Published in final edited form as:

Hypertension. 2015 August ; 66(2): 260–262. doi:10.1161/HYPERTENSIONAHA.115.05093.

ANGIOTENSINS AND THE HEART: IS ANG 1-7 CARDIOPROTECTIVE?

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Left ventricular hypertrophy is the most common cardiac complication of hypertension. While the initial adaptations associated with cardiac hypertrophy are compensatory, ultimately abnormal ventricular function including diastolic dysfunction (impaired relaxation) and often heart failure may develop.¹ Activation of the renin-angiotensin system (RAS) and its main effector peptide angiotensin II (Ang II), acting on the AT1 receptor, has been considered an important part of the cascade leading to left ventricular hypertrophy and cardiac fibrosis.^{1, 2} Recent work, however, examining the effects of Ang II infusion using cardiomyocyte and vascular smooth muscle-specific AT1 knockouts suggests that the hypertension-induced increase in afterload, rather than direct Ang II-AT1 signaling in the heart, is the key factor that promotes hypertrophic responses.² The RAS peptide Ang-(1-7), which is generated from Ang II by the action of carboxypeptidases such as ACE2,³ exhibits actions that are mainly opposite to those of Ang II, including vasodilatory and anti-fibrotic effects.⁴

In this issue, de Almeida et al.⁵ report a series of interesting observations that suggest that in an Ang-(1-7) transgenic line, TGR(A1-7)3292, there is cardioprotection from DOCA-induced hypertension which is independent of blood pressure. The latter conclusion is not unexpected considering the strong evidence against an antihypertensive effect of Ang-(1-7): a) acute infusions of supraphysiologic concentrations of this peptide do not lower blood pressure in mice,³ b) a 4-week continuous infusion of Ang-(1-7) did not decrease blood pressure in DOCA-treated Sprague-Dawley (SD) rats,⁶ c) acutely Ang-(1-7) does not attenuate the hypertensive effect of infused Ang II, and a blocker of the Mas receptor also does not worsen the blood pressure response to infused Ang II³, and d) the antihypertensive effects of an Ang II antagonist are not altered by the concomitant administration of the Ang-(1-7) receptor blocker.⁷ Notwithstanding these observations, the rat transgenic TGR(A1-7)3292 used displayed an attenuated hypertensive response to DOCA, and appropriate experiments were performed to show that the observed cardioprotective effects

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Disclosures: None

were not found in control animals with hydralazine-induced attenuated blood pressure levels⁵. Previous studies have shown cardioprotective effects of Ang-(1-7)^{6, 8, 9}. The question then arises: how does Ang-(1-7) act directly on cardiomyocytes to reduce hypertrophic responses?

de Almeida et al.⁵ examines this question in detail using the DOCA hypertension model in SD control and TGR(A1-7)3292 rats by investigating cardiomyocyte-specific molecular pathways that are associated with hypertrophy and diastolic dysfunction. As expected, SD rats treated with full-dose DOCA (SD-DOCA) developed hypertension, and echocardiography revealed concentric left ventricular hypertrophy and impaired relaxation. In addition, cardiomyocytes isolated from SD-DOCA rats expressed increased levels of cardiac stress markers; furthermore, Ca^{2+} homeostasis was disturbed in SD-DOCA cardiomyocytes with reduced peak Ca^{2+} transients and reduced expression of sarcoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2), which mediates the rapid reuptake of cytosolic Ca^{2+} . However, the rate of Ca^{2+} reuptake, which is prolonged in the settings of diastolic dysfunction and decreased SERCA2a activity, was not reported. Similar to their previous results,⁸ TGR(A1-7)3292 rats treated with DOCA demonstrated an absence or attenuation of several echocardiographic hypertrophic changes, as compared with baseline TGR(A1-7)3292 rats; importantly, however, left ventricular posterior wall and interventricular thickness were similar in TGR(A1-7)3292 and SD rats at baseline despite a 20% lower body weight in TGR(A1-7)3292 rats. SERCA2 protein levels and phospholamban signaling pathways were preserved in TGR(A1-7)3292-DOCA rats, as compared with SD-DOCA rats⁵. The relative differences between SD and SD-DOCA versus TGR(A1-7)3292 and TGR(A1-7)3292-DOCA rats overall support a protection from cardiac hypertrophy and diastolic dysfunction in TGR(A1-7)3292-DOCA rats.

As noted above, the authors administered hydralazine to one group of SD-DOCA rats and reduced DOCA dose to a second group of SD rats to match the blood pressure in TGR(A1-7)3292-DOCA rats. Despite attenuated blood pressure increase, SD-DOCA-hydralazine rats displayed more significant echocardiographic markers of cardiac hypertrophy than TGR(A1-7)3292-DOCA rats, and both SD-DOCA-hydralazine and SD-low-DOCA demonstrated diastolic dysfunction. Interestingly, cardiomyocytes isolated from SD-DOCA-hydralazine rats elicited a high peak transient Ca^{2+} that was similar to TGR(A1-7)3292-DOCA rats but was in contrast to the low peak transient Ca^{2+} in SD-DOCA; however, one would expect the SD-DOCA and SD-DOCA-hydralazine groups to be similar to each other and different from TGR(A1-7)3292-DOCA, given diastolic dysfunction in both SD control groups. Likewise, levels of phosphorylated phospholamban, which stimulates SERCA2 and promotes normal diastolic function, were similar between SD-DOCA-hydralazine and TGR(A1-7)3292-DOCA rats, and significantly higher than in SD-DOCA rats; one would expect decreased phosphorylated phospholamban in SD-DOCA-hydralazine rats, given the presence of diastolic dysfunction. These incongruous results require additional investigation to better understand the mechanism of improved echocardiographic diastolic function only in TGR(A1-7)3292-DOCA rats as compared to SD-DOCA and SD-DOCA-hydralazine rats.

What role does attenuated fibrosis play in the cardiac protection mediated by Ang-(1-7)? While the current study focused on cardiomyocytes, fibroblasts can bind Ang-(1-7) *in vitro* to reduce collagen expression¹⁰. Consistent with these data, chronic Ang-(1-7) infusion in SD-DOCA rats attenuated interstitial and perivascular collagen deposition⁶ and Ang-(1-7) co-infusion with Ang II prevented both hypertension-induced cardiac interstitial fibrosis and cardiomyocyte hypertrophy.¹¹ Furthermore, the previous study of TGR(A1-7)3292 DOCA-treated rats found attenuated expression of collagen types I and III that accompanied the improvement of cardiac function.⁸ Therefore, one could assume that the beneficial cardiac effects in the current study were not limited solely to improved myocyte function, but potentially to fibrosis reduction as well.

What other cardiomyocyte pathways might contribute to the cardioprotective effects of Ang-(1-7)? Cardiac effects of Ang-(1-7) are mediated by the Mas receptor through various signaling pathways including autocrine, MAPK/ERK, AKT, NADPH oxidase, TGF- β 1, EGFR, and NF- κ B activity.¹² Interestingly, de Almeida et al.⁵ found both elevated Mas protein levels and increased phosphoERK (activated MAPK/ERK) only in SD-DOCA cardiomyocytes, while phosphoERK was reduced in TGR(A1-7)3292-DOCA cardiomyocytes; these observations do not fit a model of increased plasma Ang-(1-7) and Mas activity as the mechanism of cardioprotection. Moreover and unexpectedly, their previous study in TGR(A1-7)3292 DOCA-treated rats revealed normal plasma Ang-(1-7) but increased local cardiac Ang-(1-7), and the mechanism underlying this phenomenon remains unknown.⁸

Several other issues deserve comment. The transgene used to generate TGR(A1-7)3292 rats consisted of a fusion protein containing the human renin signal peptide followed by the Fc portion of mouse IgG, then the prosegment of human prorenin, a cleavage site for furin (a ubiquitous protease), and finally Ang-(1-7).¹³ This fusion protein was driven by the CMV promoter with the intention to generate Ang-(1-7) in all tissues, but the transgene only expressed in testes and generated a 2-fold increase in plasma Ang-(1-7) levels.¹³ It is unclear whether control SD rats used in the present study were littermates of TGR(A1-7)3292 rats; if not, some effects in TGR(A1-7)3292 rats, including smaller size and potentially the attenuated DOCA-induced blood pressure rise, may be due to founder effects rather than increased plasma Ang-(1-7). Moreover, levels of this peptide were not reported in the present study, and therefore it is uncertain if the observed effects are directly related to increased levels of circulating and/or local cardiac Ang-(1-7). Experiments using a blocker of the Mas receptor would have provided important insight into the role of Ang-(1-7), since increased Mas receptor level in SD-DOCA rats but unchanged Mas receptor level in TGR(A1-7)3292-DOCA rats is difficult to interpret mechanistically. Finally, confirmation of the current results using alternative hypertension models would add strength to the molecular mechanisms outlined in this study; thoracic artery constriction would be a better model, as it non-pharmacologically increases ventricular afterload. Looking ahead for therapeutic application, it remains uncertain whether exogenous Ang-(1-7) or Mas receptor agonists will replicate the cardioprotection observed in the transgenic Ang-(1-7) model studied by De Almeida et al.⁵, but such studies in hypertensive models are clearly worthy of consideration.

Acknowledgments

Sources of Support: This work was supported by a grant from the National Institute of Diabetes and Digestive Kidney Diseases (grant 1R01DK080089–01A2) and a gift to Northwestern University by the Joseph and Bessie Feinberg Foundation.

Literature

1. Cacciapuoli F. Molecular mechanisms of left ventricular hypertrophy (lvh) in systemic hypertension (sh)-possible therapeutic perspectives. *Journal of the American Society of Hypertension : JASH.* 2011; 5:449–455. [PubMed: 22015318]
2. Sparks MA, Stegbauer J, Chen D, Vivekanandan-Giri A, Pennathur S, Crowley SD, Gurley SB, Coffman TM. Abstract 424: Cardiac hypertrophy in angiotensin ii-dependent hypertension: Dominant effect of blood pressure. *Hypertension.* 2014; 64:A424.
3. Wysocki J, Ye M, Rodriguez E, Gonzalez-Pacheco FR, Barrios C, Evora K, Schuster M, Loibner H, Brosnihan KB, Ferrario CM, Penninger JM, Battle D. Targeting the degradation of angiotensin ii with recombinant angiotensin-converting enzyme 2: Prevention of angiotensin ii-dependent hypertension. *Hypertension.* 2010; 55:90–98. [PubMed: 19948988]
4. Campagnole-Santos MJ, Diz DI, Santos RA, Khosla MC, Brosnihan KB, Ferrario CM. Cardiovascular effects of angiotensin-(1-7) injected into the dorsal medulla of rats. *Am J Physiol.* 1989; 257:H324–329. [PubMed: 2750946]
5. de Almeida PW, et al. Beneficial effects of angiotensin-(1-7) against doca-induced diastolic dysfunction occur independently of changes in blood pressure. *Hypertension.* 2015; 65
6. Grobe JL, Mecca AP, Mao H, Katovich MJ. Chronic angiotensin-(1-7) prevents cardiac fibrosis in doca-salt model of hypertension. *American journal of physiology. Heart and circulatory physiology.* 2006; 290:H2417–2423. [PubMed: 16415071]
7. Varagic J, Ahmad S, VonCannon JL, Moniwa N, Brosnihan KB, Wysocki J, Battle D, Ferrario CM. Predominance of at(1) blockade over mas-mediated angiotensin-(1-7) mechanisms in the regulation of blood pressure and renin-angiotensin system in mren2.Lewis rats. *Am J Hypertens.* 2013; 26:583–590. [PubMed: 23459599]
8. Santiago NM, Guimaraes PS, Sirvente RA, Oliveira LA, Irigoyen MC, Santos RA, Campagnole-Santos MJ. Lifetime overproduction of circulating angiotensin-(1-7) attenuates deoxycorticosterone acetate-salt hypertension-induced cardiac dysfunction and remodeling. *Hypertension.* 2010; 55:889–896. [PubMed: 20212262]
9. Grobe JL, Der Sarkissian S, Stewart JM, Meszaros JG, Raizada MK, Katovich MJ. Ace2 overexpression inhibits hypoxia-induced collagen production by cardiac fibroblasts. *Clin Sci (Lond).* 2007; 113:357–364. [PubMed: 17600530]
10. Iwata M, Cowling RT, Gurantz D, Moore C, Zhang S, Yuan JX, Greenberg BH. Angiotensin-(1-7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. *American journal of physiology. Heart and circulatory physiology.* 2005; 289:H2356–2363. [PubMed: 16024575]
11. Grobe JL, Mecca AP, Lingis M, Shenoy V, Bolton TA, Machado JM, Speth RC, Raizada MK, Katovich MJ. Prevention of angiotensin ii-induced cardiac remodeling by angiotensin-(1-7). *American journal of physiology. Heart and circulatory physiology.* 2007; 292:H736–742. [PubMed: 17098828]
12. Bader M, Alenina N, Andrade-Navarro MA, Santos RA. Mas and its related g protein-coupled receptors, mrgprs. *Pharmacological reviews.* 2014; 66:1080–1105. [PubMed: 25244929]
13. Santos RA, Ferreira AJ, Nadu AP, Braga AN, de Almeida AP, Campagnole-Santos MJ, Baltatu O, Ilescu R, Reudelhuber TL, Bader M. Expression of an angiotensin-(1-7)-producing fusion protein produces cardioprotective effects in rats. *Physiological genomics.* 2004; 17:292–299. [PubMed: 15039487]