Biochemical and Biophysical Research Communications 467 (2015) 645–650

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Continuous angiotensin-(1–7) infusion improves myocardial calcium transient and calcium transient alternans in ischemia-induced cardiac dysfunction rats

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ARTICLE INFO

Article history:
Received 6 October 2015
Accepted 19 October 2015
Available online 21 October 2015

Keywords:
Angiotensin-(1–7)
Heart failure
Calcium transient
Alternans

ABSTRACT

The aim of this study was to evaluate the impact of Ang-(1–7) on calcium transient (CaT) in cardiomyocytes during the pathogenesis of heart failure. Cardiac dysfunction was induced by ligation of left anterior descending coronary artery in adult SD rats. Randomly selected rats were ligated and continuously infused with Ang-(1–7) [HF + Ang-(1–7) group] or saline (HF + saline group) via osmotic minipumps. After 28 days, hemodynamic parameters, the CaT, and the heart rate threshold of CaT alternans (CaT-Alt) were measured. Continuous Ang-(1–7) treatment could attenuate the impairment of cardiac function following LAD ligation. The amplitudes (F/F_0) and 50%/90% recovery time of CaT were significantly different among HF + saline, HF + Ang-(1–7) and Sham-operated group. Compared to the Sham-operated group, the HF + saline group showed decreased CaT amplitude, and a prolonged 50%/90% CaT recovery time; Ang-(1–7) significantly improved these abnormalities. Compared with Sham-operated group, heart rate thresholds of CaT-Alt significantly reduced in HF + saline group, and Ang-(1–7) partly restored it. These findings indicate that Ang-(1–7) attenuates the CaT disturbance and increases the heart rate threshold of CaT-Alt during the pathogenesis of ischemic heart failure.

1. Introduction

Severe cardiac dysfunction and lethal arrhythmia are the two outcomes for patients with chronic heart failure (HF). Accumulating evidence has demonstrated that alteration of intracellular calcium homeostasis is an important cause of myocardial contractile dysfunction and electric activity instability [1,2]. In HF, the decreased amplitude and blunted ascending/recovery velocity of calcium transient (CaT) has been associated with depressed contractile function [3,4]. Moreover, the increased diastolic intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and sarcoplasmic reticulum (SR) calcium leak also led to delayed after-depolarization (DAD) and triggered activity (TA) [1].

In addition, it is well known that CaT alternans (CaT-Alt), which is the beat-to-beat alternation of CaT amplitude, could cause anisotropic activity conduction and promotes the incidence of malignant arrhythmias [5]. Compelling data has demonstrated that CaT-Alt is related with action potential duration alternans (APD-Alt) and T-wave alternans (TWA) [6]. And evidences showed that the heart rate threshold of cardiac alternans was lowered, and the arrhythmogenic cardiac alternans were enhanced during the pathogenesis of HF [7,8].

Angiotensin-(1–7) [Ang-(1–7)], a biologically active heptapeptide derived from Angiotensin I or Angiotensin II in renin-angiotensin system (RAS), plays a pivotal role in the cardiovascular system [9]. Interestingly, a growing number of studies have shown that Ang-(1–7) alleviates the development of HF [9], and the beneficial effects of angiotensin II receptor blockers (ARBs) and angiotensin converting enzyme inhibitor (ACEIs) on patients with HF are partly related to the regulation of Ang-(1–7) [10]. Moreover, recent evidence suggested that Ang-(1–7) affects the impulse propagation and cardiac arrhythmias in the failing heart [10,11], and treatment with Ang-(1–7) decreases the incidence and

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http://dx.doi.org/10.1016/j.bbrc.2015.10.093
0006-291X/

duration of ischemia-reperfusion arrhythmias [12]. Therefore, we hypothesized it was through ameliorating the disorder of intracellular calcium homeostasis that Ang-(1–7) could improve myocardial contractility and reduce arrhythmia in HF. Therefore, in this study, we aimed to evaluate the effects of persistent Ang-(1–7) infusion on the CaT and CaT-Alt heart rate threshold in isolated ventricular myocytes from ischemia-induced cardiac dysfunction rats.

2. Materials and methods

2.1. Animals

Twenty-one Sprague Dawley adult male rats (Experimental Animal Center, Guangdong, China) weighting 180–220 g were used in this experiment. The animals were housed at 20 °C ± 2 °C, 55 ± 20% humidity with 12-h light/dark cycles and free access to food and water in the Experimental Animal Center of Sun Yat-sen University. The animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th edition, 2011) and the Guidelines for the Human Use of Laboratory Animals of the Sun Yat-Sen University. The protocol was approved by the Animal Care and Use Committees at Sun Yat-sen University prior to initiation of the study.

2.2. Heart failure model and groups

The HF model was induced by ligation of left coronary artery as previously described [13,14]. Briefly, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (initial dose 40 mg/kg, additional 10 mg/kg was administered after 25 min if the surgery was not finished) , endotracheal intubated, and ventilated with room air (75 breaths/min, tidal volume 3 ml). A left thoracotomy was performed, and the proximal left anterior descending coronary artery (LAD) between the pulmonary artery outflow tract and the left atrium was ligated with a 6-0 silk suture. After closing the thorax, Alzet osmotic minipump (2ML4, Durect Corporation, Cupertino, Calif) was implanted subcutaneously and connected into left internal jugular vein. After the operation, buprenorphine (0.05 mg/kg) was injected intraperitoneally to recover the animals. The rats were randomly allocated and received to a continuous infusion of Ang-(1–7) (24 μg/kg per hour, HF + Ang-(1–7) group) or saline (HF + saline group) after ligation of LAD [15,16]. For the Sham-operated group, a silk suture was placed under the LAD without ligation and saline was minipumped.

2.3. Hemodynamic measurements

Twenty-eight days after the surgery, invasive hemodynamic measurements were performed. Briefly, a micro-tube (epidural anesthesia catheter, OD = 1 mm) was introduced into left ventricle through the right carotid artery. Left ventricular pressure/aortic pressure (when micro-tube was pulled back into aorta from left ventricle) and electrocardiogram were simultaneously recorded with Biopac MP150 data acquisition and analysis system (Biopac, USA). Left ventricular end diastolic pressure (LVEDP), the maximum and minimum first derivatives of LV pressure (±dp/dt), systolic aortic pressure (SAP), and diastolic aortic pressure (DAP) were measured. All measurements were recorded as the averages over 10 consecutive beats.

2.4. Isolation of cardiomyocytes

After measurement of hemodynamics, the rats were heparinized by intraperitoneal injection with heparin (3000 U/Kg), and euthanized by cervical dislocation in accordance with the UK Animals (Scientific Procedures) Act 1986. Ventricular myocytes were isolated by enzymatic dissociation according to our previous protocol [17]. Briefly, hearts were quickly excised after heparinization by intraperitoneal injection with heparin (3000 U/Kg), placed on the Langendorff apparatus, perfused with Ca^{2+}-free buffer solution (pH 7.4, 37 °C, gassed with a mixture of 95% O_2–5% CO_2), containing (in mM) NaCl 90, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 5, $NaHCO_3$ 15, taurine 30, and glucose 20. After 5 min, the perfusion was switched to the same buffer supplemented with 0.04% (w/v) collagenase, 0.1% (w/v) BSA, and 50 μM $CaCl_2$ for 30–45 min. Subsequently, the non-infarction part of left ventricles were separated and blown gently in the buffer solution with 50 μM $CaCl_2$ using a pipette. The supernatant was collected and filtered through a 200 μm nylon mesh to remove large particles. The myocytes were re-suspended in buffer with gradually increasing extracellular Ca^{2+} concentrations (250, 500, and 750 μM) to a final concentration of 1 mM. Finally, the preparation was washed twice by centrifugation at 100× *g* for 1 min to minimize the contamination from dead cells. All cells were used within 4 h after isolation.

2.5. Measurements of calcium transient and calcium transient alternans

After loaded with Fluo-4/AM (5 μM, Invitrogen, Eugene, OR, USA), the suspended myocytes were placed in a custom-built perfusion chamber mounted on the stage of a Leica SP5 laser scanning confocal microscope (LSCM; Leica Microsystems, Wetzlar, Germany), and continuously perfused with oxygenated physiologic buffer using a flow rate of 2 mL/min. Then the myocytes were field-stimulated (3 ms pulse duration, 5–10% above excitation threshold), and calcium transients were recorded through fluorescence intensity transient (F-t) by the LSCM (line scan, paralleled to long axis of myocytes, resolution pinhole 90NA; excitation/emission wavelengths: 488/525 nm). The stimulation frequencies were increased from 30 bpm by 30 bpm at a time until the CaT-Alt occurred, which was defined as the absolute difference in the amplitude of consecutive CaTs of more than 10% [18]. Then the stimulation frequency was further decreased by a stepwise of 10 bpm until the CaT-Alt disappeared. The minimum stimulation frequency that induced CaT-Alt was defined as the heart rate threshold of CaT-Alt ($T_{Cal\text{-}Alt}$). In order to minimize the bias and fluorescence bleaching, at each stimulation frequency, the cells were stimulated for 30s, and only CaTs of the last 10–15 paces were recorded. F/F_0 represented the relative intracellular Ca^{2+} concentration, where F was the fluorescence intensity at a specific time point during the cardiac cycle and F_0 was the basic diastolic fluorescence intensities. CaT dynamics were evaluated using CaT rise time (T_{peak}, the time rising from 10% to 90% CaT peak) and CaT 50%/90% of recovery time ($T_{50\%}/T_{90\%}$, the time from the onset of CaT to 50%/90% CaT peak recovery) at 60 bpm stimulation frequency.

2.6. L-type Ca^{2+} channel current detection

L-type Ca^{2+} channel currents ($I_{ca,L}$) was recorded, according to our previous reported protocols [19]. Briefly, after myocytes adhered to the bottom of a dish placed on the stage of an inverted microscope (Olympus IX70, Japan), the cells were perfused with solution (in mM, TEA-Cl 140, $CaCl_2$ 5, $MgCl_2$ 2, HEPES 10, and glucose 10, pH 7.4). Patch pipettes were fabricated from borosilicate glass capillaries (Corning 7740, 1.2 mm OD) and had a tip resistance