HHS Public Access

Author manuscript

Am J Physiol Heart Circ Physiol. Author manuscript; available in PMC 2022 July 06.

Published in final edited form as:

Am J Physiol Heart Circ Physiol. 2008 May ; 294(5): H2242–H2247. doi:10.1152/ajpheart.00175.2008.

Angiotensin-(1–12) is an alternate substrate for angiotensin peptide production in the heart

Aaron J. Trask,

Jewell A. Jessup,

Mark C. Chappell,

Carlos M. Ferrario

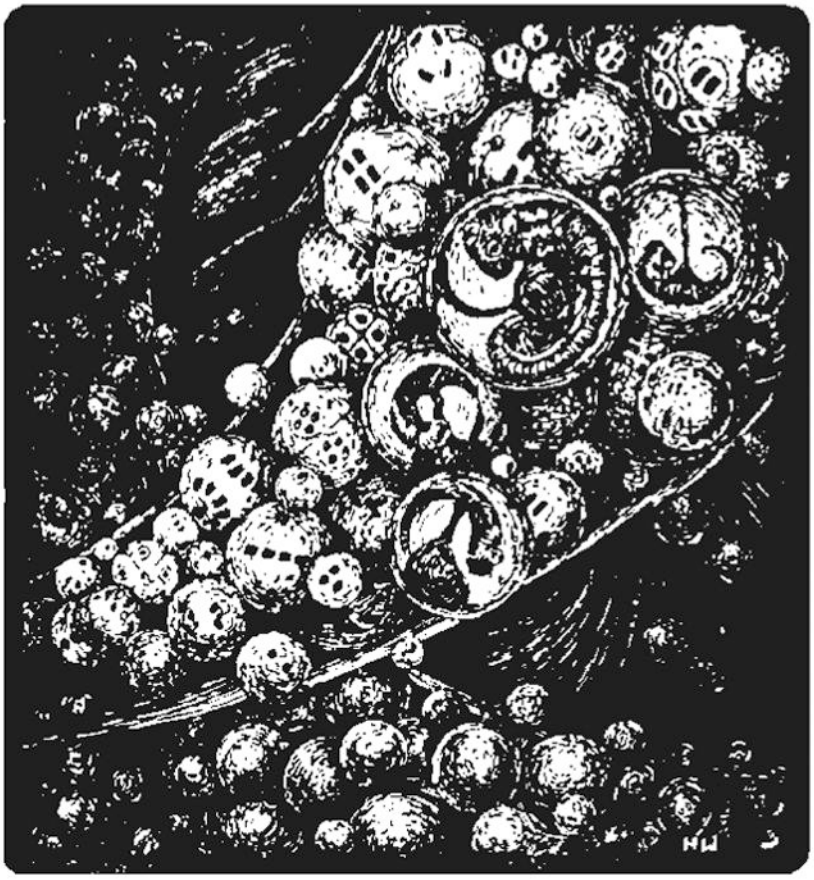
Hypertension and Vascular Research Center, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina

Abstract

Identification of angiotensin-(1–12) as an intermediate precursor derived directly from angiotensinogen led us to explore whether the heart has the capacity to process angiotensin-(1–12) into biologically active angiotensin peptides. The generation of angiotensin I, angiotensin II, and angiotensin-(1–7) from exogenous angiotensin-(1–12) was evaluated in the effluent of isolated perfused hearts mounted on a Langendorff apparatus in three normotensive and two hypertensive strains: Sprague-Dawley, Lewis, congenic mRen2.Lewis, Wistar-Kyoto, and spontaneously hypertensive rats. Hearts were perfused with Krebs solution for 60 min before and after the addition of angiotensin-(1–12) (10 nmol/l). Angiotensin-(1–12) caused the rapid appearance of both angiotensin I and angiotensin II in the perfusate that peaked between 30 and 60 min of recirculation. Production of angiotensin-(1–7) from exogenous angiotensin-(1–12) rose steadily over the course of the 60-min experiment. These data directly demonstrate that angiotensin-(1–12) is a substrate for the formation of angiotensin peptides in cardiac tissue. This finding further suggests that this angiotensinogen-derived product is a previously unrecognized important precursor peptide to the renin-angiotensin system cascade.

Graphical Abstract

Address for reprint requests and other correspondence: A. J. Trask, Hypertension and Vascular Research Center, Dept. of Physiology and Pharmacology, Wake Forest Univ. School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157 (atrask@wfubmc.edu).



Keywords

angiotensinogen; angiotensin II; angiotensin-(1–7); renin; hypertension

THE RENIN-ANGIOTENSIN SYSTEM (RAS) was originally thought to be a linear system with the cleavage of angiotensinogen by renin as the first step in the biochemical cascade leading to the production of biologically active peptides. Studies over the past 20 years have uncovered a more complex processing cascade primarily from studies that demonstrated a functional role of angiotensin-(1–7) [ANG-(1–7)], which can be generated by various peptidases from either angiotensin I (ANG I) or angiotensin II (ANG II) (1, 15, 17, 18).

In keeping with the idea of a nonlinear RAS, Nagata and colleagues (10) recently identified a propeptide hormone of the RAS, proangiotensin-12 [angiotensin-(1–12); ANG-(1–12)] in plasma and all tissues investigated. Biological actions of this propeptide as a substrate for ANG II formation were demonstrated by showing that the administration of ANG-(1–12) in isolated vessels produced a vasopressor response that could be blocked by both an angiotensin-converting enzyme (ACE) inhibitor and an angiotensin receptor blocker (ARB). The present study determined whether ANG-(1–12), a peptide upstream of the traditional RAS cascade, can lead to the generation of ANG II and ANG-(1–7) in the isolated hearts of both normal and genetically diverse hypertensive rat strains. To confirm the role of ANG-(1–

12) as a suitable substrate for angiotensin peptide formation in the heart, data were obtained in five different rat strains.

MATERIALS AND METHODS

Animals.

To examine whether the rat heart has the capacity to process ANG-(1–12) into the downstream angiotensin peptides ANG I, ANG II, and ANG-(1–7), initial experiments focused on employing 11- to 12-wk-old male Sprague-Dawley (SD) rats (Harlan Laboratories, Indianapolis, IN, $n = 6$) to the Langendorff isolated heart protocol outlined below. To determine whether ANG-(1–12) is differentially processed in hearts of hypertensive animals versus their normal counterparts, we also employed both a targeted model of ANG II-driven hypertension and a genetic model of hypertension using the Langendorff method in separate experiments. Normotensive Lewis rats (11–12 wk old, Charles River Laboratories, Wilmington, MA, $n = 4$) and hypertensive mRen2.Lewis rats (11–12 wk old, congenic, Hypertension and Vascular Research Center, Wake Forest University School of Medicine, Winston-Salem, NC, $n = 4$) served as the ANG II-driven hypertensive model. Aged-matched Wistar-Kyoto (WKY) rats (Charles River Laboratories, $n = 6$) and spontaneously hypertensive rats (SHRs; Charles River Laboratories, $n = 6$) served as the genetic model. All animals were housed in pairs in cages (12:12-h light-dark cycle) in an American Association for Accreditation of Laboratory Animal Care-approved facility with ad libitum access to rat chow and tap water. Procedures were approved by our Institutional Animal Care and Use Committee.

Langendorff procedure.

The isolated heart preparation was performed as previously described by our laboratory (15). Briefly, rats were weighed, placed under deep isoflurane (2.5–3%) anesthesia, and given heparin (300 USP units) via a catheter inserted into the jugular vein. The heart was excised and immediately placed in ice-cold Krebs buffer. Rat hearts were then perfused at a constant flow (10–12 ml/min) on a Langendorff isolated heart perfusion apparatus. Heart rates, perfusion pressures, and flow rates were monitored continuously throughout the experiment.

After a 1-h equilibration period, a baseline sample of the cardiac effluent (2.5 ml) was collected, and 60 ml of Krebs buffer with 10 nmol/l ANG-(1–12) (Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}-His^{9}-Leu^{10}-Leu^{11}-Tyr^{12}, Peptide Institute, Osaka, Japan) was then recirculated through the heart for 60 min. This dose was chosen based on a previous study by Nagata et al. (10), which showed that 10 nmol/l ANG-(1–12) was just below the concentration that elicited marked vasoconstriction in isolated rat aortae. Moreover, previous experience investigating ANG II metabolism in the isolated heart preparation is in keeping with the employed dose of ANG-(1–12) (15). All effluent samples were acid matched at 1:1 (vol/vol) with 1% heptafluorobutyric acid (HFBA) to abolish metabolism of the peptides at the following times: *1*) 5 min of recirculation, *2*) 15 min, *3*) 30 min, and *4*) 60 min. One-half of the WKY and SHR hearts received 1 μmol/l of the renin inhibitor WFML-1 (AnaSpec,

San Jose, CA) immediately after the collection of the 15-min sample. A previous study (11) from our laboratory demonstrated that WFML-1 specifically inhibits rat renin.

Biochemical procedures.

Angiotensin peptides were extracted from the acid-matched samples using C18 Sep Pak columns (Waters, Milford, MA). Each Sep Pak was conditioned with 5 ml of 80% methanol (MeOH) and 0.1% HFBA, followed by 5 ml of 0.1% HFBA. The 5-ml samples were then applied to the columns, followed by 10 ml of 0.1% HFBA. Columns were rinsed with 5 ml of MilliQ water, and peptides were eluted in 3 ml of 80% MeOH and 0.1% HFBA. The eluate was then analyzed by radioimmunoassay for ANG I, ANG II, and ANG-(1–7) as previously described by our laboratory (4, 5). The minimum detectable limits of the ANG I, ANG II, and ANG-(1–7) assays were 1.0, 0.8, and 2.5 pg/ml, respectively. The intra- and interassay coefficients of variability were 18% and 22% for ANG I, 12% and 22% for ANG II, and 8% and 20% for ANG-(1–7), respectively.

Renin assay.

Effluent collected from the hearts of WKY rats and SHRs (with no renin inhibitor) was concentrated using Amicon Ultra 10,000 molecular weight cutoff centrifugal filters (Millipore, Billerica, MA) and washed three times with HEPES buffer (25 mmol/l HEPES, 125 mmol/l NaCl, and 10 μmol/l $ZnCl_2$; pH 7.4). In addition, left ventricles from both isolated perfused ($n = 3$) and nonperfused ($n = 3$) WKY rats and SHRs were homogenized in 500 μl of HEPES buffer using the Qiagen TissueLyser for 1 min at 25 Hz. The homogenate was then centrifuged for 10 min at 28,000 *g*, and 25 μl of either the resulting supernatant or the concentrated cardiac effluent were incubated at pH 6.5 in the presence of excess exogenous angiotensinogen substrate at 37°C for 90 min. Renin activity was measured as the difference in ANG I generated at 37°C minus that present at 0°C. Additional experiments were conducted in the presence of 3 μmol/l WFML-1 to verify that the ANG I-generating activity in the heart was indeed renin. ANG I was measured by radioimmunoassay (DiaSorin, Stillwater, MN).

Statistical analyses.

All values are reported as means ± SE. Student's *t*-test and repeated-measures ANOVA followed by a Tukey's post hoc test for multiple comparisons were used to determine significant differences at a probability of <0.05 using GraphPad Prism 5.0 software (San Diego, CA). For the radioimmunoassays, values at or below the minimal detectable limits of the assays were assigned that value for statistical purposes.

RESULTS

Heart rates were not different between normal and hypertensive rat hearts at either baseline or during the 60-min experiment ($P > 0.05$). Administration of ANG-(1–12) to isolated hearts did not change heart rates throughout the experiment (Table 1) in all but SD hearts; in this strain, mild bradycardia occurred after 60 min ($P = 0.02$).

Perfusion pressures were not different between normal and hypertensive rat hearts either at baseline or over the course of the experiment ($P > 0.05$). ANG-(1–12) caused a small increase in perfusion pressures in SD and WKY hearts only at the end of the experiment (60 min) after the metabolism had stabilized (Table 2; $P = 0.004$ and $P = 0.005$, respectively), whereas perfusion pressures in Lewis, congenic, or SHR hearts did not change over the time course of the experiment ($P > 0.05$).

At baseline, the effluent from the isolated heart of all strains investigated did not contain detectable concentrations of ANG I, ANG II, or ANG-(1–7). The addition of ANG-(1–12) to the perfusate of isolated rat hearts from SD animals was associated with rapid and sustained increases in the concentrations of ANG I and ANG II; increases in these peptides were then followed by the slow appearance of ANG-(1–7) (Fig. 1). ANG I production from exogenous ANG-(1–12) peaked at 28 ± 3 min (191 ± 34 pmol/l, $P < 0.0001$), followed by ANG II at 45 ± 7 min (364 ± 81 pmol/l, $P < 0.0001$), whereas ANG-(1–7) production steadily increased to an average of 97 ± 31 pmol/l ($P = 0.0003$) at 55 ± 5 min.

In other experiments, the addition of ANG-(1–12) to isolated hearts of Lewis and congenic rats resulted in similar production of ANG I, ANG II, and ANG-(1–7) of a magnitude and time course comparable with those obtained in SD rats (Fig. 2). ANG I production in Lewis and congenic hearts peaked at 26 ± 4 and 30 ± 0 min ($P > 0.05$), respectively, followed by ANG II at 53 ± 8 and 53 ± 8 min, respectively ($P > 0.05$), whereas ANG-(1–7) production again peaked after 60 ± 0 min in both strains ($P > 0.05$).

Correlation analysis of the peptides in SD, Lewis, and congenic cardiac effluent revealed highly significant correlations between ANG I and ANG II values (Table 3). Moreover, ANG II values were highly correlated with ANG-(1–7) values in all rat hearts.

Evaluation of renin in the metabolism of ANG-(1–12).

A final set of experiments in WKY and SHR hearts expanded our characterization of ANG-(1–12) metabolism and evaluated a potential role of cardiac renin in the cleavage of ANG-(1–12) into ANG I. The application of ANG-(1–12) to the perfusate of isolated hearts from WKY rats and SHRs resulted in similar and sustained production of ANG I, ANG II, and ANG-(1–7) (Fig. 3). Similar to the previous experiments, ANG I production in WKY and SHR hearts peaked at 40 ± 10 and 25 ± 5 min ($P > 0.05$), respectively, followed by ANG II at 50 ± 10 min in both strains, respectively ($P > 0.05$), whereas ANG-(1–7) production peaked after 60 ± 0 and 50 ± 10 min, respectively ($P > 0.05$). The addition of the rat renin inhibitor WFML-1 to the perfusate did not alter the time to peak values for any of the angiotensin peptides ($P > 0.05$). The addition of WKY and SHR angiotensin peptide values to the other strains' correlation analyses did not significantly alter the Pearson correlations, although the correlations remained remarkably significant [ANG I:ANG II, 0.77, $P < 0.0001$; ANG I:ANG-(1–7), 0.61, $P < 0.0001$; and ANG II:ANG-(1–7), 0.62, $P < 0.0001$].

The addition of the renin-specific inhibitor WFML-1 to the perfusate did not alter the production of any of the angiotensin peptides measured in either WKY or SHR hearts (Fig. 3). Renin activity measured in the effluent of WKY rats and SHRs averaged 1.74 ± 0.15

and 2.24 ± 0.56 ng ANG I·ml^{-1}·h^{-1} ($P > 0.05$), verifying its presence in the cardiac effluent. In addition, tissue renin activity in perfused WKY and SHR hearts averaged 1.18 ± 0.15 and 4.43 ± 2.79 ng ANG I·mg protein^{-1}·h^{-1}, respectively ($P > 0.05$); these values were similar to those measured in freshly homogenized WKY and SHR hearts (0.656 ± 0.056 and 0.716 ± 0.096 ng ANG I·mg protein^{-1}·h^{-1}, respectively, $P > 0.05$). The addition of WFML-1 to the renin assay completely abolished ANG I-generating activity from excess exogenous angiotensinogen, confirming that the activity was indeed due to the presence of renin in these tissues. Therefore, the failure of endogenous renin inhibition in altering the pattern and magnitude of peptides generated by the addition of ANG-(1–12) demonstrates that renin has no catalytic activity on ANG-(1–12).

DISCUSSION

Building upon the initial findings by Nagata et al. (10) as well as our preliminary observations (3, 6, 16), we report here for the first time that ANG-(1–12) functions as a precursor for the downstream generation of angiotensin peptides in the hearts of both normal and hypertensive rats. The addition of the dodecapeptide to the perfusate of isolated hearts from five different rat strains revealed similar profiles of angiotensin peptide production. Both ANG I and ANG II appeared in apparent sequence at similar levels in the perfusate. Moreover, the biologically active heptapeptide ANG-(1–7) was produced from exogenous ANG-(1–12) steadily, although the overall values of ANG-(1–7) found in the perfusate were less than those found for ANG I and ANG II. The delayed appearance of ANG-(1–7) in the perfusate compared with the pattern of ANG I and ANG II production suggests that ANG-(1–7) formation did not arise directly from ANG-(1–12). In agreement with this interpretation, we (15) have previously shown that ANG-(1–7) is produced from ANG II by ACE2 in isolated rat hearts.

Because baseline levels of ANG I, ANG II, and ANG-(1–7) were not detectable in the perfusate before the addition of ANG-(1–12), endogenous production of these peptides cannot account for the findings reported here. Lindpaintner et al. (7) showed nondetectable levels of ANG I and ANG II in the effluent from the coronary sinus of the isolated perfused rat heart before the addition of purified hog renin. The addition of renin resulted in a time-dependent generation of ANG I and ANG II. Therefore, it is not surprising that in our present experiments and in those reported by us previously (15), baseline levels of angiotensins were not detectable. Their experiments also argue for the possibility that the effect of ANG-(1–12) may be accounted for by release of pools of preformed (free and/or bound) ANG I, ANG II, and ANG-(1–7). In both situations, either the addition of renin or the addition of ANG-(1–12) was required to stimulate the formation of the angiotensins.

In our experiments, a 10 nmol/l dose of ANG-(1–12) was required since Nagata and colleagues (10) found that higher concentrations (≥30 nmol/l) of ANG-(1–12) elicited significant vasoconstriction in the isolated rat aorta. Since there were no differences in any of the angiotensin peptides generated from ANG-(1–12) between Lewis and congenic nor WKY and SHR hearts, neither targeted nor genetic hypertension appear to substantially influence the processing of ANG-(1–12) in these isolated hearts compared with their background strains. The proportionally similar generation of angiotensin peptides from

ANG-(1–12) among the tested strains does not negate the possibility that endogenous generation of the peptides may not differ among various normotensive and hypertensive strains.

The capacity of cardiac tissue to use ANG-(1–12) as a substrate for the production of angiotensin peptides is further illustrated by the existence of highly significant correlations for ANG I and ANG II values. Indeed, Chappell et al. (3), in a preliminary report, demonstrated that ANG-(1–12) can be metabolized efficiently by rat serum ACE into ANG I, which can then be sequentially cleaved by ACE to form ANG II. Furthermore, we observed highly significant correlations between ANG-(1–7) and both ANG I and ANG II in all five rat strain hearts used, suggesting that ANG-(1–7) was produced from both ANG I and ANG II. Collectively, these data suggest that in the heart, ANG-(1–12) is processed into ANG I, which can then be processed into both ANG II and ANG-(1–7), although a direct cleavage of ANG-(1–12) into ANG II, particularly in SD hearts, cannot be excluded.

In the study reported by Nagata et al. (10), the cardiac content of ANG-(1–12), ANG I, and ANG II averaged 151 ± 11, 85 ± 8, and 42 ± 7 pmol/l, respectively. In other words, the content of ANG-(1–12) was about twice as large that of ANG I. We measured peak concentrations of ANG I and ANG II in the coronary effluent that averaged 531.5 ± 48.4 and 594.8 ± 72.6 pmol/l, respectively, across all strains, which represents no more than 6- to 14-fold higher than was found endogenously by Nagata and colleagues. These data suggest that studies in isolated hearts reflect to a significant degree what may be the tissue dynamics in vivo.

Because others have found that the tetradecapeptide ANG-(1–14) is cleaved by renin (8, 9, 13), we administered a rat renin-specific inhibitor concomitantly with ANG-(1–12) to the perfusate of WKY and SHR isolated hearts to determine whether ANG-(1–12), like ANG-(1–14), was a suitable substrate for renin activity. As indicated above, renin inhibition did not alter the production of any of the angiotensin peptides, nor did it affect heart rate or perfusion pressures. Additionally, we verified, in both perfused and nonperfused hearts, that renin was present in the heart by measuring its tissue activity as well as the activity in the cardiac effluent. Therefore, our data show that ANG-(1–12), unlike ANG-(1–14), is not cleaved by renin, which corroborates a recent study from our group (3). Renin specifically cleaves the Leu^{10}-Leu^{11} bond of rat angiotensinogen to form ANG I, whereas the cleavage between the two aromatic residues Tyr^{12}-Tyr^{13} liberates ANG-(1–12). A lack of differential processing of ANG-(1–12) between Lewis and congenic mRen2.Lewis rats also supports a non-renin role for the metabolism of ANG-(1–12) in the heart, as mRen2.Lewis rats express elevated cardiac renin levels (2).

Further support for a biological role of ANG-(1–12) in the heart stems from a study (6) that showed that ANG-(1–12) was robustly present in ventricular myocytes of both WKY rats and SHRs. Evidence of functionality is further illustrated by the increased cardiac content of ANG-(1–12) in SHRs compared with WKY rats. That this peptide was found in most tissues at higher levels than both ANG I and ANG II (10), in concert with the findings of the present study, asserts that ANG-(1–12) may be a readily available substrate for angiotensin peptide production.

Limitations of the study.

The data presented herein represent a critical first step to understanding the ultimate role that ANG-(1–12) may play in cardiovascular physiology. The purpose of this initial study was to determine whether the dodecapeptide could serve as a substrate for the formation of ANG I, ANG II, and ANG-(1–7) in the hearts of five different rat strains, and, as such, the enzymatic mechanisms accounting for the formation of ANG-(1–12) from angiotensinogen were outside the scope of the present study. While not designed to determine enzymatic mechanisms, the present study undertook steps to exclude renin in the metabolism of ANG-(1–12) into any of the three downstream angiotensin peptides measured. Moreover, based on a preliminary study from our group (3), the direct enzymatic conversion of ANG-(1–12) into ANG I and ANG-(1–7) appears to be mediated by serum ACE and renal neprilysin, respectively. Further studies will be required to determine how exactly ANG-(1–12) can be metabolized in not only the heart but in other tissues critical in physiological regulation.

Conclusions.

In the century since Tigerstedt and Bergman (14) first described renin, many advances have been made regarding the contributions of the RAS to the regulation of cardiovascular processes. Indeed, the effective clinical treatment of hypertension and heart failure arrived almost 100 years after renin's discovery: first, with the introduction of ACE inhibitors in 1981; later, with the advent of ARBs in 1995; and, most currently, with the development and arrival of the renin inhibitor aliskiren in 2007. The identification of an angiotensin peptide upstream of ANG I that can serve as a substrate to produce bioactive angiotensin peptides is a novel and important finding. Although it is not yet known what enzyme(s) can cleave ANG-(1–12) from its parent protein angiotensinogen, the possibility that this process may occur in a renin-independent manner holds high potential to change our evolving understanding of the RAS in the regulation of physiological processes. In support of a renin-independent pathway for angiotensin peptide formation, Oparil and colleagues (12) recently showed that in patients treated with maximal doses of both the renin inhibitor aliskiren and the angiotensin type 1 receptor antagonist valsartan, there were additive blood pressure reductions, which is an unexpected finding if renin is the sole liberator of angiotensin peptides. Indeed, we suggest that ANG-(1–12) may serve as a "quick release" substrate for the immediate production of RAS components as necessary, which may likely be more efficient than the cell making the almost 500-amino acid angiotensinogen for the production of angiotensin peptides. The unraveling of the functional significance of ANG-(1–12) as well as the pathways for its formation and degradation should bear considerable importance.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-51952 and HL-56973. A. J. Trask was supported by a fellowship award from the Mid-Atlantic affiliate of the American Heart Association, Mid-Atlantic Affiliate, Grant 0715249U. Additionally, the authors gratefully acknowledge grant support in part provided by Unifi Incorporated (Greensboro, NC) and the Farley-Hudson Foundation (Jacksonville, NC).

REFERENCES

1. Chappell MC. Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1–7)-MAS receptor axis: more than regulation of blood pressure? Hypertension 50: 596–599, 2007. [PubMed: 17785634]
2. Chappell MC, Gallagher PE, Averill DB, Ferrario CM, Brosnihan KB. Estrogen or the AT1 antagonist olmesartan reverses the development of profound hypertension in the congenic mRen2.Lewis rat. Hypertension 42: 781–786, 2003. [PubMed: 12874087]
3. Chappell MC, Westwood BM, Pendergrass KD, Jessup JA, Ferrario CM. Distinct processing pathways for the novel peptide angiotensin-(1–12) in the serum and kidney of the hypertensive mRen2.Lewis rat (Abstract). Hypertension 50: e139, 2007.
4. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 111: 2605–2610, 2005. [PubMed: 15897343]
5. Ferrario CM, Jessup J, Gallagher PE, Averill DB, Brosnihan KB, Ann TE, Smith RD, Chappell MC. Effects of renin-angiotensin system blockade on renal angiotensin-(1–7) forming enzymes and receptors. Kidney Int 68: 2189–2196, 2005. [PubMed: 16221218]
6. Jessup JA, Chappell MC, Nagata S, Kato J, Kitamura K, Ferrario CM. Localization of the novel angiotensin peptide, proangiotensin-12, in the heart and kidney of hypertensive and normotensive rats (Abstract). Hypertension 50: e101, 2007.
7. Lindpaintner K, Jin MW, Niedermaier N, Wilhelm MJ, Ganten D. Cardiac angiotensinogen and its local activation in the isolated perfused beating heart. Circ Res 67: 564–573, 1990. [PubMed: 2204498]
8. Mendelsohn FA, Johnston CI. A radiochemical renin assay. Biochem J 121: 241–244, 1971. [PubMed: 4330090]
9. Montague D, Riniker B, Brunner H, Gross F. Synthesis and biological activities of a tetradecapeptide renin substrate. Am J Physiol 210: 591–594, 1966. [PubMed: 5933212]
10. Nagata S, Kato J, Sasaki K, Minamino N, Eto T, Kitamura K. Isolation and identification of proangiotensin-12, a possible component of the renin-angiotensin system. Biochem Biophys Res Commun 350: 1026–1031, 2006. [PubMed: 17045572]
11. Nakamura S, Averill DB, Chappell MC, Diz DI, Brosnihan KB, Ferrario CM. Angiotensin receptors contribute to blood pressure homeostasis in salt-depleted SHR. Am J Physiol Regul Integr Comp Physiol 284: R164–R173, 2003. [PubMed: 12482748]
12. Oparil S, Yarows SA, Patel S, Fang H, Zhang J, Satlin A. Efficacy and safety of combined use of aliskiren and valsartan in patients with hypertension: a randomised, double-blind trial. Lancet 370: 221–229, 2007. [PubMed: 17658393]
13. Skeggs LT Jr, Lentz KE, Kahn JR, Shumway NP. The synthesis of a tetradecapeptide renin substrate. J Exp Med 108: 283–297, 1958. [PubMed: 13575666]
14. Tigerstedt R, Bergman PG. Niere und Kreislauf. Scan Arch Physiol 8: 223–271, 1898.
15. Trask AJ, Averill DB, Ganten D, Chappell MC, Ferrario CM. Primary role of angiotensin-converting enzyme-2 in cardiac production of angiotensin-(1–7) in transgenic Ren-2 hypertensive rats. Am J Physiol Heart Circ Physiol 292: H3019–H3024, 2007. [PubMed: 17308000]
16. Trask AJ, Jessup JA, Ferrario CM. Angiotensin-(1–12) is a precursor for the processing of cardiac tissue angiotensin peptides (Abstract). Hypertension 50: e154. 2007.
17. Welches WR, Brosnihan KB, Ferrario CM. A comparison of the properties and enzymatic activities of three angiotensin processing enzymes: angiotensin converting enzyme, prolyl endopeptidase and neutral endopeptidase 24.11. Life Sci 52: 1461–1480, 1993. [PubMed: 8387132]
18. Zisman LS, Meixell GE, Bristow MR, Canver CC. Angiotensin-(1–7) formation in the intact human heart: in vivo dependence on angiotensin II as substrate. Circulation 108: 1679–1681, 2003. [PubMed: 14504185]

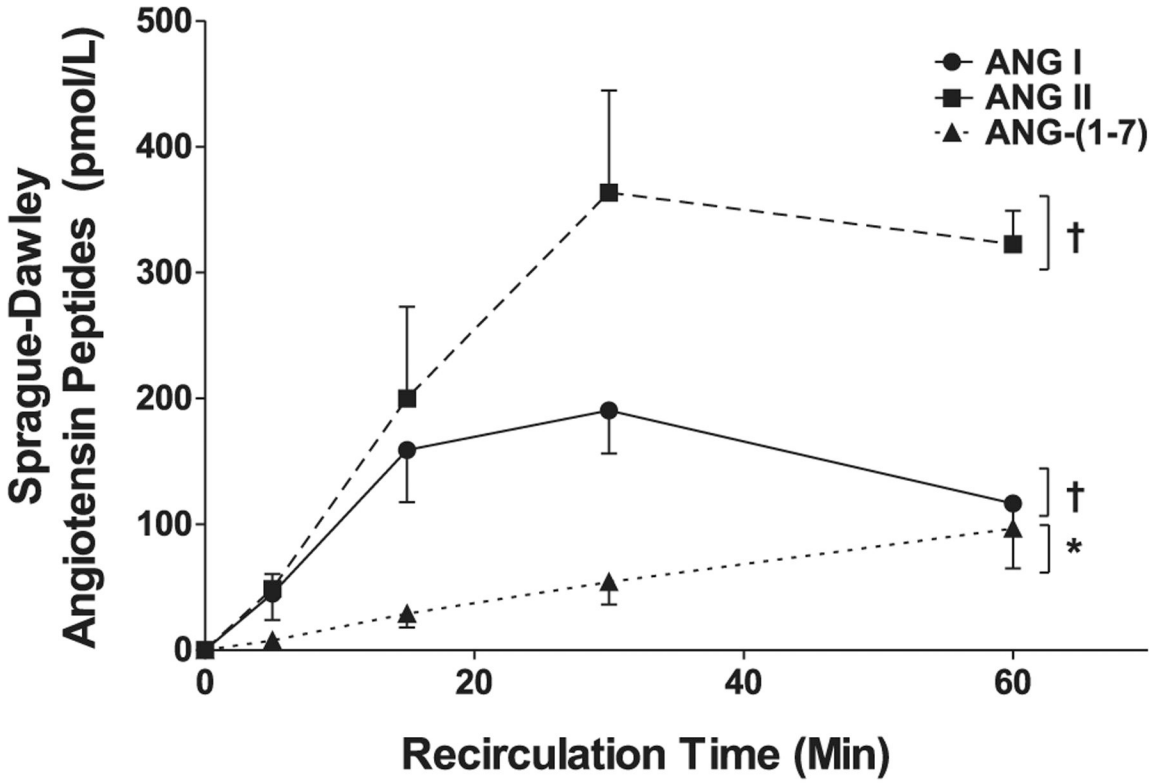


Fig. 1.

ANG I, ANG II, and ANG-(1–7) production from exogenous ANG-(1–12) in isolated Sprague-Dawley (SD) rat hearts ($n = 6$). Both ANG I and ANG II peaked at 30 min of recirculation (ANG I: 191 ± 34 pmol/l and ANG II: 364 ± 81 pmol/l), whereas ANG-(1–7) steadily increased until 60 min of recirculation (97 ± 31 pmol/l). *$P = 0.0003$ and †$P < 0.0001$ vs. baseline.

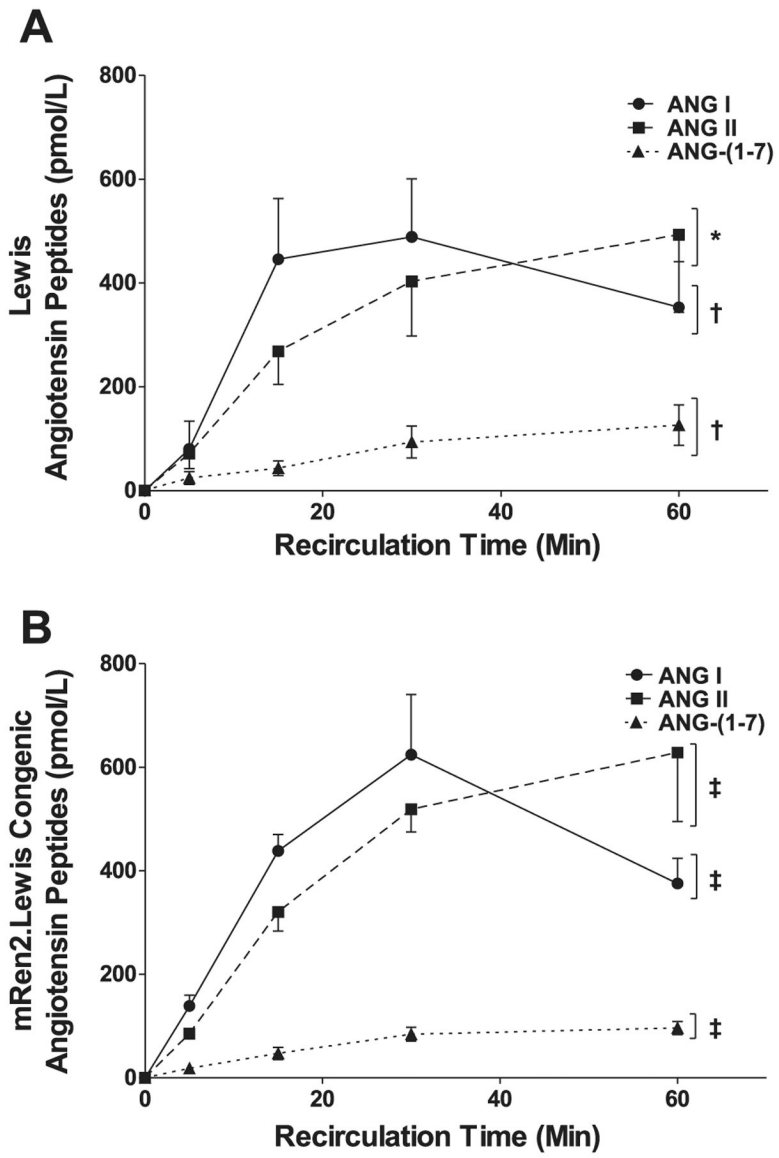


Fig. 2.

ANG I, ANG II, and ANG-(1–7) production from exogenous ANG-(1–12) in isolated Lewis (*A*; $n = 4$) and mRen2.Lewis congenic (*B*; $n = 4$) rat hearts. ANG I peaked at 30 min of recirculation in both Lewis and congenic rat hearts (489 ± 112 and 625 ± 116 pmol/l, respectively), whereas both ANG II and ANG-(1–7) steadily increased until 60 min of recirculation [ANG II: 493 ± 190 (Lewis) and 628 ± 133 pmol/l (congenic) and ANG-(1–7): 126 ± 39 (Lewis), 96 ± 13 pmol/l (congenic)]. There were no statistical differences in any of the angiotensin peptides between Lewis and congenic rat hearts. *$P < 0.01$, †$P < 0.001$, and ‡$P < 0.0001$ vs. baseline.

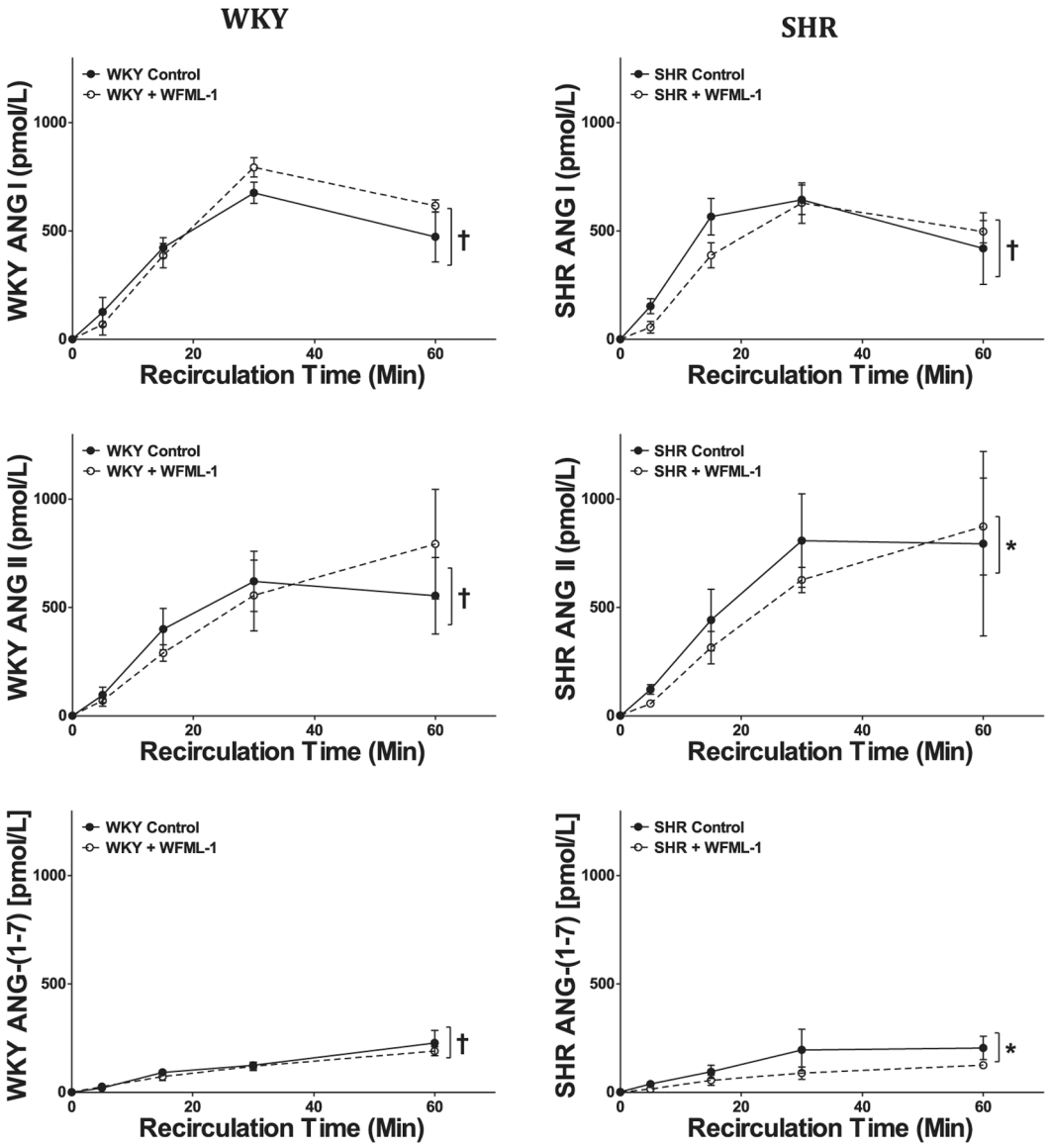


Fig. 3.

ANG I (*top*), ANG II (*middle*), and ANG-(1–7) (*bottom*) production from exogenous ANG-(1–12) in isolated Wistar-Kyoto (WKY; *left*; $n = 6$) and spontaneously hypertensive rat (SHR; *right*; $n = 6$) hearts. ANG I and ANG II both peaked at 30 min of recirculation in both WKY and SHR hearts (ANG I: 676 ± 49 and 644 ± 68 pmol/l, respectively; and ANG II: 620 ± 139 and 808 ± 216 pmol/l, respectively), whereas ANG-(1–7) steadily increased until 60 min of recirculation [228 ± 58 (WKY) and 204 ± 54 pmol/l (SHR)]. There were no statistical differences in any of the angiotensin peptides between WKY and SHR hearts, nor did renin inhibition alter the production of any of the angiotensin peptides measured (represented by open circles and dotted lines). *$P < 0.05$ and †$P < 0.01$ vs. baseline.

Table 1.

Time course of heart rates in all rat strains studied

	Time				
	5 min	**15 min**	**30 min**	**60 min**	***P* Value**
Sprague-Dawley	253 ± 10	210 ± 16	238 ± 10	228 ± 9	0.02 *
Lewis	251 ± 15	234 ± 14	223 ± 10	205 ± 26	0.06
Congenic	219 ± 8	200 ± 19	212 ± 14	211 ± 20	0.69
WKY	237 ± 24	231 ± 12	186 ± 17	181 ± 13	0.28
SHR	234 ± 9	206 ± 9	203 ± 15	195 ± 16	0.27

Values are means ± SE (in beats/min). WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat.

* Significantly different value.

Table 2.

Time course of perfusion pressures in all rat strains studied

	Time				
	5 min	**15 min**	**30 min**	**60 min**	***P* Value**
Sprague-Dawley	64 ± 9	83 ± 18	105 ± 27	170 ± 48	0.004 *
Lewis	64 ± 4	64 ± 8	74 ± 13	131 ± 48	0.13
Congenic	63 ± 5	67 ± 8	74 ± 13	109 ± 39	0.22
WKY	66 ± 5	66 ± 4	74 ± 6	113 ± 12	0.005 *
SHR	72 ± 5	73 ± 7	80 ± 14	110 ± 22	0.09

Values are means ± SE (in mmHg).

* Significantly different value.

Table 3.

Pooled angiotensin peptide correlations from Sprague-Dawley, Lewis, and congenic rat cardiac effluent

	ANG I	ANG II	ANG-(1–7)
ANG I		0.72	0.51
ANG II	0.72		0.71
ANG-(1–7)	0.51	0.71	

All Pearson correlation values were $P < 0.0001$.