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Abnormal Baroreflex Function is Dissociated from Central Angiotensin II Receptor Expression in Chronic Heart Failure

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Abstract

Neurohumoral disturbances characterize chronic heart failure (CHF) and are reflected, in part, as impairment of baroreflex sensitivity (BRS) and sympathetic function. However the mechanisms that trigger these neurohumoral abnormalities in CHF are not clear. We hypothesized that the BRS is blunted early in CHF and that the humoral effects occur later and contribute to progressive loss of cardiovascular control in CHF. We assessed the BRS (bpm/mmHg) and recorded renal sympathetic nerve activity (RSNA) in four groups of conscious rabbits at varying time intervals; control, one week CHF, two week CHF and three week CHF. CHF was induced by ventricular pacing at 360 bpm and was assessed by echocardiography. Arterial blood pressure and heart rate were recorded by an implanted telemetric device and RSNA through an implanted electrode. A significant fall in the ejection fraction, fractional shortening and an increase in LVESD & LVEDD was observed in all CHF groups. The BRS was significantly reduced in all the CHF groups with no significant change in the basal RSNA(% of maximum) after 1 week of pacing, a small but insignificant rise in RSNA was seen at 2 weeks and a significant rise in RSNA was observed at 3 weeks. AT1 receptor protein (Western Blot) and mRNA (RT-PCR) expression in the rostral ventrolateral medulla (RVLM) exhibited a progressive increase with the duration of CHF, reaching significance after 3 weeks, the same time point in which RSNA was significantly elevated. These data are the first to examine early changes in central AT1 receptors in CHF and suggest that the fall in BRS and hemodynamic changes occur early in the development of CHF followed by sympatho-excitation and over-expression of AT1 receptors with the progression of CHF causing further impairment of cardiovascular control.

Keywords

Baroreflex; heart failure; sympathetic activity; angiotensin II receptor

INTRODUCTION

Impairment of arterial baroreflex regulation of cardiovascular function (1, 2) and excitation of the sympathetic nervous system (2, 3, 4, 5, 6, 7) in chronic heart failure (CHF) have been well established. This sympatho-excitation has been linked to elevated levels of circulatory central angiotensin II (ANG II) (1, 2, 8, 9) and an attenuated arterial baroreflex (2, 10, 11, 12). ANG II, besides augmenting ROS formation also increases oxidase activity and upregulates mRNA and protein expression of most NAD(P)H oxidase subunits *in vitro* (13)

and *in vivo* (2,14) and it also increases ANG II type 1 receptors (AT1) in the RVLM (2) providing evidence that up regulation of AT1 receptors in the brain contribute to the increase in renal sympathetic nerve activity (RSNA) (2). On the other hand the increase in sympathetic nerve activity in the later stages of CHF has been associated with activation of the renin-angiotensin system (8,11,12,15). Therefore, neurohormonal disturbances characterize CHF and may be reflected as an attenuated BRS and sympatho-excitation. However, the mechanisms that trigger neurohumoral abnormalities at various time points during the development of CHF are not clear as most of the studies on animal models of CHF have been conducted later in the course of CHF, when the disease is most severe. As a consequence, the changes in sympathetic regulatory mechanisms which might occur early in the development of CHF are unknown. Because the time course of the changes in central mediators of sympathetic tone are not precisely known, we hypothesized that the BRS is affected early in the course of the progression from cardiac dysfunction to CHF and that central changes in AT1 receptors occur later thus contributing to the sustained increase in sympathetic tone and the progressive loss of cardiovascular control in CHF.

The present study was designed to determine the temporal relationship between changes in BRS and RSNA during the development of CHF. In addition we examined the temporal changes in plasma ANG II, AT1 receptors and the change in expression of the main catalytic subunit of NAD(P)H Oxidase, GP91phox in the RVLM of rabbits with developing CHF.

METHODS

General Procedure—The experimental procedures and protocols used in the present study were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee, and all procedures were performed in accordance with the American Physiological Society's "Guiding Principles for Research Involving Animals" (16) and the National Institutes of Health. Experiments were performed on male New Zealand White rabbits (Harlan, Inc, Indianapolis, IN) weighing between 3.0 – 4.0 kg. Rabbits were housed in individual cages under controlled temperature and humidity with a 12:12 h dark-light cycle and were fed standard rabbit diet (sodium content, 0.31 %; Harlan Teklad) with water available *ad-libitum*.

Production of chronic heart failure

Under isoflourothane anesthesia a sterile thoracotomy was made in the 4th intercostal space. Pacing electrodes were sutured to the left ventricle and atrium. The wires exited the chest between the ribs and were tunneled beneath the skin to exit in the mid-scapular region. The pacing wires were attached to an external pacemaker of our own design which was carried in a jacket worn by the rabbit. The rabbit was allowed to recover for approximately 2 weeks. After control measurements were made, rabbits were paced continuously at 360 bpm for a period of 1, 2 or 3 weeks.

Arterial Pressure and Heart Rate Recording

Under isoflourothane anesthesia and sterile conditions, a catheter connected to a radio-telemetry unit (Data Sciences International, St. Paul, MN) was inserted into the descending aorta via a branch of the right femoral artery for direct measurement of arterial pressure (AP) in the conscious state. Heart rate (HR) was derived from the AP pulse using a PowerLab (Model 8S; AD Instruments Inc., Colorado Springs, CO) data acquisition system.

Echocardiography

Control and weekly echocardiograms were carried out in the conscious state. The rabbits were hand held and a left parasternal 2 dimensional image was acquired using a 4 MHz probe and an Accuson Sequoia 512C echocardiograph. Left ventricular M mode images were acquired at the level of the papillary muscles. Cardiac dimensions, volumes, fractional shortening and ejection fractions were determined using standard formulas.

Plasma Angiotensin II Radioimmunoassay

Angiotensin II peptide in the plasma was measured using a specific radioimmunoassay (RIA) with a highly specific antibody provided to us by Dr. Ian Reid (University of California, San Francisco). The protocol for Ang II RIA was essentially similar to the one described by Raff *et al* (17) modified by us for final sample preparation and the assay, which improved the sensitivity of the assay to 2.5 pg/tube.

Renal Sympathetic Nerve Recording

Renal sympathetic nerve recording electrodes were implanted as we have described previously (18). In brief, under anesthesia and sterile conditions, the left kidney was exposed retroperitoneally, and a branch of the renal nerve was separated from the renal plexus and the surrounding connective tissues. A pair of stainless steel multi-stranded Teflon coated recording electrodes were placed around the nerve branch. The nerve-electrode junction was insulated electrically from the surrounding tissues and covered with a fast setting silicone compound (Kwik-Sil, World Precision Instruments, Sarasota, FL). A ground wire was secured to a nearby muscle. The recording electrodes and ground wire were tunneled under the skin and exteriorized in the midscapular area. During the experiment, the electrical signal from the electrode was amplified with a Grass P55 preamplifier (Grass Instrument; W. Warwick, RI) with high- and low-frequency cutoffs of 1000 and 100 Hz, respectively. The output from the Grass amplifier was directed to the PowerLab system sampling at 1000 samples/s. The signal was also full wave rectified and integrated. The average rectified signal (RC filtered, time constant, 0.2 s) was then recorded and stored for later analysis. The frequency of nerve discharge was counted by using a window discriminator and rate meter. The cursor of the window discriminator was set just above the electrical noise. Both frequency and integrated nerve activity were recorded continuously along with the raw nerve activity. Baseline RSNA was quantified as a percentage of the maximal renal sympathetic nerve activity (“% Max”) induced by 50 ml of smoke delivered by syringe into the face of the rabbit. RSNA was expressed as the change from baseline during construction of baroreflex curves (19).

Evaluation of Arterial Baroreflex Function

Evaluation of the arterial baroreflex was carried out as previously described (20). In brief, rabbits were studied in a quiet, dimly lit room while standing in a plexiglass box. Arterial pressure, HR, and RSNA were recorded on a Powerlab system. Intravenous infusions were carried out through a lateral ear vein. After the animal had adjusted to the environment and all hemodynamics were stable, an intravenous infusion of sodium nitroprusside (SNP) was started at a rate of 100µg/kg/min at 0.5 ml/min. When MAP reached its nadir (usually 40–50 mmHg) the SNP infusion was stopped and after the recovery of the arterial pressure and HR to the basal level an infusion of phenylephrine (PE) was started at a rate of 80µg/kg/min at 0.5 ml/min. The PE infusion continued until MAP reached approximately 110 mmHg. The baroreflex was analyzed over the pressure range from lowest to highest. These infusion rates increased arterial pressure at a rate of approximately 1 mmHg per second.

The HR and MAP data were acquired every two seconds from the threshold to the saturation points. The slope of the linear regression between MAP and HR was determined for the rise (PE) and fall (SNP) in arterial pressure separately using a linear regression program (Sigma Plot ver. 8.0, Jandel Corporation). The slope of the linear regression curves were used as an index of BRS.

Preparation of RVLM Tissue

At the end of experiment, the rabbit was euthanized with pentobarbital sodium. The brain was removed and immediately frozen on dry ice, blocked in the coronal plane and sectioned at 300 μ m thickness in a cryostat. The RVLM was punched according to the method of Palkovits and Brownstein (21) for the analysis of O_2^- production, mRNA and protein for AT₁ receptor and NAD(P)H subunits.

RT-PCR Analysis of AT₁ Receptor and NAD(P)H Oxidase Subunit mRNA in the RVLM

Total RNA of RVLM was isolated by means of the RNeasy Mini Kit Total RNA Isolation System (QIAGEN Inc., Valencia, CA, USA), after which cDNA was synthesized by means of M-MLV Reverse Transcriptase (Invitrogen Life technologies, Carlsbad, CA, USA), according to the manufacturers' instructions. RNA was treated in parallel in the presence or absence of reverse transcriptase. PCR amplification was performed by means of a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA, USA) as follows: 1 cycle at 95°C for 15 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min. The primer pairs were based on the cDNA sequences of rabbit AT₁ receptor (GenBank-S59041), gp91^{phox} (GenBank-AF323788), [only at1 and gp91 were measured here] with β -Actin (GenBank-AF309819) as an internal control. The primer pairs were 5'-TTTGGAACAGCTTGGCGGT-3' and 5'-GCCAGCCAGCAGCCAAATAA-3' for AT-1 receptor, 5'-GCTTGTGGCTGTGATAAGCA-3' and 5'-CTCCTGCATCTGTGTCTCCA-3' for gp91^{phox} and 5'-GTCGTACTCCTGCTTGGTG-3' for β -Actin. The amplification products were visualized on 2% agarose gels by the use of ethidium bromide and sequenced so that their identity could be confirmed. The bands were analyzed using UVP BioImaging Systems.

Western Blot analysis of AT₁ Receptor and NAD(P)H Oxidase Subunits Protein in the RVLM

The RVLM was homogenized with the homogenizer in RIPA buffer. The protein extraction from homogenates was used for western blot analysis for the rabbit AT-1 receptor gp91^{phox}. The protein concentration was measured using a protein assay kit (Pierce; Rockford, IL). Samples was adjusted to the same concentration of protein, mixed with equal volumes of 2X 4% SDS sample buffer, and then boiled for 5 min following by loading on the 7.5% SDS-PAGE gel (5 μ g protein/30 μ L per well) for electrophoresis using Bio-Rad mini gel apparatus at 40 mA/each gel for 45 min. Then the fractionized proteins on the gel were electrophoretically transferred onto the PVDF membrane (Millipore) at 300 mA for 90 min. The membrane was probed with primary antibody (rabbit anti human AT₁ receptor polyclonal antibody, goat anti human gp91^{phox} polyclonal antibodies, Santa Cruz, 1:1000) and secondary antibody (goat anti-rabbit IgG-HRP and rabbit anti-goat IgG-HRP, Santa Cruz, 1:2500), and then treated with enhanced chemi-luminescence substrate (Pierce; Rockford, IL) for 5 min at room temperature. The bands in the membrane were visualized and analyzed using UVP BioImaging Systems.

Experimental protocol

Rabbits were randomly divided in four groups; control, one week of pacing, two weeks of pacing and three week of pacing. The pre-paced data for each week was used as a non-paced

comparison. On the day of the experiment arterial blood samples were collected from the central ear artery for the measurement of plasma concentrations of norepinephrine (NE) and ANG II. Arterial baroreflex control of HR was then determined in response to intravenous infusion of SNP and PE in all groups prior to chronic pacing. This procedure was then repeated after specific durations of pacing in the three groups.

Renal nerve electrodes were implanted 2–3 days before experiments were carried out in the conscious state. The basal RSNA was recorded in control and CHF groups to evaluate the changes due to developing CHF. At the end of the experiment, each rabbit was euthanized with pentobarbital sodium. The brain was removed and immediately frozen on dry ice, the RVLM was punched for the analysis of mRNA and protein of AT1 receptor and NAD(P)H subunits. Heart and lungs were collected and weighed.

Statistical Analysis

Data are expressed as the mean $\pm$ SE and also as median and range values. The differences between groups were determined with a two way ANOVA followed by the Newman-Keuls test for post hoc analysis of significance. The differences before and after icv infusion treatment in each group were analyzed with a paired *t* test. A *P* value of < 0.05 was considered statistically significant

RESULTS

Hemodynamics

All hemodynamic measurements were made either in the pre-paced state or after the pacemaker had been turned off for ~20min with the rabbit resting quietly in a darkened laboratory. There were no significant changes in the body weight (Table 1) or MAP (Table 2) at each time period compared to the pre-paced state. At 3 weeks of pacing resting HR was significantly increased (Table 2). A significant fall in ejection fraction ($75.5 \pm 1.7\%$ to $64.4 \pm 3.7\%$), fractional shortening ($41.0 \pm 2.2\%$ to $33.6 \pm 1\%$) and an increase in LV end diastolic and end systolic diameter occurred even at one week of chronic pacing (Table 2). These parameters continued to be significantly altered for the remainder of the pacing periods. Pre-pace hemodynamic values in the three paced groups were not significantly different from each other. Whole heart weight and left ventricular weight was significantly increased after 3 weeks of pacing however lung water was not significantly increased (Table 1).

Plasma Angiotensin II

There was no significant change in plasma ANG II over the first 2 weeks of pacing. The increase was not found to be significant until three weeks of pacing. At this time ANG II concentration exhibited a marked increase from 16.5 ± 4.0 to 106.2 ± 51.5 pg/ml plasma (Figure 1A, Table 3).

Renal Sympathetic Nerve Activity

Figure 1B shows the effect of CHF on the basal RSNA as the % max generated by nasopharyngeal administration of smoke. In the control state, RSNA averaged $32.4 \pm 2.5\%$ of max. despite a significant reduction in cardiac function (Table 2), RSNA was not significantly increased until 3 weeks of pacing where it averaged $49.4 \pm 2.3\%$ of max ($p < 0.05$).

Baroreflex Function

Separate slopes were determined for the tachycardia response to hypotension induced by SNP and the bradycardia response to a rise in blood pressure induced by PE. Figure 2A shows a significant fall in the baroreflex sensitivity for the tachycardia response to hypotension in all the CHF groups. Similarly the sensitivity of the baroreflex for the bradycardia response to a rise in blood pressure was significantly attenuated in all the CHF groups (Figure 2B). BRS (both PE and SNP) was significantly depressed as early as 1 week following pacing (Figure 2).

mRNA expression of AT1 receptor and gp91phox in the RVLM

The mRNA expression for the AT1 receptor and gp91phox in the RVLM were measured by RT-PCR. As shown in Figure 3A there was no change in AT1 receptor expression after one or two weeks of pacing. However, a significant increase in AT1 receptor expression was found in the RVLM of three week paced rabbits (Figure 3A). We evaluated the expression of the NAD(P)H Oxidase subunit gp91phox in the RVLM of these same rabbits. A small but insignificant rise in gp91phox was observed after two weeks of pacing which became significant after three weeks of pacing (Figure 4A).

Protein expression of AT1 receptor and gp91phox in the RVLM

The protein expression of AT1 receptor and gp91phox in the RVLM were assessed by Western blot analysis. Protein expression for the AT1 receptor was significantly increased after three weeks of pacing (Figure 3B). Protein expression for gp91phox was similarly significantly increased after three weeks of pacing (Figure 4B).

DISCUSSION

The aim of this study was to assess the baroreflex sensitivity during progression of CHF and its association with sympatho-excitation in conscious rabbits. Sympatho-vagal imbalance is known to be a significant factor in neurohumoral excitation in CHF (2,10, 22). There is a fair amount of evidence for sympatho-excitation and blunting of arterial baroreflex function linked to elevated peripheral and central ANG II in CHF (2,9). Recent studies have shown that NAD(P)H Oxidase- derived reactive oxygen species are important mediators of ANGII signaling mechanisms and play an important role in the modulation of sympathetic activity and arterial baroreflex function (2,8,12,15,23,24,25). One proposed mechanism that may be responsible for sympatho-excitation in CHF is reduced baroreflex sensitivity (11, 24). With the chronic fall in arterial blood pressure there is an increase in sympathetic nerve activity (26). In this regard, interventions that reduce both ANG II and ROS have been shown to be associated with a decrease in sympathetic hyperactivity. For instance, exercise training restores cardiopulmonary reflex sensitivity and baseline RSNA in CHF animals (24).

In the current study we have demonstrated, for the first time in a conscious animal model of CHF that BRS is significantly reduced early in the pathogenesis of heart failure in the absence of sympatho-excitation. In spite of a significant fall in BRS there was no change in plasma ANG II, AT1 receptor expression, gp91^{phox} or sympatho-excitation at one or two weeks of pacing suggesting that the impairment of the baroreflex early in the development of CHF may contribute to sympatho-excitation later in the disease process and that the depressed baroreflex is not due to sympatho-excitation or to overexpression of central AT1 receptor generally observed late in CHF (2).

In a previous study from this laboratory (27) we found an increase in plasma norepinephrine concentration after 1 week of ventricular pacing in conscious dogs. The increase in norepinephrine progressed for an additional 3 weeks of pacing. It is not clear why plasma

norepinephrine would increase in this study at a time when RSNA did not exhibit an increase in the present study. There may be several explanations for these seemingly disparate observations. First, species differences may account for the differences in the time course. Second, plasma was taken only 10 minutes following termination of pacing in the dog study while we waited a longer period of time in the present study. Lastly, it is conceivably possible that plasma norepinephrine concentration reflects sympatho-excitation from circulatory beds other than the kidney. In this regard, there is substantial evidence that cardiac sympathetic nerve activity may be an important contributor to circulating norepinephrine in the CHF state (28,29,30,31,32).

AT1 receptors are widely distributed in the brain, from the forebrain to the brain stem (33). The RVLM contains a high density of these receptors (34) and is a major site of sympatho-excitation in response to central administration of ANG II (35). Beside an important role in the genesis and maintenance of sympathetic nerve activity (36,37) the RVLM is an important neural pathway of central baroreflex control (38,39). The absence of any rise in NAD(P)H oxidase subunits in RVLM at one week of pacing further supports the view that the reduced baroreflex sensitivity early in CHF is independent of humoral factors and NAD(P)H oxidase derived ROS activity and suggests that the above two roles of the RVLM function independently at least in early CHF. In another study from this laboratory (10) showed a significant elevation in NAD(P)H Oxidase subunits after 4 weeks of pacing. In the animals continuing to pace for three weeks there was a significant rise in plasma ANG II, AT1 receptor expression and elevation in NAD(P)H Oxidase subunit in the RVLM demonstrating the important role of central humoral factors in the disturbed cardiovascular regulation in severe CHF.

The role of ROS in central ANG II mediated sympatho-excitation has been demonstrated by over-expression of superoxide dismutase (SOD) in brain tissue of mice. This intervention blocked the arterial blood pressure and heart rate response to icv administration of ANG II (40). Attenuation of the sympathetic nerve activity response to central ANG II infusion was also demonstrated by administration of the SOD mimetic agent 4-hydroxyl-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol) (41).

In summary, our results demonstrated that in conscious rabbits, early in the development of CHF there was significant attenuation of the sensitivity of the arterial baroreflex and hemodynamic changes without any sympatho-excitation. With the progression of CHF there was an increase in sympathetic nerve activity and over-expression of AT1 receptors suggesting that on progressive development of CHF there is a rise in central ANG II, an up-regulation of AT1 receptors and gp91^{phox} expression in the RVLM which contributes to the sympatho-excitation and further impairment of arterial baroreceptor reflex control of HR and sympathetic nerve activity late in CHF.

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References

1. DiBona GF, Jones SY, Brooks VL. ANG II receptor blockade and arterial baroreflex regulation of renal nerve activity in cardiac failure. *Am J Physiol.* 1995; 69:R1189–R1196. [PubMed: 7503310]

2. Gao L, Wang W, Li YL, Schultz HD, Liu D, Cornish KG, Zucker IH. Sympatho-excitation by central Ang II: The roles for AT1 receptor upregulation and NAD(P)H oxidase in the RVLM. *Am J Physiol Heart Circ Physiol*. 2005; 288:H2271–H2279. [PubMed: 15637113]
3. Kaye DM, Lefkovits J, Cox H, Lambert G, Jennings G, Turner A, Esler MD. Regional epinephrine kinetics in human heart failure: evidence for extra-adrenal, nonneural release. *Am J Physiol*. 1995; 269:H182–H188. [PubMed: 7631847]
4. Svanegaard J, Johansen JB, Thayssen P, et al. Neurohormonal systems during progression of heart failure: A review. *Cardiology*. 1993; 83:21–29. [PubMed: 7903208]
5. Wollert KC, Drexler H. The renin-angiotensin system and experimental heart failure. *Cardiov Res*. 1999; 43:838–849.
6. Zucker IH, Wang W. Reflex control of renal sympathetic nervous activity in heart failure. *Herz*. 1991; 16:82–91. [PubMed: 2066056]
7. Zucker IH, Wang W, Pliquett RU, Liu JL, Patel KP. The regulation of sympathetic outflow in heart failure. The roles of angiotensin.II, nitric oxide, and exercise training. *Ann N Y Acad Sci*. 2001; 940:431–443. [PubMed: 11458699]
8. Francis GS. The relationship of the sympathetic nervous system and the renin-angiotensin system in congestive heart failure. *Am Heart J*. 1989; 118:642–648. [PubMed: 2570521]
9. Wang W, Ma R. Cardiac sympathetic afferent reflexes in heart failure. *Heart Failure Reviews*. 2002; 5:57–71. [PubMed: 16228916]
10. Rueckschloss U, Quinn MT, Holtz J, Morawietz H. Dose-dependent regulation of NAD(P)H oxidase expression by angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2002; 22:1845–1851. [PubMed: 12426214]
11. Mollnau H, Wendt M, Szocs K, Lassegue B, Schulz E, Oelze M, Li H, Bodenschatz M, August M, Kleschyov AL, Tsilimingas N, Walter U, Forstermann U, Meinertz T, Griendling K, Munzel T. Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ Res*. 2002; 90:E58–E65. [PubMed: 11884382]
12. Gao L, Wang W, Li YL, Schultz HD, Liu D, Cornish KG, Zucker IH. Superoxide mediates sympathoexcitation in heart failure: roles of angiotensin II and NAD(P)H oxidase. *Circ Res*. 2004; 95:937–944. [PubMed: 15459075]
13. Mancia G, Seravalle G, Giannattasio C, Bossi M, Preti L, Cattaneo BM, Grassi G. Reflex cardiovascular control in congestive heart failure. *Am J Cardiol*. 1992; 69:17G–23G.
14. Zucker IH, Schultz HD, Li YF, Wang Y, Wang W, Patel KP. The origin of sympathetic outflow in heart failure: the roles of angiotensin II and nitric oxide. *Prog Biophys Mol Biol*. 2004; 84:217–232. [PubMed: 14769437]
15. Zucker IH, Liu JL. Angiotensin II–Nitric oxide interactions in the control of sympathetic outflow in heart failure. *Heart Failure Reviews*. 2000; 5:27–43. [PubMed: 16228914]
16. American Physiological Society. Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol*. 2002; 383:R281–R283.
17. Raff H, Maselli J, Reid IA. Correlation of plasma angiotensin II concentration and plasma renin activity during acute hypoxia in dogs. *Clin Exp Pharmacol Physiol*. 1985; 12:91–94. [PubMed: 2988837]
18. Liu JL, Zucker IH. Regulation of sympathetic nerve activity in heart failure: a role for nitric oxide and angiotensin II. *Circ Res*. 1999; 84:417–423. [PubMed: 10066676]
19. Burke SL, Head GA. Method for in vivo calibration of renal sympathetic nerve activity in rabbits. *J Neurosci Methods*. 2003; 127:63–74. [PubMed: 12865149]
20. Liu JL, Pliquett RU, Brewer E, Cornish KG, Shen YT, Zucker IH. Chronic endothelin-1 blockade reduces sympathetic nerve activity in rabbits with heart failure. *Am J Physiol Regul Integr Comp Physiol*. 2001; 280:R1906–R1913. [PubMed: 11353699]
21. Palkovits, M.; Brownstein, M. Brain microdissection techniques. Wiley; UK: 1983.
22. Pliquett RU, Cornish KG, Peulaer JD, Zucker IH. Simvastatin normalizes autonomic neural control in experimental heart failure. *Circulation*. 2003; 107:2493–2498. [PubMed: 12695293]
23. Davisson RL. Physiological genomic analysis of the brain renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol*. 2003; 285(3):R498–R511. [PubMed: 12909574]

24. Mousa TM, Gao L, Cornish KG, Zucker IH. Effects of angiotensin II on autonomic components of nasopharyngeal stimulation in male conscious rabbits. *J Appl Physiol.* 2005; 98:1607–1611. [PubMed: 15649867]
25. Zimmerman MC, Lazartigues E, Sharma RV, Davisson RL. Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ Res.* 2004; 95(2):210–216. [PubMed: 15192025]
26. Thrasher TN. Unloading arterial baroreceptors causes neurogenic hypertension. *Am J Physiol Regul Integr Comp Physiol.* 2002; 282:R1044–R1053. [PubMed: 11893608]
27. Brandle M, Wang W, Zucker IH. Hemodynamic correlates of baroreflex impairment of heart rate in experimental canine heart failure. *Basic Res Cardiol.* 1996; 91(2):147–154. [PubMed: 8740531]
28. Kaye DM, Jennings GL, Dart AM, Esler MD. Differential effect of acute baroreceptor unloading on cardiac and systemic sympathetic tone in congestive heart failure. *J Am Coll Cardiol.* 1998; 31(3):583–587. [PubMed: 9502639]
29. Kaye DM, Lefkowitz J, Jennings GL, Bergin P, Broughton A, Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol.* 1995; 26(5):1257–1263. [PubMed: 7594040]
30. Kaye DM, Smirk B, Finch S, Williams C, Esler MD. Interaction between cardiac sympathetic drive and heart rate in heart failure: modulation by adrenergic receptor genotype. *J Am Coll Cardiol.* 2004; 44(10):2008–2015. [PubMed: 15542284]
31. Meredith IT, Eisenhofer G, Lambert GW, Dewar EM, Jennings GL, Esler MD. Cardiac sympathetic nervous activity in congestive heart failure. Evidence for increased neuronal norepinephrine release and preserved neuronal uptake. *Circulation.* 1993; 88(1):136–145. [PubMed: 8391399]
32. Motte S, Mathieu M, Brimiouille S, Pensis A, Ray L, Ketelslegers JM, Montano N, Naeije R, van de Borne L, McEntee K. Respiratory related heart rate variability in progressive experimental heart failure. *Am J Physiol Heart Circ Physiol.* 2005; 289(4):H1729–H1735.
33. Granata AR, Ruggiero DA. Evidence of dysynaptic projections from the rostral ventrolateral medulla to the thoracic spinal cord. *Brain Res.* 1998; 781:329–334. [PubMed: 9507179]
34. Allen AM, Moeller I, Jenkins TA, Zhuo J, Aldred GP, Chai SY, Mendelsohn FA. Angiotensin receptors in the nervous system. *Brain Res Bull.* 1998; 47:17–28. [PubMed: 9766385]
35. Head GA, Saigusa T, Mayorov DN. Angiotensin and baroreflex control of the circulation. *Braz J Med Biol Res.* 2002; 35:1047–1059. [PubMed: 12219176]
36. Dampney RA, Moon EA. Role of ventrolateral medulla in vasomotor response to cerebral ischemia. *Am J Physiol.* 1980; 239:H349–H358. [PubMed: 7435582]
37. Dampney RA, Goodchild AK, Robertson LG, Montgomery W. Role of ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. *Brain Res.* 1982; 249:223–235. [PubMed: 6128058]
38. Dampney RA, Blessing WW, Tan E. Origin of tonic GABAergic inputs to vasopressor neurons in the subretrofacial nucleus of the rabbit. *J Auton Nerv Syst.* 1988; 24:227–239. [PubMed: 3209808]
39. Granata AR, Enero MA, Krieger EM, Langer SZ. Norepinephrine release and vascular response elicited by nerve stimulation in rats with chronic neurogenic hypertension. *J Pharmacol Exp Ther.* 1983; 227(1):187–193. [PubMed: 6312017]
40. Zimmerman MC, Sharma RV, Davisson RL. Superoxide mediates angiotensin II induced influx of extracellular calcium in neural cells. *Hypertension.* 2005; 45(4):717–727. [PubMed: 15699459]
41. Mayorov DN, Head GA, Matteo RD. Tempol attenuates excitatory actions of angiotensin II in the rostral ventrolateral medulla during emotional stress. *Hypertension.* 2004; 44:101–106. [PubMed: 15159379]

Figure 1.

A: Plasma concentration of ANG II in control and in CHF rabbits after left ventricular pacing for 1,2, or 3 weeks. B: Basal RSNA expressed as the percentage of the maximal response to smoke inhalation in control animals and after pacing for 1, 2, or 3 weeks. Results are represented as mean $\pm$ SEM; n=6 each group. * P<0.05: significantly different from the control.

Figure 2. Mean data of maximum sensitivity of arterial baroreflex for tachycardia (increase in HR) response to fall in MAP by SNP infusion (A) and bradycardia (fall in HR) response to rise in MAP by PE infusion (B) in rabbits prior to and after CHF induced by 1,2, or 3 weeks of left ventricular pacing. Results are represented as means $\pm$ SEM; n=6 each group. * P<0.05: significantly different from the pre-pace of the same group.

Figure 3.

A: RT-PCR analysis for mRNA expression of the AT1 receptor (AT1) in the RVLM of control and chronic heart failure (after 1,2 or 3 weeks of LV pacing) rabbits. M, marker; C, control animals without LV pacing; WK, weeks of LV pacing. Top, A representative RT-PCR image showing upregulation of AT1 receptor mRNA expression in the RVLM after 2 and 3 weeks of pacing. β -Actin was used as an internal control. Bottom, results of densitometric analysis representing means $\pm$ SEM, ** $P < 0.01$ compared with control; $n = 6$ each group. B: Western blot analysis for protein expression of the AT1 receptor in the RVLM of control and chronic heart failure (after 1,2 or 3 weeks of LV pacing) rabbits. Top, Representative Western blots showing the upregulation of AT1 receptor protein expression in the RVLM after 2 and 3 weeks of pacing Bottom. Results of densitometric analysis representing means $\pm$ SEM. ** $P < 0.01$ compared with control; $n = 6$ each group.

Figure 4.

A: RT-PCR analysis for mRNA of $gp91^{phox}$ in the RVLM of control and chronic heart failure (after 1, 2 or 3 weeks of LV pacing) rabbits. M, marker; C, control animals without LV pacing, WK, weeks of LV pacing. Top: A representative RT-PCR image showing the upregulated $gp91^{phox}$ mRNA expression in the RVLM after 2 and 3 weeks of pacing. β -Actin was used as an internal control. Bottom: Results of densitometric analysis representing means $\pm$ SEM. n=6 each group. B: Western blot analysis for protein expression of $gp91^{phox}$ in the RVLM of control and chronic heart failure (after 1, 2 or 3 weeks of pacing) rabbits. Top: A representative Western blot result showing the upregulation of $gp91^{phox}$ protein expression in the RVLM after 2 and 3 weeks of LV pacing. Bottom: Results of densitometric analysis representing means $\pm$ SEM; * $P < 0.05$ compared with control; n=6 each group.

Table 1

Body weight (B.wt.), left ventricular weight (LV wt.), heart weight (Ht wt.), LV wt. to B. wt. ratio, Ht wt. to B.wt. ratio, wet lung weight and dry lung weight in control animals and after pacing for 1,2 or 3 weeks.

	Control	1 week pacing	2 weeks pacing	3 weeks pacing
B. wt. (kg)	3(3–4)	4(3–4)	4(3–4)	4(3–4)
LV wt. (gm)	5(5–6)	5(5–6)	5(5–6)	6(6–7) *
Ht wt. (gm)	9(8–9)	9(9–10)	10(9–11)	12(11–13) *
LVwt./B.wt. (gm/kg)	1(1–2)	1(1–2)	1(1–2)	2(1–2) *
Ht wt./B.wt. (gm/kg)	3(2–3)	3(2–3)	3(2–3)	3(3–4) *
Wet lung wt. (gm)	15(14–17)	13(12–15)	14(12–15)	17(15–18)
Dry lung wt. (gm)	3(3–4)	3(3–4)	3(3–4)	4(3–5)

Values are median and range from 6 rabbits in each group;

* P< 0.05 significantly different from control

Basal hemodynamic characteristics in conscious rabbits before and after induction of heart failure by left ventricular pacing for 1, 2 or 3 weeks.

Table 2

Group	1 week		2 weeks		3 weeks	
	Pre-pace	Post-pace	Pre-pace	Post-pace	Pre-pace	Post-pace
MAP (mmHg)	70 (67–74)	69 (65–74)	68 (64–72)	67(65–69)	70(67–73)	65 (61–69)
HR(beats/min)	220(214–228)	225 (219–231)	209 (204–213)	226 (221–231)	215(210–220)	243(236–249) *
EF (%)	75 (74–77)	64(61–68) *	75(74–77)	60 (57–63) *	79 (81–77)	56(52–60) *
FS (%)	41(39–43)	34(35–32) *	41(39–43)	30(29–31) *	41(39–43)	27(26–30) *
LVEDD (mm)	15(14–15)	17(16–17) *	15(15–16)	18(19–17) *	14(14–15)	18(17–18) *
LVESD (mm)	9 (8–9)	11(10–11) *	9(8–9)	13(12–13) *	8(8–9)	13(12–13) *

Values are median and range from 6 rabbits in each group

MAP, mean arterial pressure; HR, heart rate; EF, ejection fraction; FS, functional shortening; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter.

* P<0.05 significantly different from pre-pace of the respective group.

* P< 0.05 significantly different from control

Table 3

Plasma concentration of ANG II in control and in CHF rabbits after left ventricular pacing for 1,2, or 3 weeks

Angiotensin (pg/ml)	1 Week		2 Week		3 Week	
	Pre pacing	Post pacing	Pre pacing	Post pacing	Pre pacing	Post pacing
Median	26	24	26	45	18	110*
Range	38-14	27-21	36-16	57-33	20-16	135-85*

Values are median and range from 6 rabbits in each group.

* P< 0.05 significantly different from control

Baroreflex sensitivity with sodium nitroprusside (SNP) and phenylephrine (PE) in control and in CHF rabbits after left ventricular pacing for 1,2, or 3 weeks

Table 4

BRS bpm/mmHG	1 week		2 week		3 week	
	Pre pacing	Post pacing	Pre pacing	Post pacing	Pre pacing	Post pacing
Median with SNP	4	3*	5	3*	5	3*
Range	4-5	3-4	5-6	3-4	4-5	3-4
Median with PE	3	2*	3	2*	3	1*
Range	3-4	1-2	2-3	1-2	2-3	1-2

Values are median and range from 6 rabbits in each group.

* P< 0.05 significantly different from control