International Journal of Cardiology 236 (2017) 405–412

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Cellular basis of angiotensin-(1-7)-induced augmentation of left ventricular functional performance in heart failure☆,☆☆

Xiaowei Zhang [a], Heng-Jie Cheng [b], Peng Zhou [b], Dalane W. Kitzman [b], Carlos M. Ferrario [c,d,e], Wei-Min Li [a,*], Che Ping Cheng [a,b,**]

[a] Department of Cardiology, The First Affiliated Hospital of Harbin Medical University, Harbin, China
[b] Section on Cardiovascular Medicine, Wake Forest School of Medicine, Winston-Salem, NC, United States
[c] Department of Surgery, Wake Forest School of Medicine, Winston-Salem, NC, United States
[d] Department of Internal Medicine-Nephrology, Wake Forest School of Medicine, Winston-Salem, NC, United States
[e] Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC, United States

ARTICLE INFO

Article history:
Received 25 October 2016
Accepted 6 January 2017
Available online 10 January 2017

Keywords:
Heart failure
Angiotensin-(1-7)
Pressure-volume relation
Cells
Calcium transient
Contractility

ABSTRACT

Background: Angiotensin-(1-7) [Ang-(1-7)] exhibits cardiovascular effects opposite those of angiotensin II (Ang II), thus providing protection against heart disease. However, how Ang-(1-7) imparts cardioprotection is unclear, and its direct cardiac effects are controversial. Whether heart failure (HF) alters cardiac contractile responses to Ang-(1-7) remains undetermined. We tested the hypothesis that in HF, Ang-(1-7) may produce positive modulation on $[Ca^{2+}]_i$ regulation, enhancing left ventricular (LV) and myocyte contraction and relaxation via Ang-(1-7) Mas receptor coupled with nitric oxide (NO)/bradykinin (BK)-mediated mechanism.

Methods and results: We measured LV contractility changes after Ang-(1-7) (650 ng/kg, iv) and compared myocyte functional and $[Ca^{2+}]_i$ transient ($[Ca^{2+}]_{iT}$) responses to Ang-(1-7) superfusion in 24 normal rats and 34 rats with isoproterenol-induced HF (3 months after 170 mg/kg, s.q. for 2 days). To assess the mechanisms of altered HF responses to Ang-(1-7), subsets of HF myocytes were pretreated to inhibit NO synthase (L-NAME), BK (HOE-140), and Mas receptor (A-779) followed with Ang-(1-7). In normal rats, Ang-(1-7) produced no significant changes in LV and myocyte function. In HF rats, Ang-(1-7) significantly augmented LV contractility and relaxation with increased E_{ES} (51%), but decreased τ compared to baseline. Ang-(1-7) also significantly increased myocyte contraction (dL/dt_{max}, 30%), relaxation (dR/dt_{max}, 41%), and $[Ca^{2+}]_{iT}$. L-NAME increased, HOE-140 decreased, and A-779 prevented HF myocyte contractile responses to Ang-(1-7).

Conclusions: In a rat model of HF, Ang-(1-7) increases $[Ca^{2+}]_{iT}$, and produces positive inotropic and lusitropic effects in the LV and myocytes. These effects are mediated by the Mas receptor and involve activation of NO/BK pathways.

1. Introduction

Angiotensin-(1-7) [Ang-(1-7)] is an important bioactive component of the renin-angiotensin system (RAS) that can be formed by the hydrolytic action of neprilysin on angiotensin I or Angiotensin II (Ang II) degradation by angiotensin-converting enzyme 2 (ACE2) [1–5]. ACE2 and the Ang-(1-7) Mas receptor coexist in cardiomyocytes [5–7], and Ang-(1-7) can be generated directly within the myocardium. Ang-(1-7) levels increase after either ACE inhibitors (ACEI) or Ang II receptor blockers (ARB) in experimental models and humans, and Ang-(1-7) contributes to the beneficial effects of ACEI and ARB [2,8–11]. Ang-(1-7) opposes the pressor, trophic, profibrotic, and prothrombotic actions of Ang II [1,2,6,7,12–14].

Accumulating evidence suggests that Ang-(1-7) also may play a pivotal role in heart failure (HF) [1,3,8,12], in line with our previous findings of negative inotropic actions of Ang II in HF [15,16]. Ang-(1-7) and ACE2 cardiac expression as well as ACE2 activity are increased in the failing human heart and animals with experimentally induced HF [2–4,8]. The cardioprotective effects of Ang-(1-7), first shown by Loot et al. [8] in an experimental model of HF induced by coronary artery

☆ Grant Support: This study was supported by the National Institutes of Health grants [R01AG049770 (HJ Cheng); P01HL051952-21 (CM Ferrario); R01HL074318 (CP Cheng); R01AG0118915 and P30AG012332 (DW Kitzman)]; and National Natural Science Foundation of China (81270252) (WM Li).

☆☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and the discussed interpretation.

* Correspondence to: W.-M. Li, Department of Cardiology, The First Affiliated Hospital, Harbin Medical University, NO.23 Youzheng Street, Harbin 150001, China.

** Correspondence to: C.P. Cheng, Section on Cardiovascular Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1045, United States.

E-mail addresses: liweimin_2009@163.com (W.-M. Li), ccheng@wakehealth.edu (C.P. Cheng).

http://dx.doi.org/10.1016/j.ijcard.2017.01.071
0167-5273/

ligation, were characterized by Ferrario's laboratory [2] as associated with increased Ang-(1-7) expression in myocytes surrounding the infarcted tissue.

Although chronic Ang-(1-7) treatment has been associated with cardioprotection [8,9,17], no previous studies have shown its direct cardiac effects. Whether and how Ang-(1-7) may antagonize Ang II-induced depression on left ventricle (LV) contractile performance in HF, thus providing cardioprotection, is unknown. The integrated effects of Ang-(1-7) on LV contractility, independent of alterations in loading conditions, remain to be determined, and its role and mechanisms on myocyte contractile performance have not been defined. In addition, the direct effect of Ang-(1-7) on intracellular Ca^{2+} mobilization in myocytes is unclear, since previous studies provided conflicting findings [12,18,19]. Furthermore, the ever-emerging body of experimental evidence continues to support that an important subset of cardiovascular actions of Ang-(1-7) is related to its bradykinin (BK) potentiating activity and its facilitation of nitric oxide (NO) release [6,8,12,20,21]. It is reasonable to speculate that the effects of Ang-(1-7) on myocyte may via Ang-(1-7) receptors while be coupled with NO/BK-mediated mechanism. However, no previous study has specifically examined the functional effect of Ang-(1-7)/NO/BK/Mas pathway in cardiomyocytes. Whether and to what extent NO/BK pathway contributes to its protective effects in HF myocytes is unknown. It is important to understand its direct cardiac effects, especially if increasing Ang-(1-7) is a potential therapeutic strategy for patients with HF [1,13,22].

Accordingly, we assessed the direct cardiac effects and underlying cellular mechanisms of Ang-(1-7) in normal rats and rats with isoproterenol (ISO)-induced HF, an experimental paradigm mimicking many structural, functional, and hormonal changes of clinical HF [23–27]. We tested the hypothesis that after HF, Ang-(1-7) may produce positive modulation of LV function, improve myocyte contraction and relaxation, and $[Ca^{2+}]_i$ regulation via Ang-(1-7) Mas receptors, coupled with a NO/BK-mediated mechanism.

2. Methods

This study was approved by the Wake Forest School of Medicine Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication 8th edition, updated 2011). A detailed Methods section is available in the Online Supplement Materials, Briefly, we measured LV contractility changes after Ang-(1-7) (650 ng/kg, iv) and compared freshly isolated myocyte functional and $[Ca^{2+}]_i$ transient ($[Ca^{2+}]_{iT}$) responses to Ang-(1-7) (10^{-5} M) superfusion in 24 male normal rats and 34 male rats with ISO-induced HF (3 months after 170 mg/kg, s.q. for 2 days). To assess the mechanisms of altered HF responses to Ang-(1-7), subsets of HF myocytes were pretreated to inhibit NO synthase (L-NAME), BK (HOE-140), and Mas receptor (A-779) followed with Ang-(1-7). Experimental conditions, such as the duration of incubation and peptide concentrations, were based on our initial concentration-response studies and past reports by us and others.

3. Results

3.1. Verification of experimental HF

The rat model of ISO-induced HF has been studied by many investigators, including our laboratory [24–27]. Pathologic cardiac changes in ISO-treated rats resemble those of myocardial infarction (including LV structural remodeling, myocardial necrosis, eccentric-hypertrophy with reduced contractile functional performance, and decreased β-adrenergic reserve) [23–26,28].

In the present study, 34 rats in the HF group survived after ISO injection. All had clear evidence of HF (anorexia, edema, and pulmonary congestion). In line with our previous work and that of others [23,25,27], the total infarction area in ISO-injected rats averaged about 36 ± 5%. Diffuse subendocardial necrosis and pronounced LV enlargement with increased LV volume were also observed. Although body weights were similar in normal and HF rats (539 ± 31 vs. 528 ± 24 g, p = NS), cardiac weight (2.23 ± 0.07 vs. 1.66 ± 0.03 g, $p < 0.05$), ratio of heart-to-body weight (4.13 ± 0.25 vs. 3.13 ± 0.19 g/kg, $p < 0.01$), and the wet-lung-to-body weight ratio (4.24 ± 0.27 vs. 3.17 ± 0.19 g/kg, $p < 0.01$) were all significantly increased in HF rats. These findings documented the existence of established HF in this model.

Compared to normal rats, HF rats showed significant increases in LV end-diastolic volume (453.4 vs. 334.2 μl, $p < 0.01$) and LV end-systolic volume (336.1 vs. 155.3 μl, $p < 0.01$) with reduced stroke volume (SV) (151.8 ± 5.2 vs. 178.9 ± 7.8 μl, $p < 0.01$) and ejection fraction. LV contractility decreased >40% as measured by the slope of linear P_{ES}-V_{ES} relation (E_{ES}) (0.67 vs. 1.16 mm Hg/μl, $p < 0.01$) and the slope of stroke work - end-diastolic volume (V_{ED}) relation (M_{SW}) (64.4 vs. 108.0 mm Hg, $p < 0.01$). The time constant of LV relaxation (τ, 13.9 vs. 9.2 msec) was increased by 52% ($p < 0.01$). These LV abnormalities were accompanied with LV myocyte structural remodeling and functional impairment. As detailed in the Online Supplement Materials, with our well-established technique, more than an 80% yield of viable myocytes was obtained from both control and ISO-treated rats. After myocyte morphological examination, myocyte functional studies were performed. Only rod-shaped myocytes with clear edges were selected for the recording of mechanical properties or intracellular $[Ca^{2+}]_{iT}$ as we described previously [23,26,27,29]. The length of HF myocyte (HF: 147.4 ± 9.2 μm vs. Normal: 110.4 ± 6.0 μm, $p < 0.01$) and the length-width ratio (HF: 6.1 ± 0.7% vs. Normal: 4.3 ± 0.4%, $p < 0.01$) were significantly increased, which suggests a remodeling of myocyte shape in HF rats. In HF rats, LV myocyte contraction and relaxation were significantly depressed, as indicated by decreased peak velocity of shortening (dL/dt_{max}) (43%) and peak velocity of re-lengthening (dR/dt_{max}) (42%). Peak systolic $[Ca^{2+}]_{iT}$ was reduced (0.16 vs. 0.21, $p < 0.01$), and the decline of $[Ca^{2+}]_i$ was slower (details presented in supplemental data of online Tables 1 and 2).

3.2. Effects of Ang-(1-7) on hemodynamics and LV functional performance

3.2.1. Normal

Compared with normal values at baseline, Ang-(1-7) produced no change in heart rate, but significantly reduced end-systolic pressure (P_{ES}) [Ang-(1-7): 113.8 vs. baseline: 119.8 mm Hg, $p < 0.01$]. Systemic vascular resistance (SVR) (2.39 vs. 2.57 mm Hg/ml/min, p = NS), effective arterial elastance (E_A) (0.64 vs. 0.66 mm Hg/μl, p = NS), τ (9.0 vs. 9.2 msec, p = NS), end-diastolic pressure (P_{ED}) (5.5 vs. 5.7 mm Hg, p = NS) and the peak mitral flow (dV/dt_{max}, 6628 vs. 6586 μl/s, p = NS) were relatively unchanged. Ang-(1-7) did not change E_{ES} (1.17 vs. 1.16 mm Hg/μl, p = NS) and M_{SW} (109.5 vs. 108.0 mm Hg, p = NS) (Fig. 1A) or the steady-state LV P-V loops (Fig. 1B). This indicated that in normal rats, Ang-(1-7) had no inotropic effect on LV contractile performance and did not markedly alter LV diastolic filling dynamics.

3.2.2. HF

In HF rats, although Ang-(1-7) produced no marked change in heart rate, it caused significant reductions in P_{ES} (105.4 vs. 116.8 mm Hg, $p < 0.01$), SVR (2.47 vs. 3.58 mm Hg/ml/min, $p < 0.01$), E_A (0.70 vs. 1.00 mm Hg/μl, $p < 0.01$) and P_{ED} (10.7 vs. 15.9 mm Hg, $p < 0.01$) with decreased τ (11.3 vs. 13.9 msec, $p < 0.05$) compared with baseline ($p < 0.05$). Ang-(1-7) caused leftward shifts and increased E_{ES} (1.01 vs. 0.67 mm Hg/μl, $p < 0.01$) and M_{SW} (92.4 vs. 64.4 mm Hg, $p < 0.01$). This indicates that in HF, Ang-(1-7) produced a direct positive inotropic effect on LV contractile performance. LV-arterial coupling (E_{ES}/E_A) also was improved (1.45 vs. 0.68, $p < 0.01$). In addition, compared to baseline, Ang-(1-7) caused leftward and downward shifts of the early diastolic portion of the LV P-V loop in HF rats, decreasing early diastolic LV pressure. dV/dt_{max} (5360 vs. 4277 μl/s, $p < 0.01$) was significantly increased.