Central Angiotensin (1-7) Enhances Baroreflex Gain in Conscious Rabbits With Heart Failure
Sumit Kar, Lie Gao, Daniel A. Belatti, Pamela L. Curry, Irving H. Zucker

Abstract—In chronic heart failure (CHF), arterial baroreflex function is impaired, in part, by activation of the central renin-angiotensin system. A metabolite of angiotensin (Ang) II, Ang-(1-7), has been shown to exhibit cardiovascular effects that are in opposition to that of Ang II. However, the action of Ang-(1-7) on sympathetic outflow and baroreflex function is not well understood, especially in CHF. The aim of this study was to determine the effect of intracerebroventricular infusion of Ang-(1-7) on baroreflex control of heart rate and renal sympathetic nerve activity in conscious rabbits with CHF. We hypothesized that central Ang-(1-7) would improve baroreflex function in CHF. Ang-(1-7) (2 nmol/l μL per hour) or artificial cerebrospinal fluid (1 μL per hour) was infused by an osmotic minipump for 4 days in sham and pacing-induced CHF rabbits (n=3 to 6 per group). Ang-(1-7) treatment had no effects in sham rabbits but reduced heart rate and increased baroreflex gain (7.4±1.5 versus 2.5±0.4 bpm/mm Hg; P<0.05) in CHF rabbits. The Ang-(1-7) antagonist A779 (8 nmol/l μL per hour) blocked the improvement in baroreflex gain in CHF. Baroreflex gain increased in CHF+Ang-(1-7) animals when only the vagus was allowed to modulate baroreflex control by acute treatment with the β-1 antagonist metoprolol, indicating increased vagal tone. Baseline renal sympathetic nerve activity was significantly lower, and baroreflex control of renal sympathetic nerve activity was enhanced in CHF rabbits receiving Ang-(1-7). These data suggest that augmentation of central Ang-(1-7) inhibits sympathetic outflow and increases vagal outflow in CHF, thus contributing to enhanced baroreflex gain in this disease state. (Hypertension. 2011;58:627-634.) • Online Data Supplement

Key Words: angiotensin-(1-7) • heart failure • sympathetic nervous system • baroreflex • vagus nerve • blood pressure • heart rate

Chronic heart failure (CHF) is characterized by heightened sympathetic tone in compensation for reduced cardiac function. This compensation is mediated, in part, by activation of the renin-angiotensin system and increased production of angiotensin (Ang) II. Recent studies in experimental models of CHF and hypertension have suggested that, in the brain, the responses to Ang II are enhanced by increases in Ang type 1 receptors and decreases in Ang type 2 receptors, whereas the Ang-(1-7) axis is concurrently diminished. Our laboratory has found recently in rabbits with CHF that expression of Ang-converting enzyme (ACE) 2 decreases in regions of the brain that are involved in sympathetic function. However, there have been limited studies examining the effects Ang-(1-7) in the brain on the neural control of cardiovascular function in CHF.

The actions of Ang-(1-7) in the heart, as examined in many tissues, generally oppose the actions of Ang II. In the heart, Ang-(1-7) is expressed in cardiac myocytes, appears to have an inotropic effect, and possesses coronary vasodilator activity. In contrast to Ang II, Ang-(1-7) appears to inhibit the cardiac remodeling process in several rat models. In a study by Benter et al, oral administration of Ang-(1-7) prevented development of hypertension in spontaneously hypertensive rats treated with N6-nitro-L-arginine methyl ester.

Ang-(1-7) is produced in many areas of the brain, and its actions have been linked to sympathetic regulation. Potts et al showed that, in anesthetized rabbits, Ang-(1-7) evoked sympathoexcitation when injected into the rostral ventrolateral medulla. Similar results were obtained by Gomes da Silva et al and by Silva et al in rats. In a more recent study, Gomes da Silva et al injected the mas receptor antagonist A-779 in the paraventricular nucleus of anesthetized rats and observed a decrease in renal sympathetic nerve activity (RSNA), suggesting that Ang-(1-7) is sympathoexcitatory. On the other hand, other studies suggest that Ang-(1-7) may be sympathoinhibitory. In a recent study by Campagnole-Santos and Guimarães, central infusion of Ang-(1-7) reduced blood pressure and heart rate in deoxycorticosterone acetate–salt hypertensive rats. Thus, the central effect of Ang-(1-7) on sympathetic tone is unclear and, to our knowledge, has not been studied in CHF.

Our laboratory has shown previously that baroreflex function is impaired in CHF because of increased Ang II, increased sympathetic tone, and decreased vagal tone. Some studies have...
shown that Ang-(1-7) improves baroreflex function in hypertensive models. However, no studies have determined the role of central Ang-(1-7) on baroreflex control in CHF or have determined the sympathetic and vagal components responsible for the improvements in baroreflex function.

Therefore, we tested the hypothesis that central Ang-(1-7) enhances baroreflex control of heart rate (HR) and RSNA and favorably modulates sympathovagal balance in the setting of CHF. We determined the effect of central Ang-(1-7) on renal sympathetic outflow and cardiac sympathovagal balance using direct recordings of RSNA and HR in a conscious rabbit model of pacing-induced heart failure. We also determined the central effect of Ang-(1-7) on baroreflex control of HR and RSNA.

Materials and Methods
An expanded Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.

Animals
Experiments were carried out on 32 male New Zealand white rabbits weighing between 3.0 and 4.5 kg (Charles River Laboratories, Wilmington, MA). These experiments were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. Experiments were carried out in 5 experimental groups, a normal sham-operated group with central artificial cerebrospinal fluid (aCSF) infusion (sham+aCSF), a sham group with central Ang-(1-7) infusion (sham+Ang-[1-7]), a CHF group with aCSF infusion (CHF+aCSF), a CHF group with Ang-(1-7) infusion (CHF+Ang-[1-7]), and a CHF group with Ang-(1-7) and A779 infusion (CHF+Ang-[1-7]+A779).

Cardiac Baroreflex and Autonomic Blockade Protocol (13 Rabbits)
A representative time line of these studies is provided in Figure S1 (available in the online Data Supplement at http://hyper.ahajournals.org). Rabbits were instrumented with left ventricular pacing electrodes and a radiotelemetry transducer (DataSciences, Inc, Minneapolis MN) to monitor mean arterial blood pressure (MAP) and HR. Two weeks after the insertion of telemetry, an ICV brain cannula was inserted and was attached to an osmotic minipump (ALZET 2001, ALZET, Cupertino, CA) filled with aCSF, which infused at a rate of 80 L/h. On the day of the first experiment, the rabbit was placed in a box in a dimly lit room. The rabbit was allowed to rest quietly for 20 minutes before data were collected. Baseline recordings of MAP and HR were taken for 5 minutes. After baseline recording, the response to nasopharyngeal stimulation with 60 mL of cigarette smoke was measured, which our laboratory has used previously to activate and measure vagal tone.

The arterial baroreflex control of HR was determined by IV infusions of sodium nitroprusside (100 µg/kg) and phenylephrine (80 µg/kg) at a rate of 0.5 mL/min. After sodium nitroprusside lowered MAP to its lowest point (≈40 mmHg), the sodium nitroprusside infusion was stopped and replaced immediately with phenylephrine, which was infused at the same rate until MAP reached 110 mmHg. The following day, the aCSF pump was replaced with a pump filled with Ang-(1-7). Ang-(1-7) (Sigma, St Louis, MO) was dissolved in aCSF (2 nmol/µL per hour). Three days after the insertion of this pump, baseline recordings and cardiac baroreflex assessment were repeated. After the sham (prepare) experiments, the Ang-(1-7) pump was replaced with another aCSF pump, and CHF was induced in each rabbit by chronic ventricular pacing, as described previously. The experimental protocol described above was repeated in each rabbit after they reached an ejection fraction <45%.

In each rabbit, after generation of a control baroreflex curve, a second curve was constructed 10 minutes after IV administration of 0.2 mg/kg of atropine methylbromide. A new set of baseline recordings, smoke response, and baroreflex function was carried out. On the following day, the same procedures were carried out, after administration of 1 mg/kg IV of metoprolol bitartrate.

RSNA Measurement Protocol (16 Rabbits)
Additional rabbits were randomly divided into 4 groups (n=4 per group) for measurement of baseline RSNA and baroreflex control of RSNA. A representative time line for these animals is provided in Figure S1. Nerve recording electrodes were implanted as described previously along with an ICV cannula and osmotic minipump containing aCSF or Ang-(1-7). Recording electrodes and the ICV cannula plus minipump were implanted in CHF rabbits when EF fell to <45% after pacing.

RSNA was digitized at 1000 samples per second and amplified with an Animal Bio Amp (ADInstruments, Inc) with the bandwidth set between 25 Hz and 1 KHz. The spike frequency of the raw nerve activity was determined by setting a window discriminator =10% above the noise. Baseline recordings and baroreflex experiments were carried out 3 to 5 days after the implantation of the nerve electrodes. Raw nerve activity and frequency were recorded continuously during the experiments. The data are expressed as a percent age of the maximal spike frequency. The maximal activity was determined in each rabbit by observing its response to 60 mL of cigarette smoke administered into the external nares.

Statistical Analysis
Data are expressed as mean±SEM. All of the statistical analysis was performed with SigmaPlot 11 software. Differences between groups were analyzed with a 1-way ANOVA or repeated-measures ANOVA where appropriate followed by the Newman-Keuls post hoc test. P<0.05 was considered statistically significant.

Results
Cardiac Dimensions and Hemodynamics
Body weight, baseline hemodynamics, and echocardiographic data in the 5 groups studied are provided in the Table. The CHF group exhibited a significantly lower EF and fractional shortening, along with higher resting HR and left ventricular systolic diameter and volume compared with the sham group. Additional clinical signs of CHF were observed, including ascites and pulmonary edema. Central infusion of Ang-(1-7) did not alter cardiac dimensions in sham or CHF rabbits. Ang-(1-7) significantly reduced HR in CHF animals. CHF rabbits receiving central Ang-(1-7)+A779 had a significantly higher resting HR than CHF rabbits receiving only Ang-(1-7). Interestingly, however, the HR was significantly lower than CHF rabbits receiving aCSF. Similar changes in baseline HR and EF were observed in the subset of rabbits used for recording of RSNA (Table S1). All of the time domain parameters of heart rate variability were depressed in CHF+aCSF rabbits (Table S2). Central Ang-(1-7) normalized heart rate variability in the CHF state.

Baseline HR was also recorded before and after IV injections of atropine and metoprolol to determine changes in sympathovagal balance. The increase in HR in response to atropine (Figure 1A) seen in sham+aCSF rabbits was essentially abolished in the CHF+aCSF group, indicating a dramatic reduction in vagal activity in CHF. Ang-(1-7) treatment in CHF increased the tachycardic response to atropine, indicating an increase in vagal tone. The bradycardic response to metoprolol (Figure 1B) was significantly lower in CHF+Ang-(1-7) rabbits compared with CHF+aCSF, reflect-
ing a decrease in sympathetic tone in response to chronic ICV infusion of Ang-(1-7).

**Effect of Ang-(1-7) on the Response to Smoke**

Figure 2 shows the change in HR in response to nasopharyngeal stimulation. An abrupt fall in HR with little or no increase in blood pressure was seen in sham rabbits. The bradycardia was significantly diminished in CHF/aCSF. Ang-(1-7) normalized this response. In all of the groups, atropine abolished the change in HR to near 0 (data not shown).

**Resting RSNA After Ang-(1-7)**

Figure 3 shows an original recording of baseline MAP, HR, and RSNA in a CHF rabbit given aCSF (Figure 3A) and a CHF rabbit given ICV Ang-(1-7) (Figure 3B). The frequency of sympathetic bursts in CHF Ang-(1-7) appears to be lower than that in CHF/aCSF. The mean data for all of the groups (Figure 3C) shows that CHF/aCSF rabbits had significantly higher RSNA than sham rabbits. Ang-(1-7) lowered resting RSNA in CHF and A779 treatment returned RSNA to CHF/aCSF levels.

**Cardiac and Sympathetic Baroreflex Function in Heart Failure**

Representative recordings of baroreflex changes are show in Figure S2. Figure 4 shows composite baroreflex curves of HR and RSNA for all of the groups before autonomic blockade. In the unblocked conscious state, baroreflex sensitivity was

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**Table. Baseline Hemodynamics in Sham and CHF Rabbits Before and After Ang-(1-7) Infusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham + aCSF</th>
<th>Sham + Ang-(1-7)</th>
<th>CHF + aCSF</th>
<th>CHF + Ang-(1-7)</th>
<th>CHF + Ang-(1-7) + A779</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>3.6±0.1</td>
<td>3.6±0.1</td>
<td>3.7±0.1</td>
<td>3.6±0.1</td>
<td>3.5±0.1</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>198.5±4.6</td>
<td>186.8±4.5</td>
<td>248.0±5.5*</td>
<td>209.6±4.9†</td>
<td>221.5±5.5*†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>74.3±1.9</td>
<td>69.1±3.1</td>
<td>73.4±3.0</td>
<td>67.3±3.2</td>
<td>76.1±6.3</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>16.2±0.5</td>
<td>16.3±0.4</td>
<td>17.1±0.4</td>
<td>16.6±0.6</td>
<td>16.7±0.1</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>7.1±1.4</td>
<td>7.5±0.3</td>
<td>13.6±0.4*</td>
<td>13.5±0.5*</td>
<td>13.8±0.2*</td>
</tr>
<tr>
<td>LVD vol, mL</td>
<td>7.6±0.6</td>
<td>6.7±0.5</td>
<td>8.6±0.5</td>
<td>8.2±0.7</td>
<td>8.1±0.1</td>
</tr>
<tr>
<td>LVs vol, mL</td>
<td>2.2±0.2</td>
<td>2.4±0.3</td>
<td>4.7±0.3*</td>
<td>4.6±0.5*</td>
<td>4.8±0.2*</td>
</tr>
<tr>
<td>FS %</td>
<td>34.2±1.7</td>
<td>35.9±0.8</td>
<td>20.5±0.5*</td>
<td>19.0±0.9*</td>
<td>18.0±1.0*</td>
</tr>
<tr>
<td>EF %</td>
<td>70.3±0.6</td>
<td>73.2±1.0</td>
<td>45.1±1.0*</td>
<td>42.3±1.7*</td>
<td>40.4±1.9*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LV indicates left ventricle; CHF, chronic heart failure; MAP, mean arterial pressure; LVEDD, left ventricular end-diastolic diameter; LVSD, left ventricular systolic diameter; LVD vol, left ventricular diastolic diameter; LVs vol, left ventricular systolic diameter; FS, fractional shortening; EF, ejection fraction; aCSF, artificial cerebrospinal fluid; Ang, angiotensin.

*P<0.05 vs sham + aCSF.
†P<0.05 vs CHF + aCSF.
reduced in CHF+aCSF rabbits (sham+aCSF: 5.6±0.5 bpm/mm Hg; CHF+aCSF: 2.6±0.3 bpm/mm Hg; P<0.05). Ang-(1-7) infusion had no effect on BRS in sham animals but significantly improved baroreflex sensitivity in CHF animals (CHF+aCSF: 2.6±0.3 bpm/mm Hg; CHF+Ang-[1-7]: 6.6±1.0 bpm/mm Hg; P<0.05). HR range was also significantly increased in CHF rabbits given central Ang-(1-7) (236.7±4.7 bpm; P<0.05). This improvement in baroreflex function was largely attributed to an enhancement of the minimum HR achieved after administration of phenylephrine (sham+aCSF: 62.1±7.3 bpm; sham+Ang-[1-7]: 57.3±5.3 bpm; CHF+aCSF: 182.4±12.8 bpm; CHF+Ang-[1-7]: 64.2±7.2 bpm; P<0.05). There were no differences in the blood pressure at 50% of the range (BP50%) between groups.

For baroreflex control of RSNA, CHF significantly decreased baroreflex gain, which was normalized with Ang-(1-7), but no significant changes in minimum or maximum RSNA were observed (Figure 4C). A779 abolished the improvement in baroreflex gain seen after Ang-(1-7) infusion (Figure 4D). The mean curve parameters for baroreflex control of RSNA are shown in Table S4. A779 abolished the improvement in baroreflex gain (CHF+Ang-[1-7]: 6.6±1.0 bpm/mm Hg; CHF+Ang-[1-7]+A779: 3.6±0.4 bpm/mm Hg; P<0.05) and the improvement in the minimum HR (CHF+Ang-[1-7]: 129.9±15.1 bpm; CHF+Ang-[1-7]+A779: 236.7±4.7 bpm; P<0.05) seen from Ang-(1-7).

Cardiac Baroreflex Function After Autonomic Blockade
Figure 5 shows mean data for the peak baroreflex slopes in the 4 groups of rabbits before and after IV administration of atropine or metoprolol. The peak slope was significantly decreased in the CHF+aCSF group compared with sham+aCSF in control, atropine, and metoprolol states. Ang-(1-7) had no effect on baroreflex slope in sham animals after autonomic blockade. CHF+Ang-(1-7) animals showed increased baroreflex slope in the control, atropine, and metoprolol states compared with the same state in the CHF+aCSF group. Additional baroreflex curve parameters after autonomic blockade are presented in Table S3.

Discussion
The present study was carried out to determine the effects of chronic central infusion of Ang-(1-7) on baroreflex control of sympathetic outflow and cardiac sympathovagal balance in CHF. The most important findings of this study are as follows: (1) central Ang-(1-7) enhances arterial baroreflex control of RSNA in CHF; (2) Ang-(1-7) enhances baroreflex control of HR by a profound effect on vagal outflow; and (3) these effects were blocked by central administration of a mas receptor antagonist.

Chronic ICV infusion of Ang-(1-7) resulted in a decrease in resting HR and RSNA and an increase in baroreflex gain in animals with CHF. Heart rate variability was significantly increased in CHF rabbits treated with Ang-(1-7). The HR response to cigarette smoke (a vagally mediated bradycardia) was blunted in CHF animals and restored to control after central Ang-(1-7) infusion. The change in baseline HR after administration of atropine was augmented by central Ang-(1-7) infusion. An enhancement of vagal outflow in CHF rabbits receiving ICV Ang-(1-7) is also supported by the increased baroreflex slope after metoprolol. The change in HR after administration of the β-1 blocker metoprolol was also significantly reduced in CHF animals receiving central infusion of Ang-(1-7), indicating decreased cardiac sympathetic outflow. The fact that both cardiac sympathetic outflow and RSNA were decreased after ICV Ang-(1-7) in CHF suggests a global reduction in sympathetic outflow by Ang-(1-7). Thus, Ang-(1-7) exerts both a cardiac vagotonic effect and a sympathoinhibitory effect in the setting of CHF, thus restoring sympathovagal balance.

The central effects of Ang-(1-7) are not uniformly agreed on. Gomes da Silva et al16 injected the mas receptor antagonist A-779 in the paraventricular nucleus of anesthetized rats and observed a decrease in RSNA suggesting that Ang-(1-7) is sympathoexcitatory. However, this effect was transient compared with Ang II. On the other hand, other studies suggest that Ang-(1-7) may have a sympathoinhibitory role. Gironacci et al28 showed that Ang-(1-7) decreased norepinephrine release from the hypothalamus of spontaneously hypertensive rats. The current study supports the view that Ang-(1-7) opposes the actions of Ang II with a sympathoinhibitory effect in CHF.

The mechanism(s) by which Ang-(1-7) enhances arterial baroreflex function is not completely understood. There are substantial data showing that Ang II reduces baroreflex sensitivity by actions at several sites in the medulla.27 For instance, Ang II acting through the Ang type 1 receptor in the nucleus tractus solitarius (NTS) inhibits baroreflex function.29 Sakima et al29 showed that, in rats with low levels of brain angiotensinogen, inhibition of Ang-(1-7) reduced baroreflex sensitivity. Data from this same laboratory have convincingly shown that blockade of Ang-(1-7) receptors reduced baroreflex function in Sprague-Dawley rats when the
Ang-(1-7) antagonist (D-Ala<sup>7</sup>)-Ang-(1-7) was injected into the NTS. This maneuver most likely shifted the balance between Ang II and Ang-(1-7) toward the baroreflex inhibitory effects of Ang II.

A unique finding in the present study is that chronic Ang-(1-7) infusion into the brain activates the cardiac vagus in animals with CHF that have low resting vagal tone. Although these studies cannot determine the site of Ang-(1-7) activation of central nuclei that may contribute to this vagal effect, it is likely that the NTS is the site. A study by Campagnole-Santos et al<sup>30</sup> showed that microinjection of Ang-(1-7) into the NTS in anesthetized rats resulted in bradycardia. Importantly, Barnes et al<sup>31</sup> showed that Ang-(1-7) stimulated neurons in the dorsal motor nucleus of the vagus in a canine brain section preparation. Furthermore, Becker et al<sup>32</sup> have shown intense binding of Ang-(1-7) to mas receptors in neurons in the NTS. Thus, it is likely that the bradycardic effect of central Ang-(1-7), along with the enhancement in baroreflex function, is mediated by activation of the NTS. This notion would also explain the sympathoinhibition and improvement in baroreflex function after Ang-(1-7) infusion. Because mas receptors have also been identified in other nuclei known to be rich in Ang II receptors, such as the paraventricular nucleus and rostral ventrolateral medulla, it is also possible that other structures are responsible for the central effects of Ang-(1-7).

The cellular actions of Ang-(1-7) are still being defined. However, it is clear that, in neurons, working through the G protein–coupled mas receptor, Ang-(1-7) activates a neuronal NO synthase–dependent mechanism that regulates neuronal excitability. In catecholaminergic neurons in culture (CATH.a), Yang et al<sup>33</sup> showed that Ang-(1-7) increased NO formation. This effect was inhibited by both the mas receptor antagonist A779 and by selective neuronal NO synthase inhibitors.

**Figure 3.** Baseline renal sympathetic nerve activity (RSNA) in rabbits with pacing-induced chronic heart failure (CHF) with and without ICV angiotensin (Ang)-(1-7) infusion. A, An original recording of mean arterial blood pressure (MAP), heart rate (HR), raw RSNA, and RSNA frequency from a CHF rabbit receiving artificial cerebrospinal fluid (aCSF). B, A recording from a different CHF rabbit receiving ICV Ang-(1-7). C, Mean data for baseline RSNA expressed as a percentage of maximum nerve activity in each group of rabbits. Ang-(1-7) significantly reduced RSNA in CHF rabbits. This effect was abolished with A779 infusion. *P<0.05 vs sham+aCSF. †P<0.05 vs CHF+aCSF.
inhibition but not by endothelial NO synthase or inducible NO synthase inhibition. More importantly, patch clamp recordings of outward potassium current showed that Ang-(1-7) significantly increased this current. The increase in current was also blocked by A779 or specific neuronal NO synthase inhibition. These data would suggest that Ang-(1-7) contributes to a reduction in neuronal excitability. This is pertinent to the CHF state and sympathoexcitation. We have recently shown a reduction in the potassium channel protein Kv4.3 in the medulla of rats with CHF.34 Furthermore, Ang II, working through the Ang type 1 receptor, reduces potassium channel current, thus contributing to an increase in
neuronal excitability. Therefore, based on the current study and those cited above, we propose that one function of Ang-(1-7) is to limit neuronal discharge in opposition to the actions of Ang II. This effect may be mediated by enhanced NO formation.

The effects of Ang-(1-7) on baroreflex function in our study were blocked by the mas receptor antagonist A779. This suggests that the effects of Ang-(1-7) are mediated by the mas receptor. However, some studies suggest that Ang-(1-7) also has affinity for the Ang II receptors. It is also possible that the mas receptor itself may functionally interact with Ang II type 1 and type 2. It is also unknown whether endogenous mas receptor expression is altered in CHF. Thus, studies investigating the physiological changes and interactions of the mas receptor are necessary to determine its role in the 2 axes of the renin-Ang system.

Although previous studies show that central administration of Ang-(1-7) evokes hypotensive effects in both normal and disease states, the current study only shows significant effects of Ang-(1-7) in the CHF state and no change in MAP in either state. We did, however, observe a trend for a decrease in MAP and HR in the sham+Ang-(1-7) group. It is also possible that higher doses of Ang-(1-7) may show significant effects in sham animals. In addition, previous studies have been performed only in the anesthetized state or in rodent models. The lack of an effect in sham animals in the present study may also be because of the use of the conscious rabbit model. Finally, it is possible that the physiological effects of Ang-(1-7) are primarily seen in disease states after an imbalance of Ang II and Ang-(1-7).

The balance between Ang II and Ang-(1-7) is critically dependent on the production and activity of ACE and ACE2. In a previous study, we showed differential expression of ACE and ACE2 in the medulla and hypothalamus from CHF rabbits. In CHF animals, the balance between these enzymes is tipped toward ACE and the generation of Ang II thereby promoting sympathoexcitation. These data, therefore, suggest that overexpression of ACE2 and concurrent increased levels of Ang-(1-7) would provide protection from excessive sympathetic outflow.

Perspectives
In summary, these data provide strong evidence that Ang-(1-7) given centrally can reduce sympathetic function and enhance baroreflex sensitivity in a sympathoexcitatory state such as CHF. The fact that all of the components of the renin-Ang II system have been found in the central nervous system suggests that a therapy could be developed to enhance the sympathoinhibitory effects of Ang-(1-7). Precedence for this concept has been provided in other tissues. It has been shown that upregulation of ACE2 in the lung can ameliorate the pathology associated with pulmonary hypertension. Systemic infusion of ACE2 has been shown to reduce oxidative stress associated with Ang II and to reduce cardiac fibrosis in response to Ang II. Thus, this apparent "natural inhibitor" of Ang II and its deleterious effects should and most likely will be exploited for its therapeutic potential.

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Disclosures
None.

References
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CENTRAL ANG-(1-7) ENHANCES BAROREFLEX GAIN IN CONSCIOUS RABBITS WITH HEART FAILURE

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Methods

Surgical Instrumentation

Rabbits were instrumented as described previously. In brief, through a left thoracotomy, a platinum pacing electrode was sutured to the left ventricle and a reference electrode was secured to the left atrium. During the same surgery, a radiotelemetry transducer (DataSciences, Inc. Minneapolis MN) was implanted into the right femoral artery to monitor pulsatile and mean arterial blood pressure (MAP) and HR using Data Sciences and ADInstruments (Colorado Springs, CO) Powerlab data acquisition systems.

Two weeks after the insertion of telemetry, a brain cannula was inserted into the lateral cerebral ventricle and fixed with dental cement to the surface of the skull. The brain cannula was attached by a short microrenathane catheter to an osmotic minipump (Alzet 2001, Cupertino, CA) filled with aCSF which was infused at a rate of 1 µl/ hr.

Induction of CHF

Following the control (prepace) experiments, CHF was induced in each rabbit by chronic ventricular pacing as previously described. Left ventricular pacing (360-380 bpm) was performed for 2 to 3 weeks. Cardiac function was assessed weekly by echocardiography (Accuson Sequoia 512 C; Siemens, Malvern, PA). Heart failure was characterized by a reduction in ejection fraction (EF) to approximately 45%, a 2 mm dilation of the left ventricle in systole and diastole, and clinical signs of CHF such as ascites and pulmonary edema. The experimental protocols were repeated in each rabbit after they reached an EF below 45%. All experiments were begun after the pacemaker was turned off for approximately 30 min.

Heart Rate Variability

ECG was recorded directly from the cardiac pacing leads. A five minute baseline recording of ECG was used to determine heart rate variability (HRV). HRV was analyzed using the HRV module for LabChart software (ADInstruments, Inc.). The baseline recording was analyzed for the standard deviation of normal R-wave-R-wave intervals (SDNN) and the standard deviation of the differences between adjacent intervals (SD ΔRR). Ectopic beats were removed using cycle-length cutoffs of <150 and >350 ms for ectopics and <100 and >400 ms for artifacts.

Specificity of Ang-(1-7)

To document the specificity of the Ang-(1-7) effects on baroreflex sensitivity, in a subset of CHF rabbits (n=3, representative protocol shown in Figure S1) an ICV cannula was implanted attached to an osmotic minipump containing a mixture of Ang-(1-7) (2 nmol/ µl/ hr) and A779 (Bachem, 8 nmol/1 µl/ hr). This dose of A779 was chosen based on a previous study showing that a 4:1 molar ratio of A779 to Ang-(1-7) mixture was able to block the improvement in baroreflex function by ICV infusion of Ang-(1-7) in rats. Hemodynamics, baseline MAP, HR, and RSNA, the response to smoke, and baroreflex function were assessed 3-4 days following this infusion.

At the end of all experimental protocols, 1 µl of concentrated Evan’s Blue dye was injected through the ICV cannula, the animal was euthanized, and the brain was removed. The placement of the cannula was confirmed by staining of all four ventricles.

Arterial Baroreflex Analysis

Arterial baroreflex curves were constructed by taking points for HR or RSNA frequency every 5 sec from the lowest to highest MAP following infusions of SNP and PE, respectively. Individual logistic regression curves, as described by Kent et al. were fit to the data points by using the following equation:
HR or RSNA = \frac{A}{1 + \exp[B(MAP - C)]} + D \tag{1}

where $A$ is HR or RSNA range, $B$ is the slope coefficient, $C$ is the pressure at the midpoint of the range (BP₅₀), $D$ is the minimum HR or RSNA, and MAP is mean arterial pressure. The peak slope (or maximum gain) was determined by taking the first derivative of the baroreflex curve as described by

$$\text{Slope} = \frac{A \times B \times \exp[B(MAP - C)]}{\{1 + \exp[B(MAP - C)]\}^2} \tag{2}$$

The mean value of each parameter from the individual curves in each group of rabbits was used to derive composite baroreflex curves.

References


Table S1. Baseline Hemodynamics in Sham and CHF Rabbits before and after Ang-(1-7) Infusion for Rabbits Subject to RSNA analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham+aCSF</th>
<th>Sham+Ang-(1-7)</th>
<th>CHF+aCSF</th>
<th>CHF+Ang-(1-7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>3.6±0.1</td>
<td>3.6±0.1</td>
<td>3.4±0.1</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>Baseline MAP, mmHg</td>
<td>75.9±2.6</td>
<td>68.9±2.9</td>
<td>73.6±5.4</td>
<td>64.4±2.2</td>
</tr>
<tr>
<td>Baseline HR, bpm</td>
<td>208.2±1.7</td>
<td>202.6±5.3</td>
<td>234.5±11.4 *</td>
<td>210.5±2.9 †</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>16.3±0.3</td>
<td>16.6±0.5</td>
<td>16.6±1.0</td>
<td>13.6±4.0</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>7.1±1.4</td>
<td>9.4±1.4</td>
<td>13.6±1.0 *</td>
<td>11.1±3.3 *</td>
</tr>
<tr>
<td>LVd Vol, ml</td>
<td>7.5±0.4</td>
<td>7.8±0.6</td>
<td>6.2±0.9</td>
<td>8.0±1.3</td>
</tr>
<tr>
<td>LVs Vol, ml</td>
<td>2.2±0.1</td>
<td>2.6±0.4</td>
<td>6.7±1.9 *</td>
<td>5.9±0.6 *</td>
</tr>
<tr>
<td>FS %</td>
<td>34.8±2.3</td>
<td>35.1±2.0</td>
<td>18.1±1.0 *</td>
<td>17.8±1.3 *</td>
</tr>
<tr>
<td>Ejection Fraction, %</td>
<td>70.2±0.9</td>
<td>70.5±0.8</td>
<td>40.5±1.9 *</td>
<td>40.0±2.5 *</td>
</tr>
</tbody>
</table>
Values are mean±SEM. LV indicates left ventricle; MAP, mean arterial pressure; LVEDD, left ventricular end-diastolic diameter; LVSD, left ventricular systolic diameter; LVd Vol, left ventricular diastolic diameter; LVs Vol, left ventricular systolic diameter; FS, fractional shortening. *P<0.05 vs. Sham+aCSF, †P<0.05 vs. CHF+aCSF.

Table S2. Effect of Central Ang-(1-7) on Time-Domain Parameters of Heart Rate Variability

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham+aCSF</th>
<th>Sham+Ang-(1-7)</th>
<th>CHF+aCSF</th>
<th>CHF+Ang-(1-7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD ΔRR, ms</td>
<td>5.7±0.9</td>
<td>5.0±1.4</td>
<td>3.5±0.4*</td>
<td>7.1±2.8 †</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>11.6±1.7</td>
<td>8.1±1.7 *</td>
<td>7.9±1.3*</td>
<td>15.4±1.8 †</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>5.7±0.9</td>
<td>4.9±1.5</td>
<td>3.5±0.4*</td>
<td>7.1±2.7 †</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SD ΔRR, standard deviation of differences between adjacent NN (normal sinus rhythm intervals); SDNN, standard deviation of normal NN. *P<0.05 vs. Sham+aCSF, †P<0.05 vs. CHF+aCSF.
Table S3. Effect of Autonomic Blockade on Baseline MAP, HR, and Logistic Parameters of Baroreflex Curves for Control of HR following Ang-(1-7) Infusion

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHG</th>
<th>HR, bpm</th>
<th>Range, bpm</th>
<th>Min HR, bpm</th>
<th>BP50 , mm Hg</th>
<th>Peak Slope, bpm/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham+aCSF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=13)</td>
<td>74.3±1.9</td>
<td>198.5±4.6</td>
<td>284.2±11.6</td>
<td>62.1±7.3</td>
<td>101.5±1.9</td>
<td>5.6±0.5</td>
</tr>
<tr>
<td>Atropine (n=6)</td>
<td>76.3±3.5</td>
<td>239.3±3.4*</td>
<td>48.3±10.2*</td>
<td>249.6±7.4*</td>
<td>70.8±12.7</td>
<td>1.7±1.7*</td>
</tr>
<tr>
<td>Metoprolol (n=6)</td>
<td>67.2±3.9</td>
<td>187.9±10.1*</td>
<td>168.0±8.1*</td>
<td>62.6±10.5</td>
<td>94.5±11.8</td>
<td>3.9±0.2</td>
</tr>
<tr>
<td><strong>Sham+Ang-(1-7)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>69.1±3.1</td>
<td>186.8±4.5</td>
<td>285.8±17.6</td>
<td>57.33±5.3</td>
<td>99.6±4.1</td>
<td>5.0±0.5</td>
</tr>
<tr>
<td>Atropine (n=6)</td>
<td>77.5±2.5</td>
<td>226.8±6.2*</td>
<td>51.0±13.6*</td>
<td>259.3±13.4*</td>
<td>73.2±7.8</td>
<td>1.9±1.0*</td>
</tr>
<tr>
<td>Metoprolol (n=6)</td>
<td>66.9±1.2</td>
<td>192.5±4.8</td>
<td>153.5±4.5*</td>
<td>71.3±7.3</td>
<td>98.2±5.1</td>
<td>3.0±0.2</td>
</tr>
<tr>
<td><strong>CHF+aCSF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>73.4±3.0</td>
<td>248.0±5.5</td>
<td>129.9±15.1</td>
<td>182.4±12.8</td>
<td>87.5±4.5</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Atropine (n=6)</td>
<td>83.2±4.0*</td>
<td>245.2±5.3</td>
<td>50.7±17.8*</td>
<td>241.6±10.1*</td>
<td>56.9±17.2</td>
<td>0.5±0.1*</td>
</tr>
<tr>
<td>Metoprolol (n=6)</td>
<td>58.7±2.2*</td>
<td>201.1±9.6*</td>
<td>67.7±2.5*</td>
<td>150.5±8.8*</td>
<td>80.5±5.1</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td><strong>CHF+Ang-(1-7)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>73.4±3.0</td>
<td>248.0±5.5</td>
<td>129.9±15.1</td>
<td>182.4±12.8</td>
<td>87.5±4.5</td>
<td>2.6±0.3</td>
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<td>50.7±17.8*</td>
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<td>56.9±17.2</td>
<td>0.5±0.1*</td>
</tr>
<tr>
<td>Metoprolol (n=6)</td>
<td>58.7±2.2*</td>
<td>201.1±9.6*</td>
<td>67.7±2.5*</td>
<td>150.5±8.8*</td>
<td>80.5±5.1</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>Group</td>
<td>Range, % max</td>
<td>Min RSNA, % max</td>
<td>BP&lt;sub&gt;50&lt;/sub&gt;, mm Hg</td>
<td>Peak Slope, % max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
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<td>------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sham+aCSF (n=4)</td>
<td>99.8±0.2</td>
<td>1.4±1.0</td>
<td>59.9±5.3</td>
<td>5.8±1.0</td>
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<td></td>
</tr>
<tr>
<td>Sham+Ang-(1-7) (n=4)</td>
<td>98.6±1.4</td>
<td>7.3±1.0</td>
<td>58.2±2.0</td>
<td>7.3±1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF+aCSF (n=4)</td>
<td>80.6±12.4</td>
<td>18.7±11.9</td>
<td>60.2±4.0</td>
<td>3.7±0.6 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF+Ang-(1-7) (n=4)</td>
<td>99.4±0.6</td>
<td>1.3±0.8</td>
<td>48.4±1.1</td>
<td>6.8±1.0 †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF+Ang-(1-7)+A779 (n=3)</td>
<td>62.4±5.7</td>
<td>41.0±6.0</td>
<td>68.8±6.5</td>
<td>2.4±0.9 *</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs. Sham+aCSF
†P<0.05 vs. CHF+aCSF
Figure S1

A: Cardiac Baroreflex and Autonomic Blockade Protocol (13 rabbits)

<table>
<thead>
<tr>
<th>Surgery 1</th>
<th>Surgery 2</th>
<th>Metoprolol Experiment</th>
<th>Metoprolol Experiment</th>
<th>Metoprolol Experiment</th>
<th>Metoprolol Experiment</th>
<th>Metoprolol Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 d</td>
<td>3-4 d</td>
<td>3-4 d</td>
<td>2-3 weeks pacing</td>
<td>3-4 d</td>
<td>3-4 d</td>
<td>Placement &amp; cannula</td>
</tr>
</tbody>
</table>

Infusion: aCSF Ang-(1-7) aCSF Ang-(1-7)

Surgery 1: Implantation of left ventricular pacing electrodes, radiotelemetry in the right femoral artery

Surgery 2: Placement of ICV cannula attached to aCSF pump
B: RSNA Measurement Protocol (16 rabbits)

**Sham Rabbits**

- Surgery 1
- Surgery 2
- Baroreflex Experiment placement
- Euthanize & confirm cannula
- 14 d
- 3-4 d
- Infusion: aCSF or Ang-(1-7)

**CHF Rabbits**

- Surgery 1
- Start Pacing
- EF < 45%
- Surgery 2
- Baroreflex Experiment placement
- 14 d
- 2-3 weeks
- 3-4 d
- aCSF or Ang-(1-7)
- Euthanize & confirm cannula

Surgery 1: Implantation of left ventricular pacing electrodes, radiotelemetry in the right femoral artery

Surgery 2: Placement of ICV cannula attached to aCSF or Ang-(1-7) osmotic minipump and renal nerve recording electrodes
10

Figure S1: Representative time lines for the animals used in this study. A: measurement of cardiac baroreflex function with and without autonomic blockade (13 animals). B: measurement of baseline RSNA and baroreflex control of RSNA (13 animals). C: analysis of baroreflex function after mas receptor blockade with A779 (3 animals).
Figure S2. Original hemodynamic recordings following infusion of phenylephrine in a CHF rabbit with central aCSF or Ang-(1-7) infusion. Dashed line, intravenous injection of phenylephrine (80 µg/ kg). MAP, mean arterial pressure. HR, heart rate.