

2-1-2007

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Grobe, Justin L.; Mecca, Adam P.; Lingis, Melissa; Shenoy, Vinayak; Bolton, Tonya A.; Machado, Juline M.; Speth, Robert C.; Raizada, Mohan K.; and Katovich, Michael J., "Prevention of angiotensin II-induced cardiac remodeling by angiotensin-(1-7)" (2007). *HPD Articles*. 64.
https://nsuworks.nova.edu/hpd_facarticles/64

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PREVENTION OF ANGIOTENSIN II-INDUCED CARDIAC REMODELING BY ANGIOTENSIN-(1-7)

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Running Head: Ang-(1-7) prevents remodeling in Ang II hypertension

ABSTRACT

Cardiac remodeling, which typically results from chronic hypertension or following an acute myocardial infarction, is a major risk factor for the development of heart failure and ultimately death. The renin-angiotensin system (RAS) has previously been established to play an important role in the progression of cardiac remodeling, and inhibition of a hyperactive RAS provides protection from cardiac remodeling and subsequent heart failure. Our previous studies have demonstrated that overexpression of ACE2 prevents cardiac remodeling and hypertrophy during chronic infusion of angiotensin II (Ang II). This coupled with the knowledge that ACE2 is a key enzyme in the formation of Ang-(1-7) led us to hypothesize that chronic infusion of Ang-(1-7) would prevent cardiac remodeling induced by chronic infusion of Ang II. Infusion of Ang II into adult Sprague-Dawley rats resulted in significantly increased blood pressure, myocyte hypertrophy, and mid-myocardial interstitial fibrosis. Co-infusion of Ang-(1-7) resulted in significant attenuations of myocyte hypertrophy and interstitial fibrosis, without significant effects on blood pressure. In a subgroup of animals also administered [D-Ala⁷]-Ang-(1-7) (A779), an antagonist to the reported receptor for Ang-(1-7), there was a tendency to attenuate the anti-remodeling effects of Ang-(1-7). Chronic infusion of Ang II, with or without co-infusion of Ang-(1-7), had no effect on AT1 or AT2 angiotensin receptor binding in cardiac tissue. Together, these findings indicate an anti-remodeling role for Ang-(1-7) in cardiac tissue, which is not mediated through modulation of blood pressure or altered cardiac angiotensin receptor populations, and may be at least partially mediated through an Ang-(1-7) receptor.

Keywords

Angiotensin-(1-7), Cardiac Fibrosis, Myocyte Hypertrophy, TGF β , Hypertension.

INTRODUCTION

Cardiac remodeling is one of the most detrimental effects of chronic hypertension. With an increased workload during hypertension, the heart eventually undergoes hypertrophic (enlargement) and fibrotic responses. Hypertrophy of the cardiac myocytes without any accompanying fibrosis or vascular changes does not appear to have any adverse prognostic implications. Myocyte hypertrophy, when accompanied by fibrosis during remodeling, however, is a pathological process that can lead to a decrease in cardiac function. This cardiac hypertrophy and inappropriate interstitial collagen formation can contribute to increased wall stiffness and diastolic dysfunction (46). Thus, the remodeling process, which could accompany hypertension, would consist of changes in the architecture of the heart, including perivascular and myocardial fibrosis, and medial thickening of intramyocardial coronary arteries, in addition to the myocyte hypertrophy. Various clinical trials (40, 46) have demonstrated that regression of left ventricular hypertrophy is a desirable target for therapy, as it is associated with improved long-term clinical outcomes.

The renin angiotensin-system (RAS) has been implicated in the pathogenesis of cardiovascular disease (CVD), and particularly in various aspects of cardiac remodeling (2, 10, 16, 37, 42). Angiotensin II (Ang II), through its interactions with the angiotensin II type 1 receptor (AT1R) has been demonstrated to increase fibroblast gene expression (including collagen), fibroblast density and proliferation, and myocyte hypertrophy, all of which are hallmarks of myocardial fibrosis and remodeling (22, 29, 48). Inhibition of the RAS with ACE inhibitors (ACEi) or angiotensin receptor blockers (ARB's) significantly reduces cardiac fibrosis (47). Recent studies have shown that the activity of a new

component of the RAS, angiotensin converting enzyme 2 (ACE2), is enhanced with ACE inhibitors and ARB treatment, thereby suggesting a role for ACE2 in modulating remodeling (14, 51). ACE2 differs greatly in substrate specificity with the more commonly recognized ACE, and the activity of ACE2 is not altered by classic ACE inhibitors (14, 25). ACE2 has been shown to be important in the conversion of the vasoconstricting, hypertrophic/pro-fibrotic hormone Ang II directly to angiotensin-(1-7) (Ang-(1-7)) (14, 51), and ACE2 appears to be a critical regulator of cardiac development (9, 15, 36, 51). Genetic deletion of the ACE2 gene in some studies indicates important roles for ACE2 in cardiac structure and function, while others challenge this view. Crackower, *et al.* (9) originally reported that genetic knockout of the ACE2 gene in mice resulted in left ventricular dilation and hypocontractility, but Gurley, *et al.* (24) have since published conflicting data. Gurley, *et al.* (24) have also demonstrated that the effect of ACE2 knockout is strongly dependent on the genetic background, as C57BL/6 mice showed much greater phenotypic effects than 129/SvEv mice. Most groups agree, however, that deletion of ACE2 causes significant potentiation of the cardiac remodeling effects of Ang II (24, 54). Yamamoto, *et al.* (54) recently reported that deletion of ACE2 accelerates pressure overload-induced cardiac dysfunction in mice. They hypothesize that ACE2 plays an important role in dampening the hypertrophic response that is mediated by Ang II, as the concentration of cardiac Ang II and the activation of mitogen-activated protein kinase by Ang II were markedly increased in ACE2 deficient mice. Donoghue, *et al.* (15), using transgenic animals that increased cardiac ACE2 expression also observed conduction and rhythm disturbances. Thus, although

somewhat controversial, ACE2 appears to have effects on cardiac structure and function.

ACE2 may have actions that oppose the remodeling effects of angiotensin II, and this potential protection is likely mediated through its product, Ang-(1-7). We have previously reported that overexpression of ACE2 by lentiviral gene transfer prevents hypertension-induced, cardiac hypertrophy and fibrosis in chronic Ang II-infusion and spontaneously hypertensive rat models (13, 26). Chronic infusion of Ang-(1-7) also prevents the cardiac fibrosis produced in the DOCA-salt rat model (23). Collectively, these findings led us to the hypothesis that chronic infusion of Ang-(1-7) should prevent cardiac remodeling induced by chronic infusion of Ang II. We also attempted to evaluate if the actions of Ang-(1-7) are mediated through its own reported receptor by utilizing an antagonist to this receptor, [D-Ala⁷]-Ang-(1-7) (A779). Finally, we examined the hypothesis that Ang-(1-7) mediates its anti-remodeling effects through the modulation of AT1 and AT2 subtype Ang II receptor populations.

MATERIALS AND METHODS

Animals. A total of 42 male Sprague-Dawley rats, weighing between 270 and 300 g, were used for these studies. All animals were conventionally housed in standard shoebox cages and maintained on a 12:12-h light-dark cycle with free access to standard rat chow and water. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Chemicals. The peptides Ang II, Ang-(1-7), and A779 were obtained from Bachem (King of Prussia, PA). Picro-Sirius Red stain was obtained from American MasterTech

Scientific, Inc. (Lodi, CA). EDTA and bacitracin were obtained from Sigma (St. Louis, MO).

Angiotensin II Infusion Model. Hypertension and cardiac remodeling were induced by chronic subcutaneous infusion of Ang II (100 ng/kg/min) for four weeks through the use of subcutaneous osmotic minipumps (Model #2004, Alzet, Cupertino, CA). Pumps were implanted subcutaneously on the back between the shoulder blades and hips while animals were anesthetized by inhalation of halothane anesthetic. Osmotic minipumps of Ang-(1-7) (100 ng/kg/min, s.c.) were additionally implanted at this time.

Indirect Blood Pressure. Indirect blood pressure was recorded weekly by tail-cuff as previously described (23, 26). Animals were warmed by a 200-W heating lamp for five minutes before restraint in a heated Plexiglas cage to which the animals had been previously conditioned. A pneumatic pulse sensor was attached to the tail distal to an occluding cuff controlled by a Programmed Electro-Sphygmomanometer (Narco Bio-Systems, Austin, TX). Voltage output from the cuff and the pulse sensor were recorded and analyzed by a PowerLab signal transduction unit and associated Chart software (ADInstruments, Colorado Springs, CO). At least three to five separate pressures were averaged from each animal on each recording day. All pressures were recorded between 10 am and noon, by the same individual.

Cardiac Remodeling. Following four weeks of treatment, animals were anesthetized by intramuscular injection of a mixture of ketamine, xylazine, and acepromazine (30, 6, and 1 mg/kg, respectively). Heart ventricles were removed by blunt dissection and wet weights were recorded and normalized to body mass. Cross-sections of the ventricles were then fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at five

μm , and stained with Masson's Trichrome. Myocyte diameters and interstitial fibrosis were determined at 250x and 100x magnification, respectively, using the ImageJ program from NIH (41) as previously described (23). Quantification of diameters and fibrosis were carried out by three individuals who were blinded to the treatments. Each observer examined a minimum of five (to eight) separate images from different (non-overlapping) regions of the left ventricle free wall only. The results for each animal from each of the three observers were then averaged for subsequent statistical analysis.

A second group of animals was treated in parallel to the above-mentioned set, with the addition of an A779 (100 ng/kg/min) group. This second set consisted of sham (n=6), Ang II (n=6), Ang II + Ang-(1-7) (n=6), and Ang II + Ang (1-7) + A779 (n=6) treatment groups. These animals were treated in an identical manner to the first group, except that cross-sections of heart ventricles were stained with Picro-Sirius Red instead of Masson's Trichrome.

Angiotensin Receptor Binding. Assessment of ^{125}I -sarcosine¹, isoleucine⁸ angiotensin II (^{125}I -SI Ang II) binding to sections of heart was carried out as previously described (17). Briefly, frozen apexes of the heart were sectioned in a cryostat at a thickness of 20 microns, thaw mounted onto subbed chrome-alum slides, air-dried and stored at -20°C for 1-2 weeks. For incubation with ^{125}I -SI Ang II, sections were thawed to room temperature and placed in Coplin jars in AM5 buffer (150 mM NaCl, 5 mM EDTA, 0.1 mM bacitracin, and 50 mM NaPO_4 buffer at pH 7.1-7.20) for 30 minutes. The cross-sections were then incubated for 2 hours in AM5 buffer containing 500 pM ^{125}I -SI Ang II and either 3 μM AngII (non-specific), 10 μM losartan (AT2 binding) or 10 μM PD123,319 (AT1 binding). The sections were quickly rinsed 2 times with distilled

water, 5 times in AM5 buffer for one minute each and finally 2 quick distilled water changes. The rinsed sections were dried under a stream of cool air for 4 minutes and exposed to Biomax MR-1 film (Kodak) in X-ray cassettes for 4 days. A set of iodine-125 calibration standards (Microscales (RPA-522, Amersham) were included with each film for densitometric calibration.

Specific binding of ^{125}I -SI-AngII to the AT1 and AT2 receptors was determined using an AIS densitometric analysis system (Imaging Research Inc., St. Catherines, Ontario, Canada) as described previously (17). The threshold for detection for AT1 and AT2 binding was set at 85-95 fmol/g to allow for determination of binding through the entire cross section of the heart. Non-specific binding was detected from a sampling of the cross section of the heart determined without a threshold. Values for non-specific binding (which were subtracted from AT1 and AT2 sections to obtain specific binding) were 123 ± 15 (mean \pm SD) fmol/g.

Plasma TGF β . Blood was collected from the abdominal aorta at sacrifice into a syringe coated with EDTA. The tube was mixed by inversion and centrifuged for five minutes at 3500 rpm in a Triac centrifuge (Clay Adams, New York, NY). Plasma was aspirated from the tube and stored at -80°C until analysis. Plasma was then used to measure circulating levels of transforming growth factor- β (TGF β) protein. Total plasma TGF β was determined by ELISA (R&D Systems, Minneapolis, MN) according to manufacturer's instructions for plasma samples.

Statistics. Indirect blood pressures and body masses were analyzed by repeated measures two-way ANOVA, followed by the Student-Newman-Keuls method of pairwise post-hoc analysis. Myocyte diameter and plasma TGF β data were analyzed by one-way

ANOVA and a Fisher's PLSD post hoc test. Interstitial fibrosis was analyzed by non-parametric Kruskal-Wallis one-way ANOVA followed by a Mann-Whitney U tests. The use of this more stringent non-parametric analysis was deemed necessary for the fibrosis measurements, due to the use of "% area" data. All data are presented as mean \pm standard error of the mean. Differences for all examinations were considered significant with $P < 0.05$.

RESULTS

Cardiac Hypertrophy. None of the treatments significantly altered heart ventricular mass (Sham, 2.67 ± 0.05 g/kg; Ang II, 2.81 ± 0.06 g/kg; Ang II + Ang-(1-7), 2.72 ± 0.07 g/kg; Ang II + Ang-(1-7) + A779, 2.73 ± 0.10 g/kg). Body mass also was unaffected by any of the treatments ($P = 0.496$). Microscopic examination of ventricular cross-sections revealed an 11% increase in myocyte diameters ($P < 0.02$) in the left ventricle free wall, which was significantly prevented ($P < 0.001$) by co-infusion of Ang-(1-7) (Figure 1). Myocyte diameters in the Ang II + Ang-(1-7) + A779 group were not significantly different from that of the sham or the Ang II treated groups. There was a non-significant ($P < 0.06$) trend toward reversal of the protective effect of Ang-(1-7) by A779.

The difference in findings between the wet-weight and microscopic evaluations of cardiac hypertrophy may be due to the inclusion of right ventricle free wall and septum in the mass determinations versus only the inclusion of myocytes in the left ventricle free wall in the microscopic examination. Further, as determined in our previous study, the microscopic examination method appears to yield similar results, but with a greater fidelity than the wet weight method (23). Finally, it is possible that while most myocytes undergo hypertrophy, some may be replaced by cardiac fibroblasts. This could account

for an increase in myocyte diameter and simultaneous mass changes that are not mathematically identical.

Cardiac Fibrosis. Chronic infusion of Ang II resulted in a significant increase ($p < 0.03$) in mid-myocardial interstitial fibrosis when determined by Picro-Sirius Red. This increase was significantly attenuated ($P < 0.03$) by co-infusion of Ang-(1-7) (Figure 2), and co-infusion of A779 caused a non-significant reversal ($P < 0.15$) of the Ang-(1-7) effect. The degree of fibrosis in the A779 group was not different from the sham controls. Similar cardiac fibrosis results were obtained using the Masson's Trichrome stain (data not shown).

Blood Pressure. Blood pressure was determined weekly during infusion of angiotensins by indirect tail-cuff method. Repeated measures two-way ANOVA revealed significant main effects of treatment ($P = 0.008$) and time ($P < 0.001$), and the presence of a significant interaction between these factors ($P = 0.031$). Chronic infusion of Ang II caused a significant increase in systolic pressure ($P < 0.005$), which was not altered by chronic co-infusion of Ang-(1-7) or Ang-(1-7) + A779 ($P = 0.178$ and $P = 0.295$, respectively, vs. Ang II alone). These differences only became significant, though, after 22 days of infusion (Figure 3).

Angiotensin Receptor Binding. Cardiac angiotensin AT1 and AT2 receptor binding was determined by autoradiography. Chronic infusion of Ang II with or without Ang-(1-7) had no effect on AT1 specific binding (Figure 4A), and no effects on AT2 specific binding (Figure 4B). As anticipated, AT1 receptor density in cardiac tissue was greater than AT2 receptor density.

Plasma TGF β . Circulating levels of the pro-remodeling cytokine transforming growth factor- β (TGF β) in blood plasma were determined by ELISA. Chronic infusion of Ang II had no effect on TGF β levels (Ang II, 1.58 ± 0.31 ng/mL vs. Sham, 1.85 ± 0.32 ng/mL). Co-infusion of Ang-(1-7) resulted in an approximate 40% (yet nonsignificant) decrease in plasma TGF β (Ang II + Ang-(1-7), 0.95 ± 0.09 ng/mL) compared to the Ang II-treated animals ($P=0.12$), and an approximate 50% (significant) reduction when compared to the Sham group ($P=0.03$). No samples were analyzed in the Ang II + Ang-(1-7) + A779 group.

DISCUSSION

The most significant observations of this study are the determinations that chronic Ang-(1-7) can prevent hypertension-induced cardiac myocyte hypertrophy and interstitial fibrosis. These findings suggest that the beneficial effects of Ang-(1-7) are the result of direct actions upon the cardiac tissue. Another significant observation of this study was the determination that the anti-fibrotic and anti-hypertrophic effects of Ang-(1-7) were not mediated through changes in either AT1 or AT2 cardiac receptor numbers. Further, although the myocyte hypertrophy and interstitial fibrosis were not significantly decreased from the Ang II treatment group, the Ang II + Ang-(1-7) + A779 group was also not significantly different from the sham or Ang II + Ang-(1-7) groups. These data support the idea that Ang-(1-7) likely mediates some portion of its anti-remodeling effects through actions at an A779-sensitive receptor, such as Mas.

Ang-(1-7) is a biologically active peptide of the RAS that has both vasodilatory and antiproliferative activities that are opposite those of Ang II. Ang-(1-7) has been reported to act as an antagonist to the Ang II AT1 receptor, and may also work by antagonizing

ACE, which is involved in both the production of Ang II and the degradation of Ang-(1-7) (6, 19, 27). In the current study, Ang-(1-7) infusion prevented cardiac hypertrophy and fibrosis without having any effect on the elevated blood pressure induced by chronic Ang II treatment. The lack of an obvious, significant effect on blood pressure (Figure 3) would suggest that Ang-(1-7) is likely not mediating its effects by antagonizing the AT1 receptors or ACE. Additionally, although Ang-(1-7) is reported to reduce cardiac Ang II levels (34) and we did not attempt to measure either peptide, it seems unlikely in this study that Ang II was significantly reduced by Ang-(1-7) infusion due to the lack of effect of Ang-(1-7) on blood pressure. Unfortunately, chronic infusion of exogenous Ang II in this model would greatly confound interpretation of plasma peptide levels.

The lack of effect of Ang-(1-7) on blood pressure in the present study may be due to the dose of Ang-(1-7) used, its delivery, and the animal model under investigation. Here, we chronically infused Ang-(1-7) at 100 ng/kg/min into the subcutaneous space. This dose represents the lower end of concentrations typically reported in the literature, which range from approximately 100 ng/kg/min (23, 34) up to 400 ng/kg/min (30, 53), although, interestingly, the effects of Ang-(1-7) on blood pressure appear to be more dependent upon the model of hypertension than on the dose of Ang-(1-7) used. Ang-(1-7) is reported to have no effect on blood pressure in normal Wistar-Kyoto (WKY) or spontaneously hypertensive rats (SHR) (52), pressure-overloaded rats (53) and DOCA-salt treated rats (23), but it does appear to modulate blood pressure in normotensive Sprague-Dawley rats treated with losartan (8), SHR treated with the nitric oxide synthase inhibitor L-NAME (3), and in WKY rats and SHR treated with the AT1 receptor antagonist candesartan (52).

It has been well documented that Ang-(1-7) levels are elevated during pharmacological ACE inhibition and blockade of AT1 receptors (20, 21, 27), and it has been proposed that these cardioprotective inhibitors may actually work through the actions of increased Ang-(1-7) (19). Evidence presented here would support the hypothesis that Ang-(1-7) is a cardioprotective peptide. Correlative studies have shown that ACE2 and Ang-(1-7) levels are increased by cardiac myocytes in hearts following myocardial infarction in both rats and man (1, 5). Iwata *et al.* (28) recently demonstrated that Ang-(1-7) attenuates pro-fibrotic signaling within the myocardium, through direct actions on cardiac fibroblasts. In the current study, chronic *in vivo* administration of Ang-(1-7) also appears to have effects on hypertrophic actions on the cardiomyocytes that are induced by Ang II. This observation has also been observed *in vitro*, as Tallant, *et al.*, (50) showed that Ang-(1-7) acts on cultured cardiac myocytes to inhibit hypertrophic responses through the Mas receptor. Collectively, these findings suggest that elevated Ang-(1-7) may also protect against cardiac hypertrophy in some forms of hypertension.

Ang-(1-7) delivery has been shown to delay development of cardiac hypertrophy (44), inhibit vascular growth (49), attenuate development of heart failure (33), reduce cardiac Ang II levels (34), and reduce Ang II receptor populations (7). Most of these effects could be mediated through modulation of AT1 and/or AT2 receptor populations by Ang-(1-7). In the current study, however, we have demonstrated that while Ang-(1-7) significantly attenuates cardiac remodeling, it does not affect either AT1 or AT2 receptors in the heart of animals treated with chronic Ang II. We also recently determined (unpublished results) that chronic treatment with Ang-(1-7) does not effect

AT1 or AT2 alterations that occur in DOCA-salt - treated rats, even though cardiac fibrosis was significantly reduced in these animals maintained with osmotic minipumps containing Ang-(1-7) (23). These findings are very important in the field's elucidation of the mechanism of Ang-(1-7)'s cardioprotective actions, as they prompt the *rejection* of the hypotheses that Ang-(1-7) mediates its cardioprotective effects through either decreasing the population of pro-remodeling AT1 receptors (22, 29, 48), or increasing the population of anti-remodeling AT2 receptors (17).

The studies presented here were not designed determine if the effects of the Ang-(1-7) are elicited through actions of Ang-(1-7) at either of the classic angiotensin receptors, although this is the next logical step in our line of investigation. Walters, *et al.* (52) have previously suggested that in rats treated with the AT1 antagonist candesartan, Ang-(1-7) mediates vasodepressor effects through activation of the AT2 receptor and subsequently the production of nitric oxide. Whether this same mechanism exists for cardiac remodeling endpoints remains to be determined.

As suggested by Tallant *et al.* (50) in an *in vitro* study, it is likely that Ang-(1-7) may mediate its cardiac effects through its own receptor, Mas (45); however, the intracellular signaling pathway(s) has not yet been elucidated. Our data suggest that the cardioprotective effects of Ang-(1-7) are mediated by activation of its own receptor, although future experiments, designed to test the possible contributions of AT1 antagonism and AT2 agonism, will need to be performed to confirm the *sufficiency* of Mas-mediated Ang-(1-7) transduction. Likewise, larger dosages of A779 need to be employed in subsequent experiments to more fully determine the necessity of the Mas receptor for modulation of *in vivo* cardiac remodeling by Ang-(1-7). A recent study by

Santos, *et al.* (43) strongly support the hypothesis that Mas is *necessary* for Ang-(1-7) transduction, as genetic deletion of the Mas receptor caused significant deleterious effects on cardiac function and structure, particularly with regard to myocardial fibrosis.

Myocardial fibrosis is the most characteristic structural change in a myocardial infarct and this fibrosis contributes to both systolic and diastolic dysfunction (4). Multiple lines of evidence indicate that TGF β plays a critical role in myocardial fibrosis by inducing increases in production and secretion of collagen, is associated with Ang II-mediated fibrosis, and promotes the transdifferentiation of fibroblasts to myofibroblasts (12, 32, 55). Excess or prolonged signaling of TGF β contributes to the pathogenesis of fibrosis, scarring, and matrix deposition in several disease states including the heart (38). Antagonists of the RAS, such as ACE inhibitors and ARB's, attenuate the fibrotic changes and matrix deposition mediated by TGF β . Peterson (39) recently demonstrated that both cardiac hypertrophy and fibrosis in heart failure are characterized by an elevated level of circulating TGF β , and Fedulov, *et al.* (18) demonstrated a strong positive correlation between serum TGF β and cardiac fibrosis. Okada *et al.* (35) have also recently demonstrated that gene therapy directed against TGF β attenuates left ventricular remodeling and heart failure following myocardial infarction in a time-dependent manner. In the present study, we did not measure cardiac TGF β , but did demonstrate that the plasma levels of TGF β were approximately 40% lower in the Ang II group co-infused with Ang-(1-7). Although this data is preliminary, it suggests that the effects of Ang-(1-7) on cardiac remodeling may be related to a decrease in the pro-fibrotic signaling molecule, TGF β . Currently we are

evaluating this hypothesis in cell culture with both primary cardiac myocytes and fibroblasts.

In summary, chronic infusion of Ang-(1-7) provided significant anti-remodeling protection during the chronic infusion of Ang II in rats. Together with the literature, these findings strongly support a direct action of Ang-(1-7) on cardiac tissue. This protective effect of Ang-(1-7) was independent of effects on blood pressure or Ang II receptors in the cardiac tissue, thereby indirectly supporting the popular hypothesis that Ang-(1-7) acts through a novel signaling pathway, such as the Mas receptor, rather than through modulation of Ang II receptor populations or kinetics. Finally, examination of plasma levels of the pro-remodeling cytokine TGF β revealed a trend toward suppression by Ang-(1-7), suggesting a possible downstream mechanism for the anti-remodeling actions of Ang-(1-7).

ACKNOWLEDGMENTS

We gratefully acknowledge the University of Florida Molecular Pathology and Immunology Core for preparing the heart sections examined in this study. J.L. Grobe was supported by an American Heart Association Pre-Doctoral Fellowship. A.P. Mecca was supported by an Endocrine Society undergraduate summer research fellowship. J.M. Machado was supported by an undergraduate research fellowship from the University of Florida (University Scholars Program). This work was supported by a grant from the NIH, HL56921-2 to MKR and MJK.

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FIGURE LEGENDS

Figure 1.

Myocyte hypertrophy, induced by chronic infusion of Ang II, is significantly attenuated by co-infusion of Ang-(1-7). (* indicates $P < 0.05$ vs. Sham, † indicates $P < 0.05$ vs. Ang II) Sham (n=12), Ang II (n=14), Ang II + Ang (1-7) (n=10), Ang II + Ang-(1-7) + A779 (n=6).

Figure 2.

Interstitial fibrosis is induced in the mid-myocardium by chronic infusion of Ang II, and this effect is attenuated by co-infusion of Ang-(1-7). Example pictures at 100x magnification are shown in (A). Bars are 100 μm . Quantified data from all animals is presented in (B). (* indicates $P < 0.05$ vs. Sham, † indicates $P < 0.05$ vs. Ang II). Sham (n=12), Ang II (n=14), Ang II + Ang (1-7) (n=10), Ang II + Ang-(1-7) + A779 (n=6).

Figure 3.

Chronic infusion of Ang II causes significant hypertension ($P < 0.005$ vs. Sham). Co-infusion of Ang-(1-7) or Ang-(1-7) + A779 fail to reduce the blood pressure increase due to Ang II infusion ($P = 0.178$ and $P = 0.295$ vs. Ang II, respectively). (n=6 animals per group).

Figure 4.

Chronic infusion of Ang II and/or Ang-(1-7) had no effect on cardiac (A) AT1 or (B) AT2 angiotensin receptor binding. (n=6 animals per group).

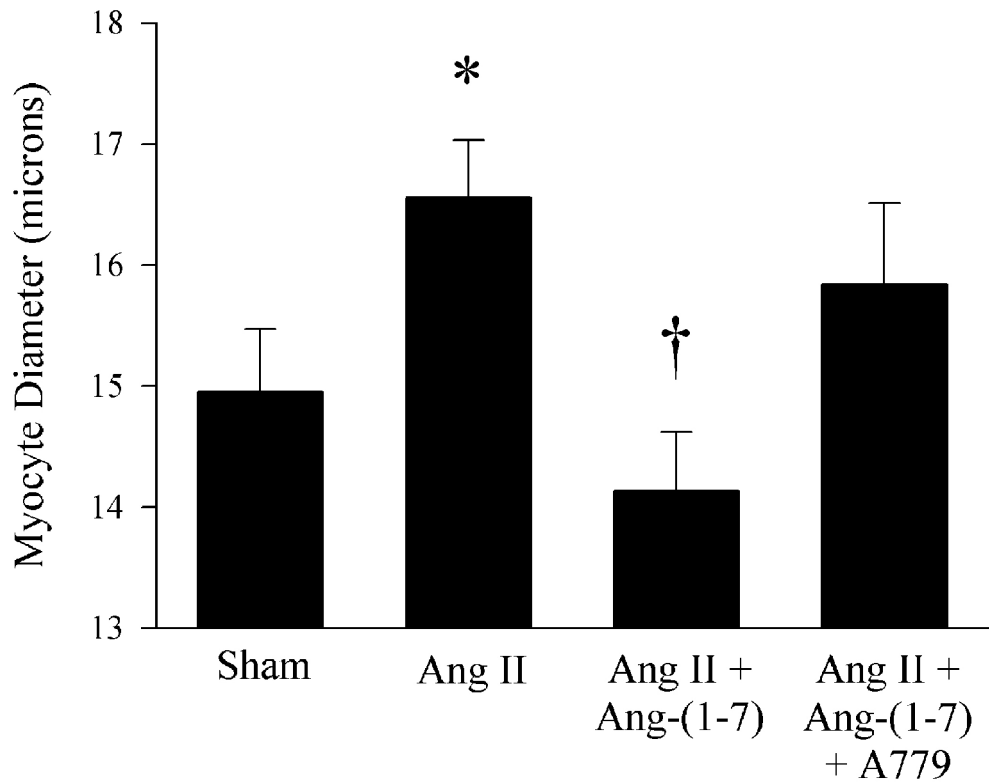
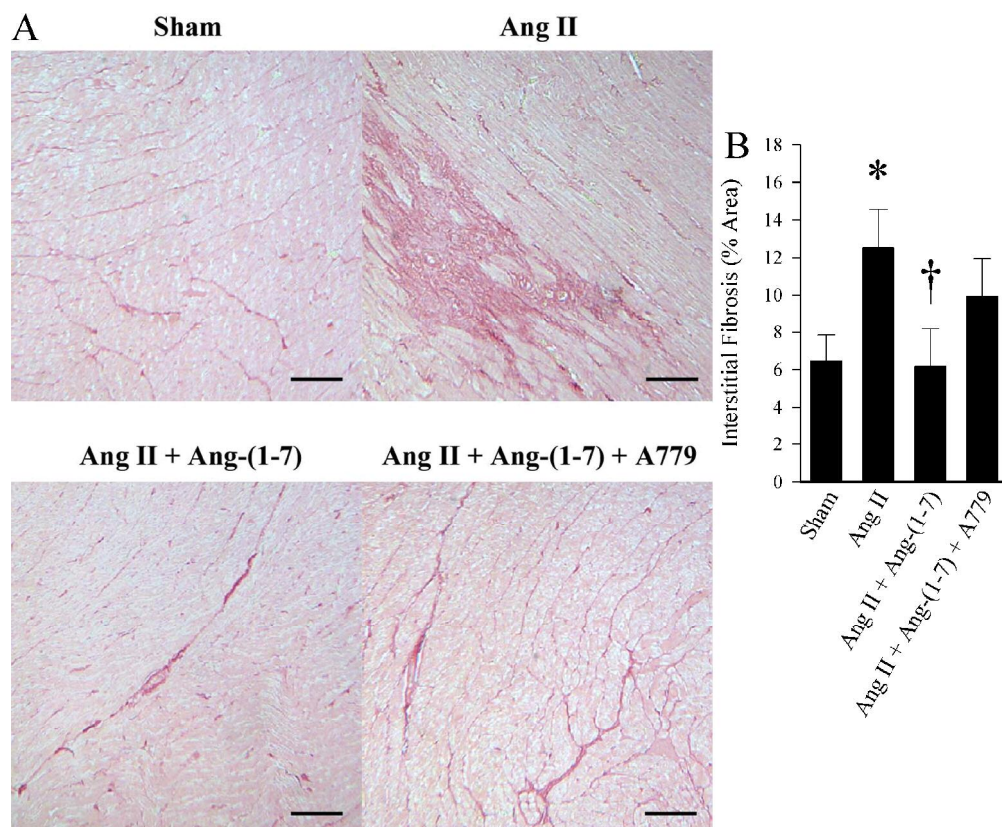


Figure 1

**Figure 2**

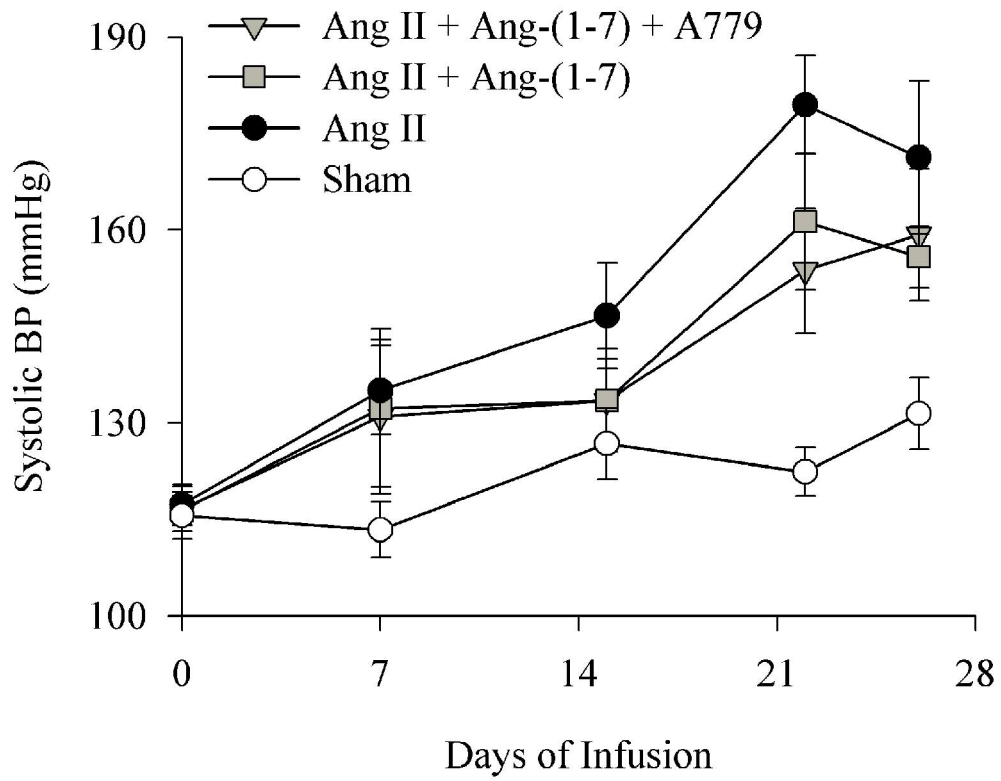


Figure 3

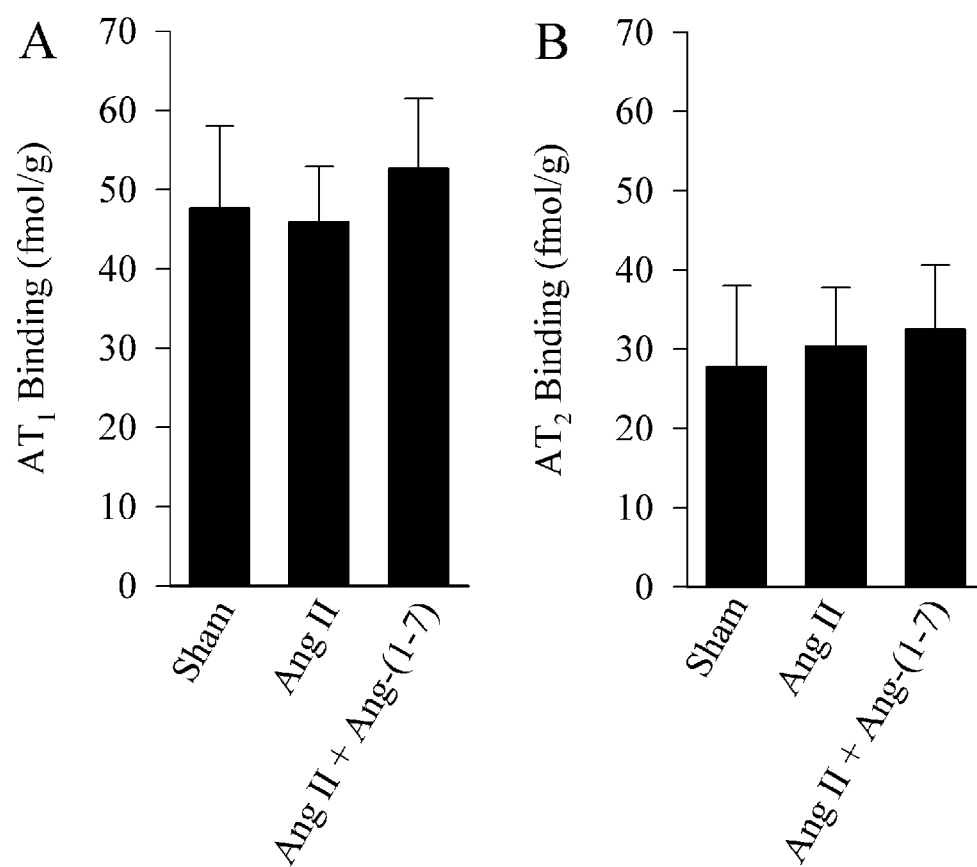


Figure 4