Inhibition of Angiotensin-Converting Enzyme 2 Exacerbates Cardiac Hypertrophy and Fibrosis in Ren-2 Hypertensive Rats

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BACKGROUND

Emerging evidence suggests that cardiac angiotensin-converting enzyme 2 (ACE2) may contribute to the regulation of heart function and hypertension-induced cardiac remodeling. We tested the hypothesis that inhibition of ACE2 in the hearts of (mRen2)27 hypertensive rats may accelerate progression of cardiac hypertrophy and fibrosis by preventing conversion of angiotensin II (Ang II) into the antifibrotic peptide, angiotensin-(1–7) (Ang-(1–7)).

METHODS

Fourteen male (mRen2)27 transgenic hypertensive rats (12 weeks old, 401 ± 7 g) were administered either vehicle (0.9% saline) or the ACE2 inhibitor, MLN-4760 (30 mg/kg/day), subcutaneously via mini-osmotic pumps for 28 days.

RESULTS

Although ACE2 inhibition had no effect on average 24-h blood pressures, left ventricular (LV) Ang II content increased 24% in rats chronically treated with the ACE2 inhibitor ($P < 0.05$). Chronic ACE2 inhibition had no effect on plasma Ang II or Ang-(1–7) levels. Increased cardiac Ang II levels were associated with significant increases in both LV anterior, posterior, and relative wall thicknesses, as well as interstitial collagen fraction area and cardiomyocyte hypertrophy in the transgenic animals chronically treated with the ACE2 inhibitor. Cardiac remodeling was not accompanied by any further alterations in LV function.

CONCLUSIONS

These studies demonstrate that chronic inhibition of ACE2 causes an accumulation of cardiac Ang II, which exacerbates cardiac hypertrophy and fibrosis without having any further impact on blood pressure or cardiac function.

Keywords: *angiotensin-converting enzyme 2; angiotensin II; angiotensin-(1-7); blood pressure; cardiac hypertrophy; hypertension*

Am J Hypertens 2010; **23**:687-693 © 2010 American Journal of Hypertension, Ltd.

Hypertension and cardiac hypertrophy are two of the most critical risk factors contributing to heart disease,[1] and the involvement of the renin–angiotensin system to the pathophysiology of hypertension and cardiac hypertrophy is undisputed.[2] Undeniably, enhanced activity of the mitogenic and pressor peptide, angiotensin (Ang) II, causes elevations in blood pressure and contributes significantly to the development of cardiac hypertrophy and fibrosis.[3,4] The heptapeptide angiotensin-(1–7) (Ang-(1–7)) acts in the heart to oppose the actions of Ang II in an antiproliferative, antiarrhythmic, antifibrotic, and antihypertrophic manner.[5–7] These Ang-(1–7) properties correct cardiac functional deficits induced by myocardial ischemia.[8–10]

An emerging key to the regulation of the balance of Ang II and Ang-(1–7) in the heart is angiotensin-converting enzyme 2 (ACE2).[11,12] This enzyme was identified as a critical regulator of cardiac function because ACE2 knockout mice exhibited severe cardiac dysfunction that was associated with thinning of the left ventricular (LV) wall.[11] Because Vickers *et al.*[13] reported that ACE2 hydrolyzes Ang II into Ang-(1–7) with high efficiency, ACE2 may limit the effects of Ang II by facilitating its conversion to the antihypertrophic peptide, Ang-(1–7).[14] Moreover, previous studies from our laboratory showed a dependence on ACE2 for cardiac Ang-(1–7) production from Ang II in Ren-2 hypertensive rats.[15] Studies investigating the *in vivo* importance of ACE2 on the heart have largely involved genetic knockout mice,[11,16,17] the results of which may have been dependent upon genetic differences in the background strains used in those studies.[18] Furthermore, ACE2 overexpression in the heart reversed cardiac hypertrophy and fibrosis,[19,20] although a recent report did not support this view given that in these experiments, ACE2 overexpression induced severe cardiac fibrosis.[21] In previous studies, no attempt was made to directly assess the effects of sustained

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Received 24 November 2009; first decision 12 January 2010; accepted 11 February 2010; advance online publication 18 March 2010. doi:10.1038/ajh.2010.51

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inhibition of endogenous ACE2 on the hemodynamic and cardiac tissue content of angiotensin peptides. Therefore, this study examined whether blockade of endogenous ACE2 in the Ren-2 hypertensive rat would shift the balance of cardiac Ang II and Ang-(1–7) toward the pressor Ang II, and to determine whether this shift was associated with structural and functional changes within the myocardium.

METHODS

Animals. Fourteen male hemizygous (mRen2)27 transgenic hypertensive rats were obtained from the colony maintained at the Wake Forest University Hypertension and Vascular Research Center (Winston-Salem, NC). All animals were pair-housed in cages until 10 weeks of age (12-h light/dark cycle) in an AAALAC International–approved facility with *ad libitum* access to rat chow and reverse osmosis water. After this time, all rats were housed singly for continuous monitoring of arterial pressure and heart rate using radiotelemetry probes implanted at 10 weeks of age. Procedures were approved by our institutional Animal Care and Use Committee.

Experimental protocol. Fourteen days after the radiotelemetry probe implant (see **Supplementary Methods** online), (mRen2)27 hypertensive rats (401 ± 7 g) were randomly divided into two treatment groups: one group (n = 7) received vehicle (0.9% saline subcutaneously) and the other group (n = 7) was administered the ACE2 inhibitor, MLN-4760 (30 mg/kg/day subcutaneously; Millennium Pharmaceuticals, Cambridge, MA) via Alzet mini-osmotic pumps (Model 2ML4; Durect, Cupertino, CA). Previous studies showed that MLN-4760 specifically inhibits ACE2 (refs. 15,22) and that *in vivo* treatment at a similar dose and duration resulted in significant renal hypertrophy in diabetic mice.[23] Moreover, preliminary studies from our laboratory showed that 30 mg/kg/day of MLN-4760 administration to hypertensive rats did not elicit a change in blood pressure. After 28 days of treatment, rat hearts were imaged by echocardiography as outlined in the **Supplementary Methods** online. Immediately following the echocardiography, (mRen2)27 rats were continued on isoflurane anesthesia (2%), and invasive cardiac function was measured using combined conductance and pressure catheterization as described in the **Supplementary Methods** online.

Biochemical analyses. Immediately following the cardiac catheterization, blood was collected in prechilled tubes containing peptidase inhibitors (25 mmol/l EDTA, 0.44 mmol/l 1,2-orthophenanthroline monohydrate, 1 mmol/l sodium *para*-chloro-mercuribenzoate, and 3 µmol/l of the rat renin inhibitor, WFML-1) as described by us previously.[24] Blood cells were isolated by centrifugation at 3,000*g* for 20 min, and aliquots of plasma were stored at −80 °C until radioimmunoassay measurements. LV tissues were rapidly collected and snap-frozen in liquid N_2 and stored at −80 °C until assayed. Angiotensin peptides were extracted from the plasma and tissue samples using C18 Sep-Pak columns (Waters, Milford, MA), and the eluate was analyzed by radioimmunoassay for Ang II and Ang-(1–7) as described.[24] The minimum detectable limits of the Ang II and Ang-(1–7) assays were 0.8 and 2.5 pg/ml, respectively. The intra- and interassay coefficients of variability were 12 and 22% for Ang II, and 8 and 20% for Ang-(1–7), respectively.

Determination of cardiac ACE2 activity. ACE2 activity was determined using a fluorometric assay as previously described by us.[25] LV tissue from Ren-2 rats, stored at −80 °C, was weighed and homogenized in 25 mmol/l HEPES (pH 7.4), 125 mmol/l NaCl, and 10 µmol/l $ZnCl_2$ and centrifuged at 28,000 *g* for 10 min. The supernatant was removed, and the membranes were resuspended in HEPES buffer containing 0.5% Triton X-100 and incubated overnight on ice at 4 °C. After centrifugation, 25 µg protein of the soluble portion was incubated with 50 µmol/l 7-methoxycoumarin-4-acetyl-alanine-proline-lysine-(2,4-dinitrophenyl)-OH, pH 7.0 in the HEPES buffer containing peptidase inhibitors in the presence and absence of MLN-4760 at 42 °C for 60 min. The reaction was terminated with 0.2% trifluoroacetic acid and the fluorescence was quantified using a Perkin Elmer LS 50B flurometer (excitation 320 nm; emission 405 nm). The reaction product was quantified by comparison with a standard curve generated by incubation with 7-methoxycoumarin-4-acetyl-alanine-proline in a dose range of 0.125–6 nmol.

Histology. A cross-section of the heart was collected at the end of 28-day treatment and placed in 4% paraformaldehyde for 24 h, after which the tissue was transferred to 70% ethanol until paraffin embedding for histological analysis. Picrosirius red staining was performed as modified from Junqueira *et al.*[26] Briefly, 4 µm heart sections were deparaffinized and rehydrated prior to placement in filtered 0.1% picrosirius red for 60 min. Sections were washed twice in 0.5% glacial acetic acid, dehydrated, cleared, and mounted. Eight interstitial and perivascular images (×200) per section were captured under both bright-field and polarized light using Spot Advanced software 4.0.9 (Diagnostic Instruments, Sterling Heights, MI) connected to an Olympus BX60 microscope (Olympus America, Center Valley, PA). The polarized RGB color images were converted to grayscale and analyzed by a blinded individual for both total interstitial and perivascular collagen using Adobe Photoshop CS2 (Adobe Systems, San Jose, CA). Hematoxylin and eosin staining was also performed using standard methods. Briefly, LV sections were deparaffinized, rehydrated and stained with hematoxylin for 5 min. After a quick dip in acid alcohol (0.5% HCl in 70% ethanol), sections were rinsed in water and placed in eosin for 2 min. Sections were dehydrated, cleared and mounted. One-hundred cardiomyocytes from each section (25 from each of LV anterior wall, posterior wall, free wall, and septum) were analyzed by a blinded individual at ×400 magnification using Simple PCI 6.0 software (Hamamatsu, Sewickley, PA) connected to a Leica DM4000B microscope (Leica Microsystems, Bannockburn, IL) for myocyte cross-sectional area.

Statistical analyses. All data analyses, expressed as mean ± s.e.m., were performed using GraphPad Prism 5.01 software

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(GraphPad Software, La Jolla, CA). Radiotelemetric data were analyzed using a two-way analysis of variance to determine differences between treatments and over time at a probability of <0.05. All other data were analyzed using a Student's t-test at a probability of <0.05. For the radioimmunoassays, values at or below the detectable limits were assigned those values for statistical purposes.

RESULTS

Body weights were not different between vehicle- and ACE2 inhibitor–treated Ren-2 rats at the end of treatment (vehicle: 504 ± 10 g vs. MLN-4760: 507 ± 6 g, $P > 0.05$). Administration of the ACE2 inhibitor in Ren-2 rats did not alter average 24-h systolic, diastolic, or pulse pressures, although ACE2 inhibition for 28 days was accompanied by mild tachycardia (Figure 1 and Table 1, $P < 0.05$). Final measurements of blood pressure and heart rate at the end of the 4-week treatment are documented in Table 1.

To determine the impact of chronic administration of MLN-4760 on ACE2, cardiac ACE2 activity was measured using a fluorescence assay. Cardiac ACE2 activity was reduced in the MLN-4760-treated animals by an average of 40% (vehicle: 0.354 ± 0.047 nmol/mg protein/min vs. MLN-4760: 0.214 ± 0.025 nmol/mg protein/min, $P < 0.05$).

Plasma Ang II and Ang-(1–7) concentrations did not change in the animals chronically treated with the ACE2 inhibitor (Figure 2a, $P > 0.05$). However, ACE2 inhibition caused a significant increase in cardiac levels of Ang II (Figure 2b, $P < 0.01$), whereas cardiac Ang-(1–7) concentrations tended to decrease compared to rats given the vehicle (Figure 2b, $P > 0.05$). Changes in the cardiac content of Ang II and Ang-(1–7) induced by MLN-4760 resulted in a significant reduction in the cardiac Ang-(1–7)/Ang II ratio (Figure 2b, right, $P < 0.05$), a finding that was not observed in the circulation (Figure 2a, right, $P > 0.05$). These data indicate that ACE2

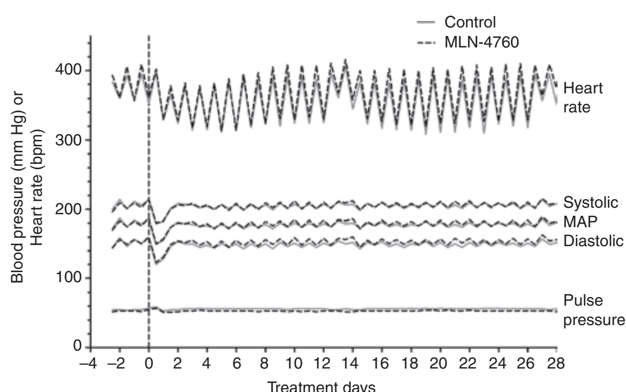

Figure 1 | Radiotelemetric blood pressure and heart rate averaged from vehicle- ($n = 7$) and MLN-4760-treated ($n = 7$) Ren-2 rats. Systolic, mean arterial pressure (MAP), diastolic, or pulse pressures were not altered as a result of ACE2 blockade ($P > 0.05$). ACE2 blockade did cause a mild elevation in heart rate ($P < 0.05$) at the end of the study. bpm, beats/min.

Table 1 | 24-hour Average blood pressures and heart rate measured by radiotelemetry on the last day of treatment

	Vehicle	MLN-4760	*P* value
Systolic BP (mm Hg)	205 ± 6	207 ± 3	>0.05
Mean arterial pressure (mm Hg)	181 ± 5	185 ± 2	>0.05
Diastolic BP (mm Hg)	153 ± 5	158 ± 2	>0.05
Pulse pressure (mm Hg)	56 ± 2	53 ± 3	>0.05
Heart rate (bpm)	376 ± 3	387 ± 2	<0.05

Data are means ± s.e.m. of vehicle- ($n = 7$) and ACE2 inhibitor– ($n = 7$) treated rats (MLN-4760) on day 28 of the infusion period.
BP, blood pressure; bpm, beats/min.

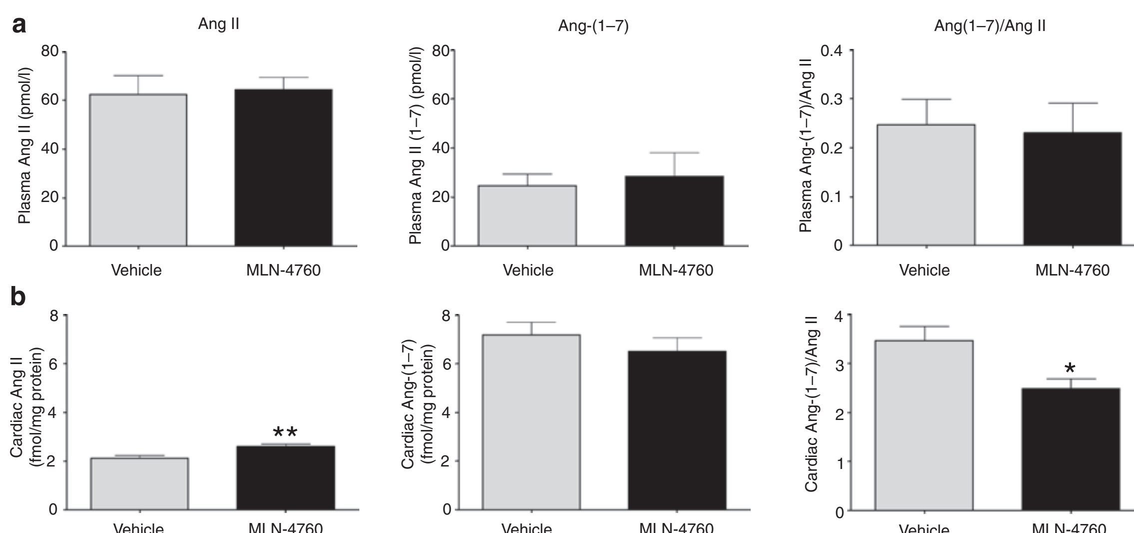

Figure 2 | (**a**) Plasma and (**b**) cardiac angiotensin peptides from vehicle- ($n = 7$) and MLN-4760-treated ($n = 7$) Ren-2 rats. MLN-4760 treatment did not change plasma Ang II or Ang-(1–7) concentrations, or the plasma Ang-(1–7)/Ang II ratio ($P > 0.05$). However, ACE2 inhibition did cause a significant accumulation of myocardial Ang II (vehicle: 2.11 ± 0.12 fmol/mg protein vs. MLN-4760: 2.61 ± 0.09 fmol/mg protein, $P < 0.01$), whereas the reduction in cardiac Ang-(1–7) was not statistically significant (vehicle: 7.19 ± 0.52 fmol/mg protein vs. MLN-4760: 6.50 ± 0.56 fmol/mg protein, $P > 0.05$). Moreover, ACE2 blockade revealed a significant decrease in the cardiac Ang-(1–7)/Ang II ratio (vehicle: 3.46 ± 0.30 vs. MLN-4760: 2.49 ± 0.19, $P < 0.05$).

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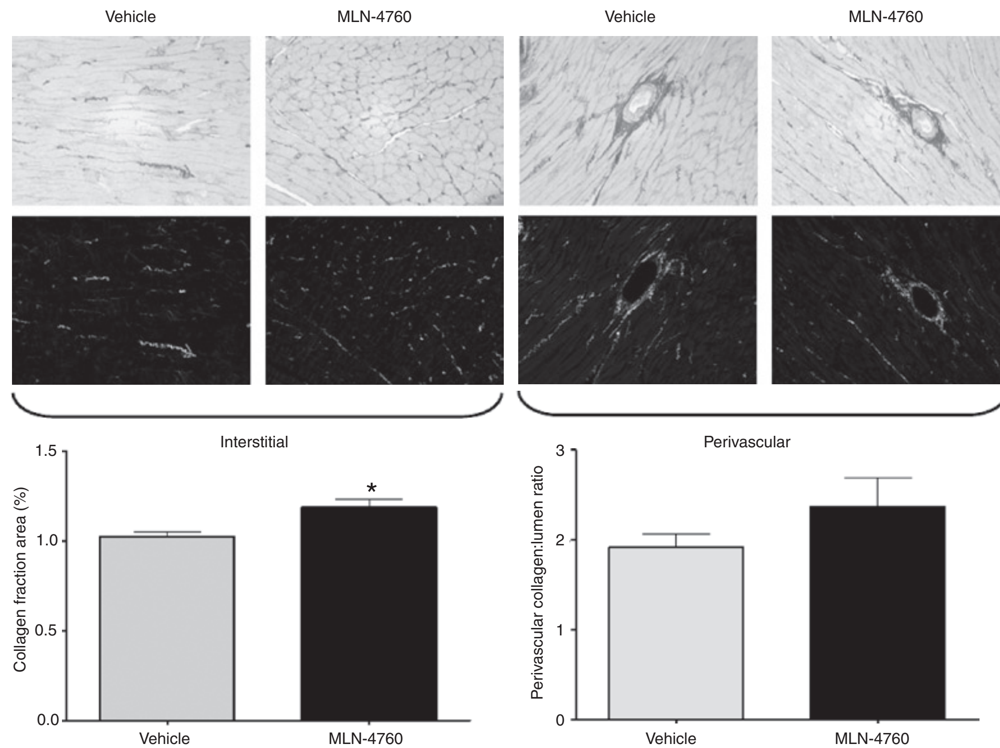

Figure 3 | Bright-field (top) and polarized (bottom) photomicrographs of collagen staining by picrosirius red in both treated ($n = 7$) and untreated ($n = 7$) Ren-2 rats. ACE2 inhibition caused a significant elevation in interstitial collagen fraction area (left, vehicle: 1.02 ± 0.03% vs. MLN-4760: 1.19 ± 0.04%, $P < 0.05$), whereas the elevation in perivascular collagen/lumen ratio did not achieve statistical significance (right, vehicle: 1.92 ± 0.15 vs. MLN-4760: 2.36 ± 0.33, $P > 0.05$).

blockade imparts an imbalance in cardiac angiotensin peptides independent of the circulation.

Because treatment with the ACE2 inhibitor resulted in an accumulation of cardiac Ang II, heart sections were stained for collagen content using picrosirius red. ACE2 blockade caused a significant elevation in interstitial collagen fraction area (**Figure 3**, left, $P < 0.05$), but the perivascular collagen/lumen ratio was unchanged (**Figure 3**, right, $P > 0.05$). Likewise, the administration of the ACE2 inhibitor increased cardiomyocyte cross-sectional area (**Figure 4**, $P < 0.01$).

Echocardiography revealed significant increases in both anterior and posterior wall thicknesses during diastole in the animals chronically treated with the ACE2 inhibitor, whereas other indexes of cardiac function were preserved (**Table 2**). Cardiac function as measured by direct cardiac catheterization revealed a tendency for increased intracardiac pressures associated with MLN-4760 treatment, although the increased trend in ESP and EDP were not statistically significant (**Table 2**; P = 0.15 and 0.10, respectively). Other indexes of direct cardiac function were unchanged between vehicle- and MLN-4760-treated Ren-2 rats (**Table 2**).

DISCUSSION

Systemic administration of a specific ACE2 inhibitor unmasked, for the first time, a contribution of endogenous cardiac ACE2 to the regulation of cardiac angiotensin peptides and structure in a renin-dependent model of hypertension. Chronic ACE2 blockade in (mRen2)27 hypertensive rats resulted in a 40% reduction in cardiac ACE2 activity, which was associated with a 24% augmentation in cardiac Ang II, increased LV wall thickness, a 17% increase in the cardiac interstitial collagen fraction area, and a 26% increase in cardiomyocyte cross-sectional area. The observed biochemical and structural abnormalities were independent of changes in blood pressure, although load-independent cardiac function was preserved.

Our approach in studying the critical importance of myocardial ACE2 in regulating cardiac structure and function differs from currently published reports in part because our study was carried out in a transgenic rat model of renin-dependent hypertension characterized by severe hypertension, increased cardiac expression of renin, and cardiac hypertrophy. Crackower *et al.*[11] showed that ACE2 deletion in mice impaired cardiac contractility associated with ventricular wall thinning, whereas Yamamoto *et al.*,[17] also in mice, observed cardiac structural and functional deficits in response to pressure overload induced by transverse aortic constriction. Furthermore, Gurley *et al.*[16] observed no overt cardiac abnormalities in ACE2-null mice produced on different genetic backgrounds, although a significant elevation in LVEDDd suggested mild LV dilatation. Conversely, lentiviral delivery of ACE2 to the heart partially ameliorated cardiac hypertrophy and fibrosis in spontaneously hypertensive rats.[19,20] A recent study[21] reported that adenoviral delivery of ACE2 to the heart produced exaggerated cardiac fibrosis in stroke-prone spontaneously hypertensive rats—an unexpected finding given the beneficial

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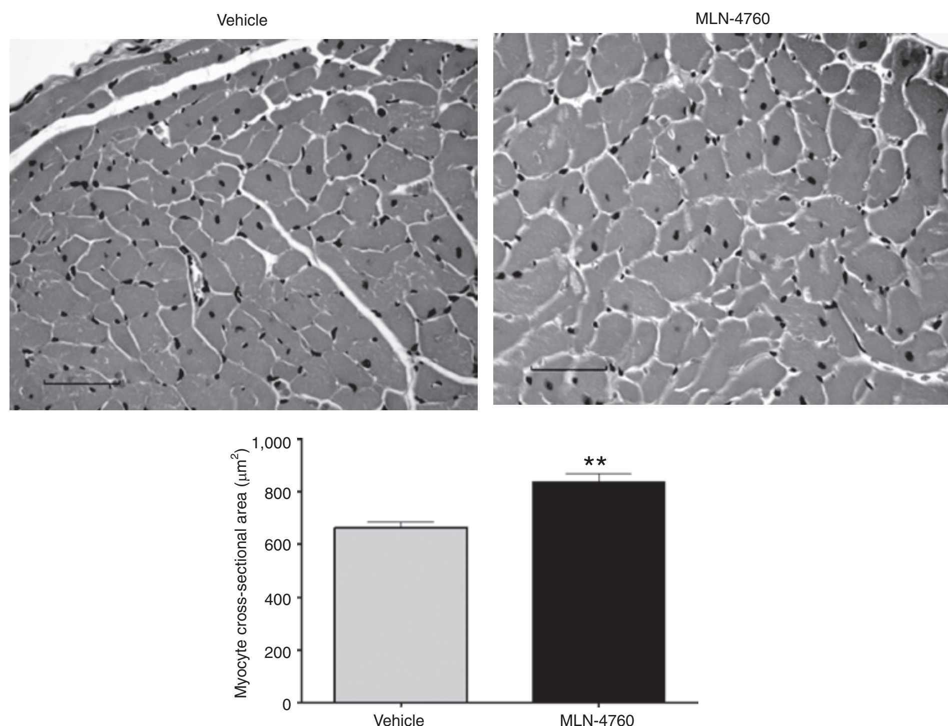

Figure 4 | Photomicrographs of cardiac sections showing cardiomyocyte cross-sectional area. ACE2 blockade elicited significant cardiomyocyte hypertrophy (vehicle: 664 ± 23 μm^2 vs. MLN-4760: 836 ± 33 μm^2, $P < 0.01$, $n = 7$ per group). Bar = 50 μm.

findings of preceding studies. This study by Masson *et al.* used a 10,000-fold higher ACE2 viral particle delivery concentration (3×10^{12}) than those in previous studies (3×10^8).[19,20] This may have produced high concentrations of Ang-(1–7), which at high doses, is known to bind to the AT_1 receptor.[27] Our current study aimed to investigate the role of native, endogenous cardiac ACE2 in the regulation of cardiac structure and function in hypertensive transgenic rats. Our data show that ACE2 inhibition worsens cardiac remodeling in hypertensive rats, a finding that is compatible with the observation of a reduction in cardiac fibrosis and hypertrophy as a result of cardiac ACE2 overexpression[19,20] or ventricular dilatation in the ACE2 knockout mice.[11,17]

Genetic ablation of ACE2 results in increased cardiac Ang II[11,17] and enhanced oxidative stress.[28] The deleterious actions of high cardiac Ang II expression on structure as observed in our study are well documented. Ang II promotes cardiac fibrosis and hypertrophy,[3,4,29,30] possibly mediated by elevated oxidative stress,[31] whereas cardiac Ang-(1–7) reverses cardiac fibrosis and hypertrophy.[5–7] Studies by us[7] and others[32] have shown that the antifibrotic and antihypertrophic actions of Ang-(1–7) are mediated by its binding to the mas receptor. Grobe *et al.*[5] showed that 100 ng/kg/min of Ang-(1–7) administration prevented cardiac fibrosis induced by Ang II in Sprague–Dawley rats. Likewise, cardiac ACE2 overexpression in Sprague–Dawley rats protected hearts from Ang II–induced cardiac hypertrophy.[20] Additionally, Loot *et al.*[10] showed that chronic administration of Ang-(1–7) 2 weeks after the induction of myocardial infarction in rats preserved cardiac function. A very recent study also showed that targeted overexpression of cardiac Ang-(1–7) was protective against Ang II–induced cardiac hypertrophy and fibrosis by a direct action on the heart.[33] In the same study, the authors also showed that mice with chronic targeted elevations in cardiac Ang II did not exhibit altered cardiac contractility. These data are consistent with our current study in that the augmentation of cardiac Ang II in response to ACE2 blockade elicits adverse cardiac remodeling, without marked functional changes in global contractility. The alteration in cardiac Ang II in our study was independent of the circulating system, as plasma Ang II and Ang-(1–7) remained unchanged in response to ACE2 blockade. Moreover, the lack of change in blood pressure in response to ACE2 blockade is likely due to the lack of change in circulating Ang II between the two treatment groups and the fact that hypertension is severe at this stage of adulthood. These findings add to the growing body of evidence supporting tissue renin–angiotensin system regulation, independent from the circulating system. Shifting the balance of these two regulatory Ang peptides toward the mitogen, Ang II has deleterious actions on the myocardium.

Although a 40% reduction in endogenous ACE2 activity caused an accumulation of cardiac Ang II, the reduction in cardiac Ang-(1–7) did not achieve statistical significance. Failure of cardiac Ang-(1–7) to decrease may stem from compensatory activities of other enzymes, such as neprilysin, which convert Ang I into Ang-(1–7). Neprilysin is expressed in the heart and is elevated in heart failure.[34] Moreover, neprilysin has been shown to be an Ang-(1–7)-forming enzyme in the systemic and renal vasculature.[35,36] Therefore, it is possible that neprilysin activity maintained, at least in part, Ang-(1–7)

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Table 2 | Echocardiographic and hemodynamic analysis of cardiac function in vehicle- (*n* = 7) and ACE2 inhibitor–treated (*n* = 7) Ren-2 rats

	Vehicle	MLN-4760	*P* value
Heart rate (bpm)	384 ± 11	387 ± 8	0.83
AWTd (cm)	0.224 ± 0.002	0.245 ± 0.006	**0.009
PWTd (cm)	0.231 ± 0.003	0.257 ± 0.006	**0.003
RWT	0.524 ± 0.011	0.611 ± 0.032	*0.02
ESDd (cm)	0.564 ± 0.012	0.547 ± 0.034	0.65
EDDd (cm)	0.885 ± 0.009	0.849 ± 0.031	0.28
FS (%)	36 ± 1	36 ± 2	0.86
E_{max} (cm/s)	98 ± 4	92 ± 5	0.39
e′ (cm/s)	6.13 ± 0.39	5.77 ± 0.30	0.47
E/e′	16.24 ± 0.28	16.35 ± 1.35	0.94
ESP (mm Hg)	144 ± 10	164 ± 6	0.15
EDP (mm Hg)	17 ± 4	28 ± 5	0.10
ESV (µl)	135 ± 16	140 ± 19	0.87
EDV (µl)	212 ± 15	209 ± 18	0.88
SV (µl)	83 ± 7	80 ± 5	0.79
dP/dt_{max} (mm Hg/s)	8,654 ± 487	9,177 ± 154	0.36
dP/dt_{min} (mm Hg/s)	−7,855 ± 506	−8,102 ± 302	0.70
τ (Weiss, ms)	8.6 ± 0.2	8.2 ± 0.2	0.16
ESPVR	0.87 ± 0.27	1.25 ± 0.31	0.98
EDPVR	0.13 ± 0.05	0.14 ± 0.08	0.65

Data are means ± s.e.m.
τ, time constant of relaxation; AWTd, anterior wall thickness during diastole; bpm, beats/min; dP/dt_{max}, maximal change in pressure as a function of time; dP/dt_{min}, minimal change in pressure as a function of time; e′, early mitral annular descent; E_{max}, maximal transmitral early filling velocity; EDDd, end-diastolic dimension during diastole; EDP, end-diastolic pressure; EDPVR, end-diastolic pressure–volume relationship; EDV, end-diastolic volume; ESDd, end-systolic dimension during diastole; ESP, end-systolic pressure; ESPVR, end-systolic pressure–volume relationship; ESV, end-systolic volume; FS, fractional shortening; PWTd, posterior wall thickness during diastole; RWT, relative wall thickness; SV, stroke volume.
$^{*}P < 0.05$; $^{**}P < 0.01$.

concentrations in response to ACE2 blockade. Further studies are warranted to dissect these mechanisms.

Another important observation in our current study relates to the relative uncoupling of structural changes and cardiac function elicited by ACE2 inhibition. The direction of change in echocardiographic indexes of diastolic dysfunction (E_{max}, e′, ESP, EDP) in (mRen-2)27 transgenic rats exposed to chronic ACE2 inhibition is in keeping with our demonstration of increased cardiac interstitial fibrosis. Concentric cardiac hypertrophy is a pre-existing compensatory response of Ren-2 rats as shown both in our[15] and other laboratories.[37,38] However, global cardiac function in the hypertrophied hearts of the (mRen2)27 strain remains normal or slightly improved when compared to normal age-matched Sprague–Dawley rats at 18 weeks of age.[37]

It should not be ignored that ACE2 can act on other substrates affecting cardiac function. Apelin can impart cardioprotective actions on the heart,[39] and ACE2 has been shown to inactivate apelin by cleaving the C-terminal phenylalanine.[13] Because apelin has cardioprotective actions and ACE2 overexpression downregulates connexins 40 and 43 (ref. 40), a reduction in ACE2 may lead to improved cardiac function and electrical conductance. Because inhibition of ACE2 was associated with clear changes in cardiac tissue concentrations of Ang II but no alterations in cardiac contractility, we cannot rule out whether apelin may have attenuated changes in cardiac function in our current study.

In summary, we show that chronic ACE2 blockade imparts a significant accumulation of cardiac Ang II, resulting in an imbalance in the two known bioactive Ang peptides, Ang II and Ang-(1–7). The accumulation of myocardial Ang II in response to ACE2 blockade was associated with significant interstitial collagen deposition and cardiomyocyte hypertrophy while cardiac function was preserved. The findings in our current study show that ACE2 is crucial in maintaining the conversion of Ang II into Ang-(1–7) in the heart. These studies illustrate the ability of ACE2 blockade to exacerbate cardiac remodeling in the absence of changes in blood pressure or cardiac function. Our research supports an increasing role of cardiac ACE2 in compensated cardiac hypertrophy.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ajh

Acknowledgments: This work was supported, in part, by American Heart Association Mid-Atlantic Affiliate grants 0715249U (to A.J.T.) and 0765308U (to J.V.), National Institutes of Health grants HL-51952 (to C.M.F.), HL-56973 (to M.C.C.), a KO8-AG026764-04 Paul Beeson Award (to L.G.), and a WFUSM Venture Fund grant (to J.V.). Additionally, we gratefully acknowledge grant support, in part, provided by Unifi, Inc., Greensboro, NC, and the Farley-Hudson Foundation, Jacksonville, NC. We thank Drs Callahan and Delo for their helpful suggestions for pressure–volume assessment of cardiac function. We also acknowledge the technical assistance of Jessica VonCannon. The ACE2 inhibitor MLN-4760 was obtained from Millennium Pharmaceuticals (Cambridge, MA).

Disclosure: The authors declared no conflict of interest.

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