mmol/l) 140 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES, and 5 Na_2ATP , at pH 7.2. I_{to} was elicited from a holding potential of -80 mV by voltage steps of 150 ms from -50 to 60 mV in 10-mV increments every 6 s. Standard pulse protocols were used to assay the biophysical properties of I_{to} .

Statistics

Results were expressed as mean \pm SD. Statistical analyses were performed using SPSS 10.0 (SPSS, Chicago, IL, USA). Differences between the mean values of multiple

Table 2 Comparison of VERP and VFT between three group rats

Group	Number of rats	VERP (ms)	VFT (mA)
Control	8	62.25 \pm 1.04	25.50 \pm 1.31
Non-PD123319	8	63.75 \pm 2.38	16.06 \pm 0.86*
PD123319	8	64.38 \pm 1.85	14.75 \pm 0.65* [#]

VERP ventricular effective refractory period, VFT ventricular fibrillation threshold

* $P < 0.01$ —compared with the control group; [#] $P < 0.05$ —compared with the non-PD123319 group

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 systolic blood pressure was significantly higher in the non-PD123319 and PD123319 groups compared with the control group (165.33 \pm 2.99 and 167.17 \pm 3.21 vs. 93.17 \pm 3.69 mmHg; $P < 0.01$). The LVMI was significantly higher in the non-PD123319 and PD123319 groups compared with the control group (3.60 \pm 0.19 and 3.83 \pm 0.11 vs. 2.46 \pm 0.11 mg/g; $P < 0.01$). In addition, the LVMI was significantly higher in the PD123319 group compared with the non-PD123319 group (3.83 \pm 0.11 vs. 3.60 \pm 0.19 mg/g; $P < 0.01$) (Table 1).

Comparison of VERP and VFT in rats

The VFT was significantly lower in the non-PD123319 and PD123319 groups compared with the control group (16.06 \pm 0.86 and 14.75 \pm 0.65 vs. 25.50 \pm 1.31 mA; $P < 0.01$). The VFT was lower in the PD123319 group compared with the non-PD123319 group (14.75 \pm 0.65 vs. 16.06 \pm 0.86 mA; $P < 0.01$) (Table 2).

Table 1 Comparison of SBP and LVMI between three group rats

Group	Number of rats	SBP (mmHg)	Number of hearts	LVMI (mg/g)
Control	12	93.17 \pm 3.69	8	2.46 \pm 0.11
Non-PD123319	12	165.33 \pm 2.99*	8	3.60 \pm 0.19*
PD123319	12	167.17 \pm 3.21*	8	3.83 \pm 0.11* [#]

SBP systolic blood pressure, LVMI left ventricular mass index

* $P < 0.01$ —compared with the control group; [#] $P < 0.01$ —compared with the non-PD123319 group

Table 3 Comparison of the ionic channels in the left ventricular myocytes between three group rats ($n = 4$, $N = 6$)

Group	C_m (pF)	I_{Na} (pA/pF)	I_{to} (pA/pF)	I_{CaL} (pA/pF)
Control	127.13 \pm 2.23	-17.57 \pm 1.31	15.71 \pm 1.05	-5.68 \pm 0.28
Non-PD123319	276.50 \pm 4.28*	-17.49 \pm 1.43	12.23 \pm 0.36*	-6.13 \pm 0.30*
PD123319	283.63 \pm 5.80* [#]	-17.59 \pm 1.29	11.49 \pm 0.50* [#]	-6.76 \pm 0.48* [#]

C_m membrane capacitance, n number of rats, N number of myocytes

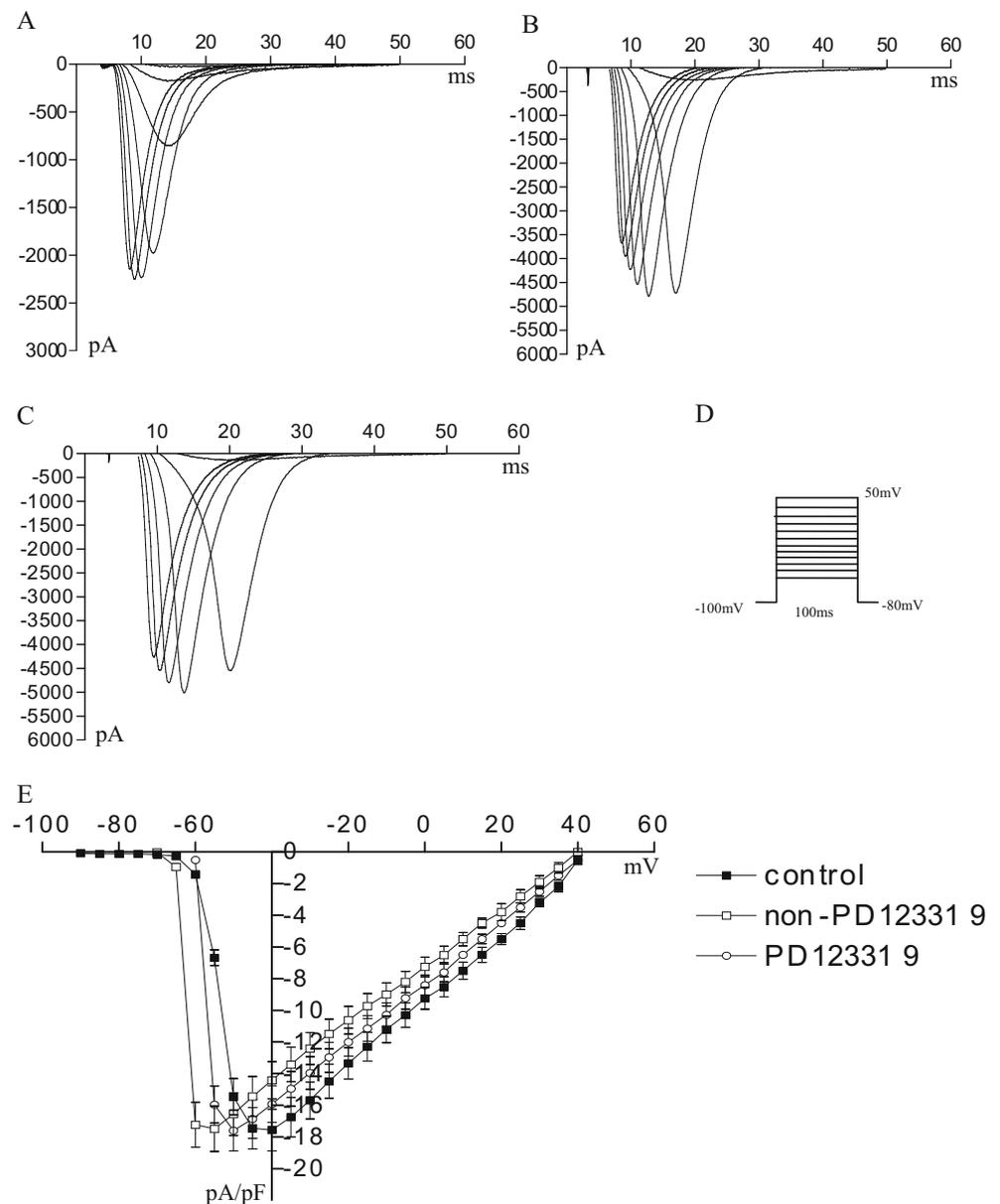
* $P < 0.05$ —compared with the control group; [#] $P < 0.05$ —compared with the non-PD123319 group

Ionic channels in the left ventricular myocardium

The membrane capacitance of the non-PD123319 and PD123319 groups was significantly larger compared with

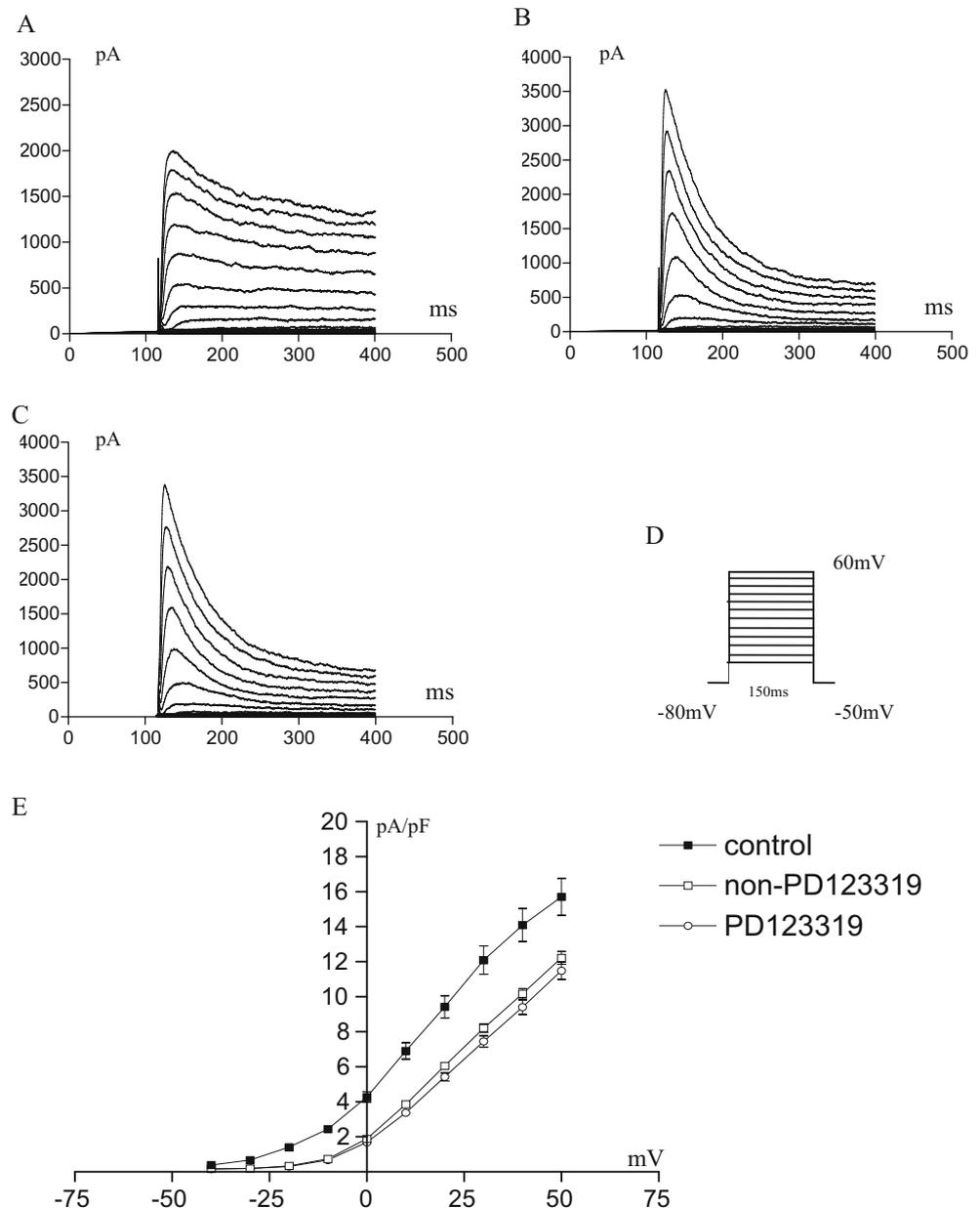
the control group (276.50 \pm 4.28 and 283.63 \pm 5.80 vs. 127.13 \pm 2.23 pF; $P < 0.01$). In addition, the membrane capacitance of the PD123319 group was significantly higher compared with the non-PD123319 group (283.63 \pm 5.80 vs.

Fig. 1 Effect of PD123319 treatment on sodium current (I_{Na}). Typical recordings of I_{Na} in cells from control (a), non-PD123319 (b), and PD123319 (c). *x*-axis: time (ms mini-second); *y*-axis: current volume (pA). The voltage clamp protocol is shown in (d). e Average I-V relationships of I_{Na} density (pA/pF) as a function of step potential (mV), obtained in control (filled squares), non-PD123319 (empty squares), and PD123319 (empty circles)



276.50 ± 4.28 pF, $P < 0.01$). The density of I_{CaL} in the non-PD123319 and PD123319 groups was higher compared with the control group (-6.13 ± 0.30 and -6.76 ± 0.48 vs. -5.68 ± 0.28 pA/pF; $P < 0.05$). The density of I_{CaL} in the PD123319 group was higher compared with the non-PD123319 group (-6.76 ± 0.48 vs. -6.13 ± 0.30 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-PD123319 and PD123319 groups was significantly lower compared with the control group (12.23 ± 0.36 and 11.49 ± 0.50 vs. 15.71 ± 1.05 pA/pF; $P < 0.05$). However, the density of I_{to} in the PD123319 group was lower compared with the non-PD123319 group (11.49 ± 0.50 vs. 12.23 ± 0.36 pA/pF; $P < 0.05$) (Table 3; Figs. 1, 2, and 3).

Fig. 2 Effect of PD123319 treatment on transient outward current (I_{to}). Typical recordings of I_{to} in cells from control (a), non-PD123319 (b), and PD123319 (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). The voltage clamp protocol is shown in (d). e Average I-V relationships of I_{to} density (pA/pF) as a function of step potential (mV), obtained in control (filled squares), non-PD123319 (empty squares), and PD123319 (empty circles)

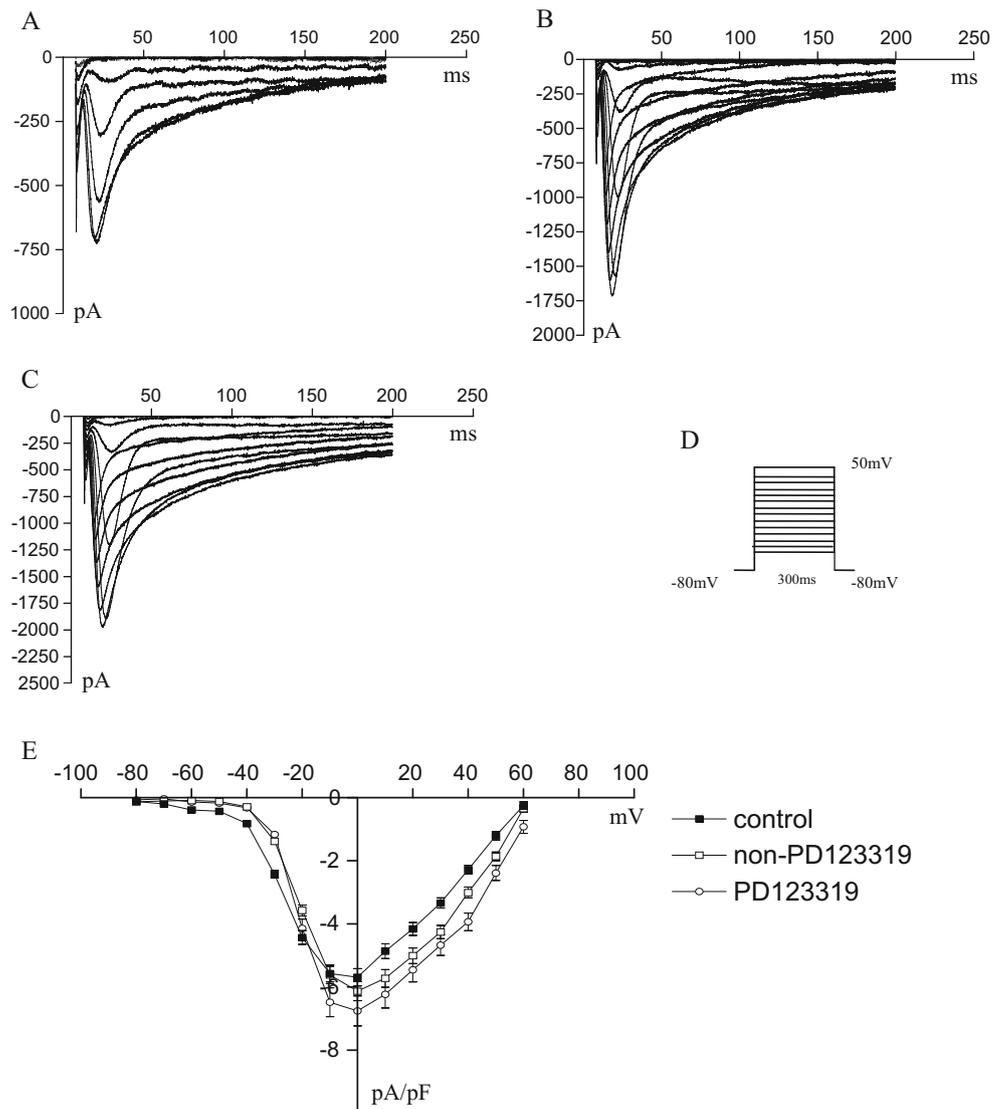


Discussion

In this study, we evaluated the effect of long-term treatment with PD123319 on the electrophysiological remodeling occurring in SHR. The main and novel finding of this study was that long-term treatment with PD123319 not only affected development of cardiac and cellular hypertrophy but also affected the electrophysiological alterations, which characteristically occurred in the hypertrophic myocardium.

Previous studies (Buisson et al. 1992; Martens et al. 1996; Chorvatova et al. 1996; Dimitropoulou et al. 2001; Zhu et al. 1998) had demonstrated that AT2 receptors modulate T-type calcium current and K^+ currents in neurons, coronary artery-smooth muscle cells, and other cells. To our knowledge, this

Fig. 3 Effect of PD123319 treatment on L-type calcium current (I_{CaL}). Typical recordings of I_{CaL} in cells from control (a), non-PD123319 (b), and PD123319 (c). x-axis: time (ms mini-second); y-axis: current volume (pA). The voltage clamp protocol is shown in (d). e Average I-V relationships of I_{CaL} density (pA/pF) as a function of step potential (mV), obtained in control (filled squares), non-PD123319 (empty squares), and PD123319 (empty circles)



was the first demonstration that chronic blockade of AT2 receptors affected cardiac ionic currents and ventricular fibrillation threshold in hypertrophic myocardium. Our results indeed clearly demonstrated that I_{to} was lower, I_{CaL} was higher, and the ventricular fibrillation threshold was lower in PD123319-treated SHR. Previous studies (Cerbai et al. 1994; Cutler et al. 2011; Huang et al. 2014a) had demonstrated that the main ionic alteration of hypertrophic myocardium caused by chronic hypertension was a specific decrease in I_{to} . The decrease in I_{to} in ventricular myocytes in hypertrophic myocardium associated with an increased propensity for ventricular arrhythmias strongly suggested their role in arrhythmogenesis (Cerbai et al. 2000b). A decrease in I_{to} may cause a dispersion of repolarization, which was per se an arrhythmogenic mechanism (Huang et al. 2014b). I_{to} has an important influence on the electrical driving force for systolic Ca^{2+} entry into the cardiac myocyte. Decrease of I_{to} density may therefore significantly contribute to the pathogenesis of

excitation contraction abnormalities and cardiac arrhythmias (Zhi-Bin et al. 2014). Previous study had demonstrated that angiotensin II induced increase in frequency of cytosolic and nuclear calcium waves of heart cells via activation of AT1 and AT2 receptors (Bkaily et al. 2005). The density of I_{CaL} was increased in hypertrophied myocytes, which resulted in increases in $[Ca^{2+}]_i$ (Zhi-Bin et al. 2014). Ca^{2+} -dependent signal pathways were likely activated, which led to myocardial hypertrophy and decrease of I_{to} . The myocardial hypertrophy, increase of I_{CaL} , and decrease of I_{to} were the underlying mechanisms of decrease in VFT (Zhi-Bin et al. 2014). The present results added novel information on the effect of pharmacological treatment on electrophysiological remodeling, showing for the first time that long-term treatment with PD123319 was able to worsen the electrophysiological alterations associated with cardiac and cellular hypertrophy.

Interestingly, the effect of PD123319 was observed for a daily dosage, which did not significantly affect systolic blood

pressure. This was not surprising since deterioration of cardiac hypertrophy in the absence of significant highing of blood pressure. AT2R was involved in vasodilation via release of bradykinin and nitric oxide, anti-inflammation, and healing from injury. Interestingly, the *in vivo* vasodilation effect of the AT2R activation did not lead to reduction in blood pressure (Bosnyak et al. 2010). This finding could be explained by the counter regulatory vaso-constrictive effects of the highly expressed AT1R. The concomitant administration of a low-dose AT1R antagonist with an AT2R agonist was shown to cause further reduction in blood pressure in rats. Blockade of AT1R unmasks the vasodilatory effects of the AT2R in acute as well as chronic *in vivo* experiments (Foulquier et al. 2012; Katada and Majima 2002; Cosentino et al. 2005; Widdop et al. 2002). These observations suggest that AT2R stimulation might potentiate vasodilation during concomitant AT1R blockade. In these studies, AT2R-mediated vasodilation was inhibited by its antagonist PD123319. In the study, to investigate the effects of chronic AT2R stimulation with compound 21 (C21) on the heart, 7-day treatment with C21 improved post-myocardial infarction systolic and diastolic ventricular function, accompanied by reduction in cardiac scar size, and diminished levels of inflammatory and apoptotic markers in the peri-infarct zone (Kaschina et al. 2008). C21 was also reported to reduce deposition of interstitial collagen in myocardial fibronectin content (Rehman et al. 2012). Interestingly, preconditioning of the bone marrow mononuclear cells with the AT2R agonist CGP42112A before transplanting into a post-myocardium infarction zone, improved global cardiac function by reducing infarct size, cardiomyocyte apoptosis, and inflammation (Xu et al. 2013). Direct stimulation of the AT2R may account for preserved heart function and attenuation of disease-associated cardiopathophysiology by restoring the balance of the RAS axes and anti-inflammatory and anti-fibrotic effects (Namsolleck et al. 2014). In the study of hearts from SHR treated with valsartan combined with the AT2 antagonist PD123319 for 1 or 2 weeks, valsartan significantly increased ventricular DNA fragmentation, increased apoptosis in epicardial mesothelial cells, decreased DNA synthesis, and significantly reduced cardiomyocyte cross-sectional area. These valsartan-induced changes were attenuated by PD123319 co-administration (Der Sarkissian et al. 2013). AT2 receptor antagonist inhibited collagen degradation by increasing the activity of matrix metalloproteinases 2 and 9 and decreasing the level of tissue inhibitor of metalloproteinases (Qi and Katovich 2014). AT2R blockade enhanced Ang II levels, leading to AT1R stimulation, which promoted the cardiac and cellular hypertrophy (Dolan and O'Brien 2016). Previous study had demonstrated that stimulation of cardiac AT2R exerts a novel anti-pressor action by inhibiting AT1R-mediated chronotropic effects, and that application of AT1R antagonists to patients with cardiovascular diseases has beneficial pharmacotherapeutic effects of stimulating cardiac

AT2R (Masaki et al. 1998). Taken together, these may be the underlying mechanism of long-term treatment with PD123319 worsening the cardiac and cellular hypertrophy.

Long-term treatment with PD123319 not only affected development of cardiac and cellular hypertrophy but also affected the electrophysiological alterations.

Limitations

One limitation of the study was that the use of urethane as anesthetic was disputed. Furthermore, the way to induce fibrillation was controversial. Finally, the receptor-ion channel signaling, gating kinetics of ion channel, and action potential of myocytes were not checked.

Compliance with ethical standards

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Conflict of interest None declared.

References

- Bkaily G, El-Bizri N, Nader M, Hazzouri KM, Riopel J, Jacques D, Regoli D, D'Orleans-Juste P, Gobeil F Jr, Avedanian L (2005) Angiotensin II induced increase in frequency of cytosolic and nuclear calcium waves of heart cells via activation of AT1 and AT2 receptors. *Peptides* 26(8):1418–1426
- Bosnyak S, Welungoda IK, Hallberg A, Alterman M, Widdop RE, Jones ES (2010) Stimulation of angiotensin AT2 receptors by the non-peptide agonist, compound 21, evokes vasodepressor effects in conscious spontaneously hypertensive rats. *Br J Pharmacol* 159(3):709–716
- Buisson B, Bottari SP, de Gasparo M, Gallo-Payet N, Payet MD (1992) The angiotensin AT2 receptor modulates T-type calcium current in non-differentiated NG108-15 cells. *FEBS Lett* 309(2):161–164
- Cerbai E, Barbieri M, Li Q, Mugelli A (1994) Ionic basis of action potential prolongation of hypertrophied cardiac myocytes isolated from hypertensive rats of different ages. *Cardiovasc Res* 28(8):1180–1187
- Cerbai E, Crucitti A, Sartiani L, De Paoli P, Pino R, Rodriguez ML, Gensini G, Mugelli A (2000a) Long-term treatment of spontaneously hypertensive rats with losartan and electrophysiological remodeling of cardiac myocytes. *Cardiovasc Res* 45(2):388–396
- Cerbai E, Crucitti A, Sartiani L, De Paoli P, Pino R, Rodriguez ML, Gensini G, Mugelli A (2000b) Long-term treatment of spontaneously hypertensive rats with losartan and electrophysiological remodeling of cardiac myocytes. *Cardiovasc Res* 45(2):388–396
- Chorvatova A, Gallo-Payet N, Casanova C, Payet MD (1996) Modulation of membrane potential and ionic currents by the AT1 and AT2 receptors of angiotensin II. *Cell Signal* 8(8):525–532
- Cosentino F, Savoia C, De Paolis P, Francia P, Russo A, Maffei A, Venturelli V, Schiavoni M, Lembo G, Volpe M, Angiotensin II (2005) Type 2 receptors contribute to vascular responses in

- spontaneously hypertensive rats treated with angiotensin II type 1 receptor antagonists. *Am J Hypertens* 18(4 Pt 1):493–499
- Cutler MJ, Jeyaraj D, Rosenbaum DS (2011) Cardiac electrical remodeling in health and disease. *Trends Pharmacol Sci* 32(3):174–180
- Der Sarkissian S, Tea BS, Touyz RM, deBlois D, Hale TM (2013) Role of angiotensin II type 2 receptor during regression of cardiac hypertrophy in spontaneously hypertensive rats. *J Am Soc Hypertens* 7(2):118–127
- Dimitropoulou C, White RE, Fuchs L, Zhang H, Catravas JD, Carrier GO (2001) Angiotensin II relaxes microvessels via the AT(2) receptor and Ca(2+)-activated K(+) (BK(Ca)) channels. *Hypertension* 37(2):301–307
- Dolan E, O'Brien E (2016) Comparing the therapeutic merits of angiotensin receptor blockers. *J Hypertens* 34(6):1052–1054
- Foulquier S, Steckelings UM, Unger T (2012) Impact of the AT(2) receptor agonist C21 on blood pressure and beyond. *Curr Hypertens Rep* 14(5):403–409
- Gómez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ (1997) Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science* 276(5313):800–806
- Huang ZB, Deng CY, Lin MH, Yuan GY, Wu W (2014b) Fosinopril improves the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rats. *Naunyn Schmiedeberg's Arch Pharmacol* 387(11):1037–1044
- Huang ZB, Fang C, Lin MH, Yuan GY, Zhou SX, Wu W (2014a) Effect of fosinopril on the transient outward potassium current of hypertrophied left ventricular myocardium in the spontaneously hypertensive rat. *Naunyn Schmiedeberg's Arch Pharmacol* 387(5):419–425
- Isenberg G, Klöckner U (1982) Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude. *Pflugers Arch* 395(1):30–41
- Kaschina E, Grzesiak A, Li J, Foryst-Ludwig A, Timm M, Rompe F, Sommerfeld M, Kemnitz UR, Curato C, Namsolleck P, Tschöpe C, Hallberg A, Alterman M, Hucko T, Paetsch I, Dietrich T, Schnackenburg B, Graf K, Dahlöf B, Kintscher U, Unger T, Steckelings UM (2008) Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with the renin-angiotensin system in myocardial infarction? *Circulation* 118(24):2523–2532
- Katada J, Majima M (2002) AT(2) receptor-dependent vasodilation is mediated by activation of vascular kinin generation under flow conditions. *Br J Pharmacol* 136(4):484–491
- Levy D, Anderson KM, Savage DD, Balkus SA, Kannel WB, Castelli WP (1987) Risk of ventricular arrhythmias in left ventricular hypertrophy: the Framingham heart study. *Am J Cardiol* 60(7):560–565
- Martens JR, Wang D, Summers C, Posner P, Gelband CH (1996) Angiotensin II type 2 receptor-mediated regulation of rat neuronal K⁺ channels. *Circ Res* 79(2):302–309
- Masaki H, Kurihara T, Yamaki A, Inomata N, Nozawa Y, Mori Y, Murasawa S, Kizima K, Maruyama K, Horiuchi M, Dzau VJ, Takahashi H, Iwasaka T, Inada M, Matsubara H (1998) Cardiac-specific overexpression of angiotensin II AT2 receptor causes attenuated response to AT1 receptor-mediated pressor and chronotropic effects. *J Clin Invest* 101(3):527–535
- Matavelli LC, Siragy HM (2015) AT2 receptor activities and pathophysiological implications. *J Cardiovasc Pharmacol* 65(3):226–232
- Messerli FH, Ventura HO, Elizardi DJ, Dunn FG, Frohlich ED (1984) Hypertension and sudden death. Increased ventricular ectopic activity in left ventricular hypertrophy. *Am J Med* 77(1):18–22
- Namsolleck P, Recarti C, Foulquier S, Steckelings UM, Unger T (2014) AT(2) receptor and tissue injury: therapeutic implications. *Curr Hypertens Rep* 16(2):416–425
- Patel BM, Mehta AA (2012) Aldosterone and angiotensin: role in diabetes and cardiovascular diseases. *Eur J Pharmacol* 697(1–3):1–12
- Qi Y, Katovich MJ (2014) Is angiotensin II type 2 receptor a new therapeutic target for cardiovascular disease? *Exp Physiol* 99(7):933–934
- Rehman A, Leibowitz A, Yamamoto N, Rautureau Y, Paradis P, Schiffrin EL (2012) Angiotensin type 2 receptor agonist compound 21 reduces vascular injury and myocardial fibrosis in stroke-prone spontaneously hypertensive rats. *Hypertension* 59(2):291–299
- Rials SJ, Xu X, Wu Y, Liu T, Marinchack RA, Kowey PR (2001) Restoration of normal ventricular electrophysiology in renovascular hypertensive rabbits after treatment with losartan. *J Cardiovasc Pharmacol* 37(3):317–323
- Steckelings UM, Paulis L, Namsolleck P, Unger T (2012) AT2 receptor agonists: hypertension and beyond. *Curr Opin Nephrol Hypertens* 21(2):142–146
- Summers C, de Kloet AD, Krause EG, Unger T, Steckelings UM (2015) Angiotensin type 2 receptors: blood pressure regulation and end organ damage. *Curr Opin Pharmacol* 21:115–121
- Widdop RE, Matrougui K, Levy BI, Henrion D (2002) AT2 receptor-mediated relaxation is preserved after long-term AT1 receptor blockade. *Hypertension* 40(4):516–520
- Xu Y, Hu X, Wang L, Jiang Z, Liu X, Yu H, Zhang Z, Chen H, Chen H, Steinhoff G, Li J, Wang J'a (2013) Preconditioning via angiotensin II type 2 receptor activation improves therapeutic efficacy of bone marrow mononuclear cells for cardiac repair. *PLoS One* 8(12):e82997
- Yang BC, Phillips MI, Ambuehl PE, Shen LP, Mehta P, Mehta JL (1997) Increase in angiotensin II type 1 receptor expression immediately after ischemia-reperfusion in isolated rat hearts. *Circulation* 96(3):922–926
- Zhi-Bin H, Chang F, Mao-Huan L, Gui-Yi Y, Shu-Xian Z, Wei W (2014) Valsartan improves the electrophysiological characteristics of left ventricular hypertrophic myocardium in spontaneously hypertensive rats. *Hypertens Res* 37(9):824–829
- Zhu M, Gelband CH, Moore JM, Posner P, Summers C (1998) Angiotensin II type 2 receptor stimulation of neuronal delayed-rectifier potassium current involves phospholipase A2 and arachidonic acid. *J Neurosci* 18(2):679–686