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Cellular Basis of Angiotensin-(1–7)-Induced Augmentation of Left Ventricular Functional Performance in Heart Failure ☆,☆☆

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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

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X.W.Z., H.J.C., and C.P.C. contributed to the conception and design of research; C.P.C. provided direction and supervision of the project; X.W.Z., H.J.C., and P. Z performed experiments; X.W.Z., P. Z., and H.J.C. analyzed data and the statistical analysis; X.W.Z., P.Z., H.J.C., C.M.F., D.W.K., W.M. L., and C.P.C. interpreted results of experiments; X.W.Z., and C.P.C. prepared figures; X.W.Z. drafted manuscript; C.M.F., D.W.K., W.M.L., and C.P.C. edited and revised it critically for important intellectual content; X.W.Z., H.J.C., P. Z., C.M.F., D.W.K., W.M. L., and C.P.C. approved final version of the manuscript.

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.

Keywords

Heart Failure; Angiotensin-(1–7); Pressure-volume relation; Cells; Calcium transient; Contractility

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 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 $36 \pm 5\%$. 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 more than 40% as measured by the slope of linear P_{ES} - V_{ES} relation (E_{ES}) (0.67 vs. 1.16 mmHg/ μL , $p < 0.01$) and the slope of stroke work - end-diastolic volume (V_{ED}) relation (M_{SW}) (64.4 vs. 108.0 mmHg, $p < 0.01$). 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 $[\text{Ca}^{2+}]_{\text{IT}}$ 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: $6.1 \pm 0.7\%$ vs Normal: $4.3 \pm 0.4\%$, $p < 0.01$) 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 $[\text{Ca}^{2+}]_{\text{IT}}$ was reduced (0.16 vs. 0.21 , $p < 0.01$), and the decline of $[\text{Ca}^{2+}]_{\text{i}}$ 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

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 mmHg, $p < 0.01$]. Systemic vascular resistance (SVR) (2.39 vs. 2.57 mmHg/ml/min, $p = \text{NS}$), effective arterial elastance (E_{A}) (0.64 vs. 0.66 mmHg/ μL , $p = \text{NS}$), τ (9.0 vs. 9.2 msec, $p = \text{NS}$), end-diastolic pressure (P_{ED}) (5.5 vs. 5.7 mmHg, $p = \text{NS}$) and the peak mitral flow (dV/dt_{max} , 6628 vs. 6586 $\mu\text{L/s}$, $p = \text{NS}$) were relatively unchanged. Ang-(1–7) did not change E_{ES} (1.17 vs. 1.16 mmHg/ μL , $p = \text{NS}$) and M_{SW} (109.5 vs. 108.0 mmHg, $p = \text{NS}$) (Figure 1A) or the steady-state LV P-V loops (Figure 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.

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 mmHg, $p < 0.01$), SVR (2.47 vs. 3.58 mmHg/ml/min, $p < 0.01$), E_{A} (0.70 vs. 1.00 mmHg/ μL , $p < 0.01$) and P_{ED} (10.7 vs. 15.9 mmHg, $p < 0.01$) 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 mmHg/ μL , $p < 0.01$) and M_{SW} (92.4 vs. 64.4 mmHg, $p < 0.01$). 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 $\mu\text{l/s}$, $p<0.01$) was significantly increased.

3.3. Effects of Ang-(1–7) on Myocyte Functional and $[\text{Ca}^{2+}]_{iT}$ Responses

Normal—In normal myocytes, Ang-(1–7) superfusion caused no significant changes in percent shortening (SA), dL/dt_{max} (152.9 vs. 150.3 $\mu\text{m/s}$), dR/dt_{max} (112.0 vs. 110.6 $\mu\text{m/s}$) and $[\text{Ca}^{2+}]_{iT}$ (0.22 vs. 0.21) ($p=\text{NS}$), indicating that Ang-(1–7) had no direct functional effects on normal myocytes (Figure 2). In HF myocytes, Ang-(1–7) significantly augmented cell contraction and relaxation with improved $[\text{Ca}^{2+}]_{iT}$.

HF—Compared with HF at baseline, Ang-(1–7) produced significant increases in SA, dL/dt_{max} (30%, 111.0 vs. 85.3 $\mu\text{m/s}$, $p<0.01$) and dR/dt_{max} (41%, 91.1 vs. 64.6 $\mu\text{m/s}$, $p<0.01$) accompanied by increased $[\text{Ca}^{2+}]_{iT}$ (0.20 vs. 0.16, $p<0.01$). The positive modulation occurred 2–3 minutes after the addition of Ang-(1–7), peaked 4 to 6 minutes later during superfusion of Ang-(1–7), and reverted to baseline values after Ang-(1–7) washout (Figure 2 and online Table 2).

3.4. Ang-(1–7)-Mediated Signal Pathways in Altered Myocyte Functional Responses in HF

Figure 3 shows superimposed tracings recorded from pre-treated LV myocytes with L-NAME (a), HOE-140 (b), losartan (LOS) (c), or A-779 (d), respectively, and then exposure to Ang-(1–7). Figure 4A summarizes the group means of effects of L-NAME, HOE-140, LOS and A-779 on myocyte functional responses to Ang-(1–7) in HF. Figure 4B shows group means of Ang-(1–7) induced percentage changes in myocyte functional performance in HF with and without pretreatment with L-NAME, HOE-140, LOS, or A-779, respectively.

3.4.1. Effects of NOS Blockade—Compared with HF control myocytes at baseline, after L-NAME incubation, baseline values of dL/dt_{max} (88.1 ± 2.5 vs. 85.3 ± 2.5 $\mu\text{m/s}$, $p=\text{NS}$) and dR/dt_{max} (67.2 ± 2.7 vs. 64.6 ± 2.4 $\mu\text{m/s}$, $p=\text{NS}$) increased, but did not reach statistical significance (Figures 3 and 4). However, L-NAME incubation significantly enhanced Ang-(1–7)-mediated positive inotropic effects in HF. In HF control myocytes, Ang-(1–7) caused 30% and 41% increases in dL/dt_{max} and dR/dt_{max} , respectively. After HF myocytes were pretreated with L-NAME, Ang-(1–7) caused significantly greater increases in dL/dt_{max} (42%, 125.0 ± 3.5 vs. 88.1 ± 2.5 $\mu\text{m/s}$, $p<0.01$) and dR/dt_{max} (50%, 101.1 ± 3.5 vs. 67.2 ± 2.7 $\mu\text{m/s}$, $p<0.01$) (Figure 4).

3.4.2. Effects of BK Blockade—Compared with HF control myocytes at baseline, after HOE-140 incubation, the baseline values of dL/dt_{max} (83.0 ± 4.7 vs. 85.3 ± 2.5 $\mu\text{m/s}$, $p=\text{NS}$) and dR/dt_{max} (62.4 ± 3.6 vs. 64.6 ± 2.4 $\mu\text{m/s}$, $p=\text{NS}$) were relatively unchanged (Figures 3 and 4). However, HOE-140 significantly attenuated Ang-(1–7)-mediated positive inotropic effects. Compared with HF control myocytes, Ang-(1–7) caused significantly less increase in dL/dt_{max} (23%, 101.7 ± 5.9 vs. 83.0 ± 4.7 $\mu\text{m/s}$, $p<0.01$) and dR/dt_{max} (22%, 76.4 ± 4.8 vs. 62.4 ± 3.6 $\mu\text{m/s}$, $p<0.01$) (Figure 4).

3.4.3. Effects of AT₁ or Ang-(1–7)-Mas Receptor Blockade—Mas receptor blockade completely prevented HF myocyte contractile responses to Ang-(1–7), while the Ang II AT₁ blocker had no effect. As shown in Figures 3 and 4, compared with HF control myocytes responses to Ang-(1–7), in the HF myocytes pretreated with LOS, the baseline was relatively unchanged. However, Ang-(1–7)-caused sustained increases in dL/dt_{max} (114.3 ± 3.2 vs. 87.6 ± 3.0 $\mu\text{m/s}$, $p < 0.01$) and dR/dt_{max} (93.9 ± 3.2 vs. 66.6 ± 2.9 $\mu\text{m/s}$, $p < 0.01$). In contrast, Ang-(1–7)-induced increases in dL/dt_{max} and dR/dt_{max} were eliminated after incubating myocytes with the Ang-(1–7) Mas receptor blocker A-779. Compared with baseline values, Ang-(1–7) superfusion caused no significant changes in dL/dt_{max} (84.8 ± 3.4 vs. 85.1 ± 3.2 $\mu\text{m/s}$, $p = \text{NS}$) or dR/dt_{max} (62.2 ± 3.5 vs. 62.6 ± 3.4 $\mu\text{m/s}$, $p = \text{NS}$).

4. Discussion

Our study demonstrates, for the first time, the direct Ang-(1–7) effects on LV myocytes in a rat model of HF. In myocytes obtained from normal rats, Ang-(1–7) has no effect on cardiac contractility, relaxation, or $[\text{Ca}^{2+}]_{\text{IT}}$. On the contrary, in HF rats, Ang-(1–7) increased $[\text{Ca}^{2+}]_{\text{IT}}$ and produced positive inotropic and lusitropic effects in the LV and in myocytes, accompanied with significantly better LV filling, LV-arterial coupling and LV mechanical efficiency. These effects were mediated by the Ang-(1–7) Mas receptor and involved downstream activation of NO/BK pathways.

This work reveals a key role for the Ang-(1–7)/Mas axis as a positive modulator of LV myocyte function and $[\text{Ca}^{2+}]_{\text{i}}$ regulation, in the presence of adverse cardiac remodeling associated with HF. These novel findings are significant and timely, because it not only by expanding the knowledge of how Ang-(1–7) may act in the heart as an autocrine/paracrine hormone to affect cardiac structure and function,(1;13;22) but for potentially uncovering new approaches for improving the efficacy of current therapeutic regimens. Accumulating evidence shows that Ang II intracellular effects are not inhibited by AT₁ blockade, while ACEIs or ARBs have limited efficacies in reversing or halting the progression of cardiovascular disease.(1;30) This investigation suggests that drug-treatment approaches that increase Ang-(1–7) activity within currently existing HF therapies may provide added benefit to patients with HF.

4.1. Positive Modulation of Ang-(1–7) on LV and Myocyte Functional Performance in HF

We previously demonstrated that HF alters LV and myocyte contractile behavior to Ang II stimulation.(15;16) In HF, acute Ang II administration causes direct decreases in LV, myocyte contractility, and relaxation rate, and impairs $[\text{Ca}^{2+}]_{\text{i}}$ regulation.(15) Whether Ang-(1–7) antagonizes Ang II-induced cardiac depression, thereby contributing to protective action in HF, is unknown. To answer this important open question in the field, the current investigation compared simultaneously intact animal and isolated cardiomyocyte functional responses to acute administration of Ang-(1–7) in normal and HF rats. In addition, to avoid the potentially confounding effects of Ang-(1–7) caused changes in loading conditions on conventional measures of LV performance, for the first time, we evaluated the effect of Ang-(1–7) on LV contractile performance using pressure-volume analysis, a load-independent measure of LV contractility.(15;31)

LV Functional Responses to Ang-(1–7)—We found that acute administration of Ang-(1–7) counteracted Ang II-related vasoconstriction and caused vasodilation in normal and HF rats.(1;9). Ang-(1–7) had no direct inotropic effects on LV functional performance in normal rats. Compared with normal rats, Ang-(1–7) produced more marked decreases in P_{ES} and significantly reduced SVR, E_A and V_{ED} in HF rats. The rate of LV relaxation depends on the systolic load. The dependence of relaxation on systolic LV pressure is enhanced in HF.(32) Ang-(1–7)-induced reduction in P_{ES} could improve both systolic LV performance and the rate of relaxation. SV was significantly augmented in HF rats due to the improved LV relaxation and diastolic filling. The reduced systolic load may be caused by Ang-(1–7)'s suppression of Ang II and endothelin-1 and their stimulation of NOS. This would increase NO or prostaglandin release and activation of guanylyl cyclase, thereby increasing cyclic guanosine monophosphate (cGMP), or affecting other biologically active peptides such as BK.(1;6;8;9;12) Importantly, we found that only in HF, Ang-(1–7) significantly increased the slopes and caused a leftward shift of the LV P_{ES} - V_{ES} relations, demonstrating increases in LV contractility. In addition to afterload reduction, Ang-(1–7) caused increased LV contractility also improved the rate of relaxation. These positive effects directly enhance LV systolic and diastolic functional performance in HF.

Moreover, we found that in HF, Ang-(1–7) also significantly improved LV-arterial coupling. We showed previously that LV-arterial coupling, which regulates SV at a given preload and closely relates to LV mechanical efficiency, is a key determinant of optimal cardiovascular function.(31) In HF, E_{ES} is significantly decreased, but E_A is increased, reducing the E_{ES}/E_A ratio and resulting in less maximal stroke work (SW).(31;32) We showed that this impaired LV-arterial coupling was exacerbated after Ang II administration.(15;16) Compared with HF rats at baseline, E_A significantly decreased and LV-arterial coupling (E_{ES}/E_A ratio) significantly improved, which resulted in near-maximum SW in HF rats.(31;32) Thus, LV mechanical efficiency was significantly augmented in HF rats after Ang-(1–7). This new finding is further strengthened by observations that Mas-deficient mice have impaired heart function improved by oral administration of Ang-(1–7).(14;33) These findings extend our understanding of the cardioprotective role of chronic Ang-(1–7) treatment in several cardiovascular diseases, such as cardioprotection against isoproterenol, improving myocardial performance and survival following ischemia/reperfusion injury, and blunting of cardiac remodeling in response to chronic Ang II infusion.(1;9;17;18;33)

Myocyte Functional Responses to Ang-(1–7)—We also found direct effects of Ang-(1–7) on cardiac myocytes isolated from the LV in normal and HF rats. These studies removed the effects of extra-cardiac factors, which may influence contractility. Similar to the findings in the intact LV, the response of the LV myocytes to Ang-(1–7) was altered after HF. In normal myocytes, Ang-(1–7) did not change the rate or extent of contraction or the speed of relengthening. But in HF myocytes, Ang-(1–7) significantly increased myocyte contraction and relaxation and peak systolic $[Ca^{2+}]_T$, indicating that acute Ang-(1–7) antagonizes Ang II-induced cardiac depression and reversed Ang II-mediated effects to positively modulate cardiac contractile performance in HF.(15;16)

4.2. Basic Cellular Mechanisms of Altered Cardiac Functional Responses to Ang-(1–7) in HF

The current study presents the additional novel finding that HF alters cardiac functional responses to Ang-(1–7). In HF, Ang-(1–7) caused cardiac positive modulation. Why doesn't Ang (1–7) have any effect on normal heart and cells but show the beneficial effect on ISO-induced HF animals? What is the mechanism of Ang-(1–7)'s positive modulation on LV contraction and relaxation in HF? The intracellular pathway coupling Ang-(1–7) stimulation is still incompletely characterized. The mechanisms on Ang-(1–7)-induced cardiac positive modulation are not entirely clear. Past observations suggest that factors such as altered Ang-(1–7) Mas receptor-mediated $[Ca^{2+}]_i$ regulation, increased Ang-(1–7)/ACE2 expression, and an altered signal transduction might contribute to these changes.(5;6) Our present study indicates that up-regulation of cardiac Ang-(1–7)/ACE2/Mas-mediated pathways may be responsible for the Ang-(1–7)-induced positive modulation of cardiac function in our HF animals. However, the exact contributions of upregulation of cardiac Ang-(1–7) Mas receptor *versus* altered NO- or BK-mediated pathways are unclear.

In the current study, we found that Ang-(1–7)-evoked cardiac positive modulation in HF may be at least partially mediated through improved $[Ca^{2+}]_i$ regulation. Past reports regarding the direct effects of Ang-(1–7) on intracellular Ca^{2+} mobilization in HF myocytes are conflicting.(12;18;19) One previous study indicated that acute Ang-(1–7) treatment does not modulate intracellular Ca^{2+} handling in normal wild-type cardiomyocytes.(12) Consistent with that work, in the current investigation, Ang-(1–7) failed to alter the $[Ca^{2+}]_{iT}$ in normal myocytes of SD rats. Importantly, we found that acute Ang-(1–7) improved Ca^{2+} signaling dysfunction in LV myocytes from rats with isoproterenol-induced HF. In addition, Ang-(1–7)-induced augmented myocyte contraction in HF was accompanied by significant increases in peak systolic $[Ca^{2+}]_{iT}$, consistent with a past report(34) and supported by our earlier finding that similar concentrations of Ang-(1–7) significantly increased L-type Ca^{2+} current in HF myocytes.(27)

Up-regulation of cardiac Ang-(1–7)/ACE2/Mas-mediated pathway in HF may be responsible for the Ang-(1–7)-induced positive response of HF myocyte.(1;3;4;8;30;35) Zisman et al.(3) found that ACE2 upregulation accompanied increased Ang-(1–7)-forming activity in failing human heart ventricles. It has been shown that both tissue and plasma levels of Ang-(1–7) were significantly increased in HF subsequent to myocardial infarction.(4;8) Past report also documented that Ang-(1–7)/ACE2 axis was induced in pressure overloaded rat model, and the levels of plasma ACE2 and Ang-(1–7) from HF group were significantly upregulated compared with control.(35)

Enhanced response to Ang-(1–7) stimulation in HF may also be related to up-regulation of Mas receptors or improved signal transduction.(1;12;36–39) In the current study, we found that the increased myocyte contraction and relaxation persisted in the presence of AT_1 blockade with losartan, but was fully prevented by the selective Ang-(1–7) Mas receptor antagonist, A-779. These data confirm that the positive inotropic effects in the intact LV of HF rats were due to a direct Mas receptor-mediated effect on LV myocytes. Mas receptors are localized in cardiac myocytes, and their activation by Ang-(1–7) improves cardiac function and remodeling, as well as attenuating HF.(12;14;36) Mas expression patterns vary

in different animal strains, disease models, and even at different stages for the same disease. (5) We speculate that increased expression of Mas receptor density on the membrane of HF cells and/or the sensitivity of the Mas receptor may contribute to our current findings.(3) Further studies are currently underway to elucidate this point in our laboratory.

The altered NO- or BK-mediated pathways resulted from the changes of NOS or BK expression and function in HF may also contribute to the enhanced cardiac functional responses to Ang-(1–7) stimulation in HF.(27;40–42) Important subsets of cardiovascular actions of Ang-(1–7) are related to its BK potentiating activity and its facilitation of NO release.(8;9;12) Past reports showed that the stimulatory effect of Ang-(1–7) on NO production was inhibited by the Mas receptor antagonist (A-779), or a nonspecific NOS inhibitor (L-NAME).(11) In the current study, we found that BK activation increased, but NOS activation decreased, Ang-(1–7)-caused positive modulation on myocyte contraction and relaxation in HF. Our previous study demonstrated that endogenous bradykinin acting through BK B2-receptors (B2R) improved LV relaxation and contractile performance in HF. (40) Pretreatment of HF myocytes with BK blockade by HOE-140 decreased Ang-(1–7)-stimulated myocyte contraction and relaxation, which suggested cross-talk between Mas and B2R in response to Ang-(1–7). On the other hand, pretreatment of HF myocytes with the non-selective NOS inhibitor, L-NAME, increased Ang-(1–7)-stimulated myocyte contraction and relaxation. These findings indicate that BK activation increases, but NOS activation decreases, Ang-(1–7) positive modulation on myocyte contraction in HF. It is noted that the Ang-(1–7)-induced increased in LV myocytes functional performance was further augmented by L-NAME. This indicates that Ang-(1–7)-stimulated NO release evokes negative inotropic action. This is consistent with the past reports that NO attenuated myocyte contraction.(43–45) This effect alone may have opposite action on Ang-(1–7)-caused positive modulation of myocyte contraction in HF. However, Ang-(1–7) also stimulates BK-mediated pathways. BK-activation increases myocyte function, thus enhances Ang-(1–7) positive modulation on myocyte contraction in HF. It appears that the cardio-stimulatory action of Ang-(1–7) mediated BK-activation overcomes NO negative inotropic effects, resulting in net improvement of cardiac performance in HF. Thus the direct cardiodepressant effects of NO should not limit potential Ang-(1–7) usefulness in HF. In addition, the beneficial effects of NO on vascular function may further override the negative inotropic action due to Ang-(1–7)-activated NO release in HF myocytes. It is increasingly recognized that NO can have a variety of effects, sometimes opposing, depending on the dose and site of action within the cell.(46) In fact, the inotropic actions of NO are controversial. NO has been reported variably to have different inotropic effects. Low (submicromolar) doses of NO may exert a small positive inotropic effect on basal contractile function, and higher (micromolar and above) doses of NO have been reported to induce negative inotropic effects.(43;44) Our current findings are supported by the past observations showing a key role for NO/cGMP as a mediator of Ang-(1–7) beneficial effects in cardiac cells.(1;6;11;13;34) In HF, NO/cGMP signaling and the expression and/or activity of BK may be changed, thereby altering HF myocyte response to Ang-(1–7) stimulation.(47) Further studies are needed to fully characterize the intracellular pathway modulating Ang-(1–7) stimulation.

5. Study limitations

Our study has several limitations. Although pathologic changes in ISO-treated rats resemble those of myocardial infarction(24) and ISO-induced HF mimics many structural, functional, and neurohormonal changes of clinical HF,(24;25) we cannot ascertain that these results would apply to clinical HF or HF from other causes, such as pure pressure overload cardiomyopathy or pure volume overload. Second, we chose the Ang-(1–7) doses used based on our initial concentration-response studies, similar to those in past reports. (39;48;49) However, these doses are higher than those reported in the blood circulation. Concentrations of hormones such as Ang-(1–7) are in the nanomolar range in interstitial tissue compartments.(38;39) Because Ang-(1–7) is generated in rat myocardium(4) and in the intact and failing human heart,(3) Ang-(1–7) tissue levels may exceed Ang-(1–7) plasma levels, in analogy to the situation with Ang II.

6. Conclusions

HF alters LV and myocyte functional responses to Ang-(1–7). In HF, Ang-(1–7) increase $[Ca^{2+}]_{iT}$, and produce positive inotropic and lusitropic effects in both LV and myocytes, accompanied by significantly augmented LV filling and improved LV-arterial coupling and LV mechanical efficiency. These effects are mediated by the Ang-(1–7) Mas receptor and involve the activation of NO/BK pathways. This study provides new evidence for a critical counter-regulatory role of the ACE2/Ang-(1–7) axis on the activated renin-angiotensin system, and support the view that Ang-(1–7) is a key protective component against HF. These results point to a potential new molecular target for HF, which is sharply rising in incidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Examples of the effects of Angiotensin-(1-7) [Ang-(1-7)] on left ventricular (LV) systolic and diastolic functional performance in normal and after heart failure (HF)

1A. Effects of Ang-(1-7) on LV end-systolic pressure (P_{ES})-end-systolic volume (V_{ES}) relations. Shown are LV pressure-volume (P-V) loops produced by inferior vena cava occlusions obtained from one control rat and one HF rat at baseline (dotted line) and after Ang-(1-7) infusion (solid line). P_{ES} - V_{ES} relation is indicated by the line. The slope and position of this line provide a load-insensitive measure of LV contractility. In normal rat, compared with baseline, Ang-(1-7) did not markedly affect P_{ES} - V_{ES} relations. In contrast, in HF rat, compared with baseline, Ang-(1-7) produced a leftward shift of P_{ES} - V_{ES} relations with increased slopes, indicating increased LV contractility.

1B. Effect of Ang-(1-7) on LV diastolic filling. Shown are the steady-state LV P-V loops obtained from the same rats as shown in Figure 1A. Each loop was generated by averaging the data obtained during a 12 to 15-second recording period, spanning several respiratory cycles. In normal rat, compared with baseline, Ang-(1-7) did not alter the P-V loops. In HF

rat, Ang-(1–7) caused a leftward and downward shift of the P-V loops with a greater stroke volume and improved LV diastolic filling.

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Figure 2.

Examples of LV myocyte functional responses and $[Ca^{2+}]_i$ regulation at baseline, and response to Ang-(1-7) stimulation. Analog recordings in the electrically-stimulated myocytes obtained from one normal rat and one HF rat. Freshly-isolated myocytes were tested after being superfused with a modified HEPES solution and field stimulated at 0.5 Hz and then after superfusion of Ang-(1-7). The percent shortening (SA), peak velocity of shortening (dL/dt_{max}), peak velocity of relengthening (dR/dt_{max}) and peak $[Ca^{2+}]_i$ transient ($[Ca^{2+}]_{iT}$) were assessed in myocytes. Only in HF myocytes, Ang-(1-7) caused markedly increases in SA, dL/dt_{max} , dR/dt_{max} and the peak $[Ca^{2+}]_{iT}$.

Figure 3.

Examples of the effects of nitric oxide synthase (NOS), bradykinin (BK), Angiotensin II type 1 receptor (AT_1) and Ang-(1-7) Mas receptor blockers on myocyte contractile functional responses to the exposure of Ang-(1-7) in HF. The subsets of HF myocytes were pretreated to inhibit NOS (L-NAME) (a), BK (HOE-140) (b), AT_1 [Losartan (LOS)] (c) or Ang-(1-7) Mas receptor (A-779) (d) followed with Ang-(1-7) continuous superfusion. Superimposed tracings were recorded before and after exposure to Ang-(1-7). Compared with corresponding baselines, L-NAME increased, HOE-140 decreased, LOS unaltered, and A-779 prevented HF myocyte contractile responses to Ang-(1-7).

B

Figure 4. Different effects of L-NAME, HOE-140, Losartan and A-779 on Ang-(1-7) induced changes to LV myocyte functional performance in HF

4A. Group means of L-NAME, HOE-140, Losartan (LOS) and A-779 on Ang-(1-7) induced changes in myocyte contraction dL/dt_{max} and relaxation dR/dt_{max} of HF rats. Compared with untreated HF control myocytes, after HF myocytes were pretreated with L-NAME, Ang-(1-7) caused significantly greater increases in dL/dt_{max} and dR/dt_{max} . In contrast, after HF myocytes incubation of HOE-140, the Ang-(1-7) produced significantly less increases in dL/dt_{max} and dR/dt_{max} . The HF myocytes pretreated with LOS, Ang-(1-7)-caused increases in dL/dt_{max} and dR/dt_{max} persisted. In contrast, after incubating HF myocytes with A-779, Ang-(1-7)-induced increased in dL/dt_{max} and dR/dt_{max} were eliminated. * $P < 0.05$, Ang-(1-7) vs. corresponding baselines in HF; ** $P < 0.05$, Ang-(1-7)-induced changes after drug incubation vs. Ang-(1-7) response without drug incubation in HF.

4B. Group means of Ang-(1-7) induced percentage changes in myocyte functional performance in HF with and without pretreatment with L-NAME, HOE-140, Losartan or A-779, respectively. L-NAME increased, HOE-140 decreased, Losartan unaltered, and

A-779 prevented Ang-(1–7) induced percentage changes in $dL/dt_{\max}$ and $dR/dt_{\max}$. * $P < 0.05$, Ang-(1–7)-induced percentage changes after drug incubation vs. HF control & Ang-(1–7).

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