Regulation of Sympathetic Nerve Activity in Heart Failure
A Role for Nitric Oxide and Angiotensin II

Jun-Li Liu, Irving H. Zucker

Abstract—The mechanisms by which sympathetic function is augmented in chronic heart failure (CHF) are not well understood. A previous study from this laboratory (Circ Res. 1998;82:496–502) indicated that blockade of nitric oxide (NO) synthesis resulted in only an increase in renal sympathetic nerve activity (RSNA) when plasma angiotensin II (Ang II) levels were elevated. The present study was undertaken to determine if NO reduces RSNA in rabbits with CHF when Ang II receptors are blocked. Twenty-four New Zealand White rabbits were instrumented with cardiac dimension crystals, a left ventricular pacing lead, and a pacemaker. After pacing at 360 to 380 bpm for approximately 3 weeks, a renal sympathetic nerve electrode and arterial and venous catheters were implanted. Studies were carried out in the conscious state 3 to 7 days after electrode implantation. The effects of a 1-hour infusion of sodium nitroprusside (SNP; 3 μg · kg⁻¹ · min⁻¹) on RSNA and mean arterial pressure (MAP) were determined before and after Ang II blockade with losartan (5 mg/kg) in normal and CHF rabbits. Changes in MAP were readjusted to normal with phenylephrine. Before losartan, SNP evoked a decrease in MAP and an increase in RSNA in both groups that was baroreflex-mediated, because both MAP and RSNA returned to control when phenylephrine was administered. In the normal group, losartan plus SNP caused a reduction in MAP and an increase in RSNA that was 152.6 ± 9.8% of control. Phenylephrine returned both MAP and RSNA back to the control levels. However, in the CHF group, losartan plus SNP evoked a smaller change in RSNA for equivalent changes in MAP (117.1 ± 4.1% of control). On returning MAP to the control level with phenylephrine, RSNA was reduced to 65.2 ± 2.9% of control (P < 0.0001). These data suggest that endogenous Ang II contributes to the sympathoexcitation in the CHF state and that blockade of Ang II receptors plus providing an exogenous source of NO reduces RSNA below the elevated baseline levels. We conclude that both a loss of NO and an increase in Ang II are necessary for sustained increases in sympathetic nerve activity in the CHF state. (Circ Res. 1999;84:417-423.)

Key Words: renal nerve activity ▪ nitric oxide synthase ▪ angiotensin ▪ sympathoexcitation ▪ heart failure

It is generally well accepted that chronic heart failure (CHF) is characterized by abnormalities in autonomic control.¹,² In severe CHF, sympathetic nervous function is augmented.¹⁻³ The chronic sympathoexcitatory state may contribute to further hemodynamic deterioration. In fact, it has been clearly shown that plasma norepinephrine concentration is positively correlated with 5-year mortality rates in patients with CHF.³ The origin of sympathoexcitation has not been clearly defined. Earlier work suggested that abnormal arterial baroreflex and cardiopulmonary reflex control of sympathetic outflow were responsible for the enhanced sympathoexcitation of CHF.⁶,⁷ However, recent studies from this laboratory found similar changes in plasma norepinephrine in chronically sinoaortic baroreceptor denervated dogs paced into CHF.⁸ Furthermore, Levett et al.⁹ showed similar increases in plasma norepinephrine in paced dogs after chronic cardiac denervation. Therefore, the sustained state of sympathoexcitation in CHF may not be completely due to abnormal cardiovascular inhibitory reflex regulation.

A variety of humoral substances have been shown to be elevated in the CHF state.¹⁰⁻¹² These include angiotensin II (Ang II), atrial natriuretic peptides, endothelin-1, and vasoressin. Ang II has been considered a prime candidate for a substance that modulates sympathetic outflow, because it has been known for some time that Ang II can alter sympathetic function at several sites from the central nervous system to the periphery.¹³,¹⁴ Indeed, much of the current therapeutic targets in the treatment of CHF relate to reducing Ang II generation or blocking the effects of Ang II at its receptor site. A good deal of evidence has now demonstrated that the action of Ang II in many systems is naturally antagonized by the vasodilator substance nitric oxide (NO).¹⁵⁻¹⁸ NO has also been shown to modulate sympathetic outflow by an inhibitory effect in several brain areas such as the nucleus tractus solitarius (NTS),¹⁹ the paraventricular nucleus (PVN),²⁰ and the rostral ventrolateral medulla (RVLM).²¹ It has been demonstrated that the capacity to generate NO from nitric oxide synthase (NOS) is depressed in aortic and coronary
artery endothelium from dogs with pacing-induced heart failure, and the vascular response to NO-dependent substances is depressed in patients with CHF. In addition to a decrease in the endothelial isoform of NOS, we have reported a decrease in the mRNA and activity for the neuronal isoform of NOS (nNOS) in the PVN of rats with CHF. In a previous study from this laboratory carried out in conscious, normal rabbits, we found that blockade of NO synthesis resulted only in an increase in sympathetic nerve activity when Ang II levels were elevated. In that study, administration of Nω-nitro-L-arginine methyl ester (L-NAME) alone increased arterial pressure and reduced renal sympathetic nerve activity (RSNA). However, L-NAME increased RSNA when accompanied by a sustained infusion of Ang II. These data demonstrated an important modulating effect of Ang II on the sympathetic response to blockade of NO synthesis in normal animals.

In the present study, we reasoned that because central NO synthesis is depressed in the CHF state and because Ang II may contribute to sympathoexcitation CHF, it may be possible to reduce sympathetic nerve activity in CHF by combining NO replacement with Ang II receptor blockade. If this is the case, it should provide support for the hypothesis that elevated Ang II is a necessary requirement for the sympathoexcitation that occurs after reduced NO synthesis in the CHF state.

Materials and Methods

Animals and Surgical Instrumentation

Studies were carried out on 24 male New Zealand White rabbits ranging in weight between 2.5 and 3.5 kg. All surgical procedures and protocols were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. Experiments were carried out under the Guidelines for the Care and Use of Experimental Animals of the American Physiological Society and the National Institutes of Health. Rabbits were kept in individual cages in a temperature-controlled room (23°C) and fed standard rabbit chow (Harlan Teklad). This chow contains 0.29% NaCl and 1.4% KCl. For surgical instrumentation, rabbits were anesthetized with a cocktail consisting of 1.2 mg/kg acepromazine, 5.9 mg/kg xylazine, and 58.8 mg/kg ketamine given as an intramuscular injection. Supplemental anesthesia was provided by intravenous pentobarbital sodium at a dose of 1.7 mg/kg as needed. After tracheal intubation, sterile surgery was carried out to implant a pair of piezoelectric crystals across the heart (2 mm; Sonometrics, Inc) to evaluate progressive changes in cardiac dimensions over time. Crystals were positioned on the epicardial surface at the base of the heart. At the same time, a stainless steel electrode was secured to the left ventricle, and an indifferent electrode was placed in the subcutaneous tissue. The electrodes were exteriorized in the back of the neck for subsequent attachment to a small pacemaker of our own design. The rabbits were allowed to recover from this surgery for 10 to 14 days before initiating pacing or the sham period. After the surgery, the rabbits were treated for 3 days postoperatively with enrofloxacin (2.3 mg/kg IM, twice per day; Baytril, Miles).

Heart Failure Model

A rapid pacing model was used in these studies. In brief, rabbits were paced as previously described. After control measurements of cardiac diameter and heart rate were taken in the awake state, the pacemaker was programmed to 320 bpm. The animal was paced at this rate for 2 to 3 days to ensure that it would tolerate this level of tachycardia. The rate was gradually increased to between 360 and 380 bpm over the next 7 to 10 days and then left at its final rate for 2 to 3 weeks. Cardiac dimensions were recorded once per week with the pacemaker turned off. Animals were instrumented for the final study when their cardiac dimensions had increased by approximately 2 mm.

Hemodynamic and Sympathetic Nerve Recording

Rabbits were anesthetized as described above. After tracheal intubation, sterile surgery was carried out to implant a renal sympathetic nerve electrode and arterial catheter as previously described. In brief, a left subcostal incision was made and the kidney was approached in the retroperitoneal space. A bundle of renal nerves was identified and gently freed from surrounding tissue using glass rods. A pair of Teflon-coated stainless steel wire electrodes (outer diameter, 0.124 mm; A-M Systems) were placed around the dissected renal nerves. To insulate the electrodes and the nerve from the surrounding tissue and to prevent the nerves from dessication, the electrodes and the nerve assembly were covered with a 2-component silicone gel (Wacker Sil-Gel). A ground lead was sutured to the fat close to the electrodes. The electrodes and the ground lead were tunneled beneath the skin to the back and fixed between the shoulder blades. The flank incision was closed.

Through a midline cervical incision, a Micro-Renathane catheter (outer diameter, 1.65 mm; inner diameter, 0.07 mm; Braintree Scientific) was inserted into the left carotid artery for the measurement of arterial pressure and heart rate. Another catheter was placed in a jugular vein for the measurement of central venous pressure (CVP) and used as a venous access. The catheters were tunneled beneath the skin and brought out the back of the neck. The catheter was flushed daily with heparin sodium (1000 U/mL; Elkins-Sinn). After the surgery, the rabbits were treated with antibiotics as described above.

Arterial blood pressure was recorded with a Hewlett-Packard pressure transducer and a Gould bridge amplifier. Heart rate (HR) and mean arterial pressure (MAP) were derived by the data acquisition software (MacLab) using the arterial pressure pulse. The renal sympathetic nerve electrode wires were attached to a Grass P16 preamplifier with the band-pass filters set between 100 and 1 kHz. The amplified signal was displayed on a storage oscilloscope and passed through an audio amplifier and loudspeaker. The raw nerve activity was full wave–rectified and integrated using the MacLab software. In addition to integrated nerve activity, the frequency of discharge in spikes per second was recorded using the frequency function of the MacLab. A window discriminator was set above the noise level to use the rate meter function of the MacLab system. Background noise was determined when arterial pressure was increased with phenylephrine. The integrated noise level was subtracted from the integrated nerve activity.

Experimental Protocols

Experiments were carried out 3 to 7 days after renal nerve electrode implantation. Rabbits were trained to sit quietly in a Plexiglas box of our own design. On the day of the experiment, the arterial catheter was connected to a pressure transducer, and MAP and HR were measured. The renal sympathetic nerve electrode wires were attached to the preamplifier. Raw RSNA, integrated RSNA, and the frequency of RSNA were recorded. After the rabbit was attached to the recording equipment, the pacemaker was turned off and the rabbit was allowed to rest for approximately 30 minutes before any data were taken. Initially, the response to a bolus injection of nitroglycerin (25 μg/kg IV) was recorded to determine the maximum sympathic response (see Table). Four groups of rabbits were studied (2 CHF and 2 sham groups; n=6 per group). After a 20 minute control period during which baseline measurements of arterial pressure, CVP, HR, and RSNA were taken, 1 of 2 interventions was begun. Either a 1-hour intravenous infusion of sodium nitroprusside (SNP) was begun at a dose of 3 μg · kg⁻¹ · min⁻¹ or losartan was administered at a dose of 5 mg/kg IV followed 15 minutes later by a 1-hour infusion of SNP. This dose of losartan completely inhibited the pressor response to 100 ng of Ang II given intravenously. At the end of the SNP infusion, arterial pressure was

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readjusted to the control level (before SNP or losartan) with an infusion of phenylephrine. Hemodynamic and RSNA recordings were made continuously throughout the experiment. In 3 additional rabbits with CHF, losartan was given without subsequent injections of SNP or phenylephrine.

**Drugs**

SNP was obtained from Sigma Chemical Co. Losartan was a gift from Merck and Co. Phenylephrine was obtained from American Regent Laboratories. All drugs were made fresh on the day of the experiment.

**Data Analysis**

Changes in RSNA were similar when quantified using either voltage integration or spike discharge rate. The discharge data in the present study are presented using the rate meter method. All measurements were averaged every 30 seconds. Changes in RSNA were expressed as the percentage of resting nerve activity before each protocol. Control RSNA was set at 100%. The data are presented as mean±SEM. Multivariate analysis and repeated-measures analysis of variance procedures were used in conjunction with specific orthogonal contrasts for post hoc analysis. The probability values reported are after adjustments using the Greenhouse-Geisser correction for multisample sphericity. All statistical analysis was done using the Statistical Analysis Systems (SAS) software. A P value of <0.05 was considered statistically significant.

**Results**

**Effects of Chronic Pacing on Baseline Hemodynamics and Heart Weight**

The Table and Figure 1 present a comparison between normal and CHF rabbits and the time course of cardiac dilatation during the pacing period, respectively. The average time of pacing was 19.2±1.5 days. Although MAP tended to be lower and HR to be slightly higher in the CHF group, the differences did not reach statistical significance. On the other hand, CVP was significantly higher in the CHF group compared with the normal group. Baseline RSNA was expressed as the absolute frequency and as a percentage of the maximum activity achievable as defined by a reduction in arterial pressure to ≈40 mm Hg with a bolus injection of nitroglycerin (25 μg/kg IV). As can be seen in the Table, CHF rabbits exhibited a significantly higher level of RSNA compared with the normal group. Both heart weight to body weight and left ventricular to body weight ratios were significantly increased in rabbits with CHF. In addition, CHF rabbits exhibited pulmonary edema and/or ascites. As shown in Figure 1, paced rabbits exhibited a significant decrease in contractility as evidenced by a progressive decline in change in diameter with respect to time (dD/dt) and percentage of shortening that were significant at 1 week. Cardiac diameters were significantly increased by 2 weeks of pacing. Normal rabbits were studied an average of 23.3±0.9 days after thoracotomy and exhibited no change in cardiac dimensions.

**Effects of SNP and Losartan in Normal Rabbits**

A 1-hour infusion of SNP lowered MAP and raised RSNA. Phenylephrine returned both MAP and RSNA to control levels (Figure 2). SNP reduced MAP ‐13% from 78.1±1.2 mm Hg. After phenylephrine, MAP was returned to 78.6±1.3 mm Hg. RSNA increased by ≈42% after SNP infusion and was returned to baseline by phenylephrine. In normal rabbits, losartan did not alter either MAP or RSNA. The mean data for this group are shown in Figure 3. When SNP was infused after losartan, MAP was reduced ≈17% from 80.7±1.9 mm Hg, and RSNA was increased ≈41% (from the level that existed after losartan). Phenylephrine returned both MAP and RSNA to control levels.

**Effects of SNP and Losartan in CHF Rabbits**

Figures 4, 5, and 6 show the responses in rabbits with CHF. The original recording shown in Figure 4 illustrates several...
important features of this response. First, as was the case for the normal rabbits, SNP reduced MAP and increased RSNA. These parameters were restored to baseline by phenylephrine. Second, losartan alone did not alter arterial pressure or RSNA. Third, SNP after losartan lowered MAP and increased RSNA. However, in contrast to the normal rabbit, when MAP was restored to control with phenylephrine, RSNA was reduced substantially below the control level. The mean data are shown in Figures 5 and 6. The responses without losartan in this group were much the same as the normal rabbits. That is, the changes in MAP and RSNA in response to SNP and SNP plus phenylephrine were not significantly different from the normal group ($P < 0.05$). As can be seen in Figure 5, SNP reduced MAP by $\approx 10\%$ from a control of 74.9 $\pm$ 1.6 mm Hg and increased RSNA by $\approx 26\%$. Phenylephrine restored these parameters to control levels. Figure 6 shows the average responses after losartan treatment in the CHF group. SNP evoked a 13$\%$ decrease in MAP from 74.3 $\pm$ 2.3 mm Hg. However, in contrast to the normal group, RSNA only increased by 7.4$\%$ relative to the postlosartan period and only 17$\%$ relative to the control period. In comparison to the normal rabbits, this increase in RSNA was significantly less (152.6 $\pm$ 9.8$\%$ of control versus 117.1 $\pm$ 4.1$\%$ of control, $P < 0.01$). When MAP was returned to control level with phenylephrine, RSNA was significantly reduced below the control level by $\approx 35\%$ to 65.2 $\pm$ 2.9$\%$ of control ($P < 0.0001$).

In 3 CHF rabbits, losartan by itself caused a slight reduction in MAP over a 75-minute observation period (from
Discussion

The mechanism by which sympathetic function is enhanced in the CHF state has been a topic of intense investigation for many years. The precise cause of the sympathoexcitatory state in CHF has eluded investigators most likely because of its multifactorial nature. The findings in the present study, which was carried out in rabbits with CHF, complement those reported by us previously in normal rabbits.28 These data support the hypothesis that Ang II not only contributes to sympathoexcitation in the CHF state but does so in concert with a reduction in NO production. The important new finding of the present study is that providing a systemic source of NO in the face of blockade of Ang II type 1 (AT$_1$) receptors can evoke a reduction in sympathetic nerve activity in rabbits with CHF. These data are consistent with the idea that in the CHF state, a reduction in the ability to synthesize NO in the central nervous system is due to a reduction in nNOS gene expression and protein along with an increase in a central Ang II stimulus that results in a profound increase in sympathetic outflow. Replacement of NO using a common NO donor may provide an inhibitory central stimulus, thus reducing sympathetic outflow.

The experimental paradigm used in the present study allowed the evaluation of the role played by Ang II and NO on sympathetic nerve activity when the confounding influence of the baroreflex was controlled for by adjusting arterial pressure to the control level with the vasoconstrictor phenylephrine. These data support the hypothesis that the loss of the sympathoinhibitory effect of NO is amplified by a simultaneous increase in Ang II in the setting of CHF. Replacement of a source of NO causes sympathoinhibition if, and only if, it is accompanied by blockade of the action of Ang II.

Although we have no direct evidence from the present study that central NO production is depressed in rabbits with CHF, previously published data from the rat suggests that central NO production is depressed in the CHF state.26 In rats with chronic coronary artery ligation, we found both nNOS mRNA and protein are reduced in the CHF state.26 In rats with chronic coronary artery ligation, we found an approximate 30% reduction in the nNOS mRNA in the dorsal pons and the hypothalamus compared with sham rats.

DiBona et al.34 have shown that intracerebroventricular administration of losartan to rats with coronary artery ligation induced CHF-augmented arterial baroreflex control of RSNA. In addition, Ma et al.35 have shown that central administration of losartan reduced the sensitivity of the cardiac sympathetic afferent reflex in anesthetized dogs with CHF. These 2 studies strongly suggest that central Ang II plays a major role in altering cardiovascular reflex function in CHF. In contrast to these studies, we did not observe an effect on baseline RSNA with systemic administration of losartan alone nor did we observe a significant fall in arterial pressure in rabbits with CHF. Although we did not measure plasma levels of Ang II systematically in the present study, we did measure Ang II levels in 7 normal and 2 CHF rabbits that were used in another study but which were instrumented and paced in an identical fashion to those reported in the present study. Normal rabbits had a plasma Ang II concentration of 12.1±2.0 pg/mL whereas the 2 CHF rabbits had plasma Ang II levels of 23 and 85 pg/mL, respectively. Therefore, in spite of the fact that losartan did not evoke significant hypotension, it is likely that Ang II was elevated. We, of course, do not know what the tissue levels of Ang II were in these rabbits. Several studies suggest that local Ang II concentrations may be exceedingly high in the CHF state.36,37 In our previous study,28 the effect of Ang II and L-NAME to augment RSNA in normal rabbits was completely abolished by losartan and could not be evoked by D-NAME. In another study carried out in rabbits with CHF, we showed that the AT$_1$ antagonist L-158,809 augmented baroreflex function when given intravenously.33 Therefore, it is highly likely that the effects of losartan in the present study were due to specific AT$_1$ blockade.

It is of interest to speculate where the interaction between Ang II and NO may be taking place. Assuming both substances can pass the blood-brain barrier when administered systemically, several sites are prime candidates for this interaction. Strong evidence has now accrued that implicates the area postrema as an important neuron group in the brain stem for peptidergic modulation of sympathetic outflow and baroreflex function.38,39 Ang II appears to modulate baroreflex function via receptors in the area postrema and, in part, through its projections to the NTS.40–44 Lesions of the area postrema in rats that overexpress the renin gene (Ren-2) attenuate the development of hypertension by a decrease in sympathetic outflow.45 In a recent study from this laboratory, it was demonstrated that arterial baroreflex function was not normalized by an AT$_1$ antagonist in conscious CHF rabbits with lesions of the area postrema compared with nonlesioned rabbits.46 Although the NO pathway has been found to be weak in the area postrema47,48 because of the close association of the area postrema with the NTS, it is likely that Ang II can modulate NO responses in the NTS via pathways between these nuclei. Both Ang II and glutamate can act as excitatory neurotransmitters in the central nervous system.49–51 These effects are especially prevalent in, but not limited to, the RVLM. Finally, the NTS itself is a good candidate for modulation by NO and Ang II.19,52

Exactly how Ang II and NO interact at the cellular level is not completely clear. In some peripheral systems, this interaction may be a simple algebraic summation of the effects of NO and Ang II. However, in the central nervous system as...
well as in the periphery, a more complex interaction appears to exist. Ang II has been shown to cause the release of NO from neurons and endothelial cells. Whether it be through a mobilization of intracellular calcium or by other, more specific mechanisms, both excitatory neurotransmitters Ang II and glutamate acutely increase the synthesis of NO, which can constitute a negative feedback system to limit the stimulation by these substances. However, chronically the interaction between Ang II and NO appears to be organized at the cellular level in such a way as to constitute a mutually inhibitory pathway in which Ang II causes a decrease in nNOS gene expression.

Limitations of the Study
Several potential limitations to the present study should be addressed. First, we only evaluated changes in RSNA in conscious rabbits. Although studying these responses in the conscious state is clearly an advantage, selective recording from renal nerves may be a disadvantage. We are assuming that RSNA reflects global changes in sympathetic nerve activity. This, in fact, may not be the case. It has been shown that differences occur in sympathetic outflow to various beds after blockade of NO synthesis. On the other hand, it has also been shown that changes in RSNA not only reflect changes in the renal release of norepinephrine but also correlate well with changes in nerve activity to other beds. Changes in RSNA most likely reflect sympathetic outflow to many beds; however, it is acknowledged that differences may exist to selective vascular beds.

A second potential concern is the fact that both losartan and NO were given intravenously rather then directly into the central nervous system. We did not measure plasma or central levels of losartan in the present study. However, we can reasonably assume that losartan gains access to the brain on the basis of previous reports. The NO released by a sustained infusion of SNP should gain access to the brain. Both substances should easily pass through blood vessels surrounding the various circumventricular organs that have a low or no blood-brain barrier and are located near various nuclei involved in autonomic control.

Lastly, although we did not construct full baroreflex function curves in the present study, it is likely that the combination of Ang II blockade and NO donation enhanced baroreflex function because RSNA was substantially reduced at control arterial pressures (returned with phenylephrine) in rabbits with CHF.

Conclusion
In summary, the present study shows, for the first time, an important interaction between Ang II and NO in the setting of CHF. A possible scenario for the augmentation of sympathetic nerve activity in CHF is that the loss of the capacity of specific central neurons to synthesize NO results in an unimpeded and amplified Ang II signal that acts as a central sympathoexcitatory stimulus. It is intriguing to speculate that increases in central Ang II are responsible for the downregulation of nNOS in the brain. Data exist in peripheral tissues to support this notion.

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