Progression of heart failure after myocardial infarction in the rat

J. FRANCIS,1,2 R. M. WEISS,1,2 S. G. WEI,2 A. K. JOHNSON,3 AND R. B. FELDER1,2

1Research Service, Veterans Affairs Medical Center and Departments of Internal Medicine and 3Psychology, University of Iowa, Iowa City, Iowa 52242

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Francis, J., R. M. Weiss, S. G. Wei, A. K. Johnson, and R. B. Felder. Progression of heart failure after myocardial infarction in the rat. Am J Physiol Regulatory Integrative Comp Physiol 281: R1734–R1745, 2001.—This study examined the early neurohumoral events in the progression of congestive heart failure (CHF) after myocardial infarction (MI) in rats. Immediately after MI was induced by coronary artery ligation, rats had severely depressed left ventricular systolic function and increased left ventricular end-diastolic volume (LVEDV). Both left ventricular function and the neurohumoral indicators of CHF underwent dynamic changes over the next 6 wk. LVEDV increased continuously over the study interval, whereas left ventricular stroke volume increased but reached a plateau at 4 wk. Plasma renin activity (PRA), arginine vasopressin, and atrial natriuretic factor all increased, but with differing time courses. PRA declined to a lower steady-state level by 4 wk. Six to 8 wk after MI, CHF rats had enhanced renal sympathetic nerve activity and blunted baroreflex regulation. These findings demonstrate that the early course of heart failure is characterized not by a simple “switching on” of neurohumoral drive, but rather by dynamic fluctuations in neurohumoral regulation that are linked to the process of left ventricular remodeling.

left ventricular function; echocardiography; neurohumoral regulation; sympathetic nerve activity; paraventricular nucleus of hypothalamus

CONGESTIVE HEART FAILURE (CHF) is a multisystem disorder characterized by an exaggerated state of neurohumoral excitation. The CHF syndrome is initiated by impaired left ventricular systolic function and left ventricular chamber dilatation, with a requirement for high left ventricular filling pressures to sustain adequate cardiac output and tissue perfusion. The neurohumoral compensatory mechanisms activated in CHF are the same ones that are activated in hypovolemic states (3): the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) act to restore arterial pressure through vasoconstriction and fluid retention. In heart failure, however, homeostasis cannot be achieved because of the persistent impairment of left ventricular function. Thus, in the absence of the usual negative-feedback signals that terminate their activity, these seemingly appropriate neurohumoral compensatory mechanisms become part of a vicious cycle that precipitates clinical deterioration.

The neurohumoral response to CHF is typically presented as a binary phenomenon, i.e., either “on” or “off,” with the implication that this response persists unabated throughout the course of CHF. Previous studies of experimental heart failure (1, 12, 23, 27) have tended to reinforce that concept by focusing on the state of neurohumoral regulation at a single point in time, usually at least several weeks after induction of CHF. Typically, these studies have relied on a single indicator of left ventricular dysfunction to define heart failure, e.g., m-mode echocardiography, a terminal measurement of end-diastolic pressure, or postmortem anatomy. Neither the evolving pattern of neurohumoral excitation nor its interplay with the progressive changes in cardiac structure and function that result from left ventricular remodeling has been systematically examined in experimental or clinical CHF.

The present study addresses these shortcomings by gathering information on neurohumoral regulation and left ventricular function at multiple time points during the development of CHF after myocardial infarction (MI) induced by coronary ligation in the rat. This model simulates the most common cause of CHF in humans and permits precise timing of the inciting event and of the changes in neurohumoral systems and left ventricular function as CHF progresses. In a neurohumoral study group, sequential weekly measurements of blood-borne peptide markers of heart failure and metabolic cage indicators of volume regulation were obtained over the course of 6 wk after MI. Recordings of renal sympathetic nerve activity (RSNA) were obtained 6–8 wk after MI. In this group of rats, a single quantitative echocardiographic assessment of left ventricular mass, volume, and systolic function was made 2 wk after MI. In a separate remodeling study group, the echocardiographic assessment of left ventricular structure and function was obtained 24 h after MI and at 2-wk intervals over a 6-wk study interval.

The findings demonstrate a heretofore unappreciated temporal divergence among the humoral changes...
METHODS

Experiments were carried out in adult (250–350 g) male Sprague-Dawley rats obtained from Harlan Sprague Dawley (Indianapolis, IN). The animals were housed in the Animal Care Facility at the Veterans Affairs Medical Center in Iowa City, IA. The study was approved by the institution’s Animal Care and Use Committee and conformed to the guidelines of the American Veterinary Association.

Neurohumoral Study Protocol

A unique aspect of this study is the repetitive measurement of neuroendocrine and volume-regulatory indexes in the same rats over a 6-wk study protocol. For 2 wk before initiation of the study protocol, rats became adapted to a metabolic cage environment in which they had ad libitum access to food, water, and a 1.8% NaCl solution. A normal 12:12-h light-dark cycle was maintained. These conditions were then maintained for the 6 wk of the study protocol. At the beginning of the first protocol week, all rats underwent surgery for implantation of a jugular venous catheter for venous sampling. Each week, measurements were made of food intake, fluid intake (from the 1.8% NaCl and tap water burettes), urine volume, and body weight. Urine samples were collected for analysis of sodium content, and jugular venous samples were collected for measurement of plasma renin activity (PRA), arginine vasopressin (AVP), and atrial natriuretic factor (ANF). Early in the first week, all rats underwent coronary artery ligation (CL) and then at 2, 4, and 6 wk. No other data were acquired from these rats.

Procedures

Rats in the neurohumoral study protocol were subjected to three survival surgeries and one echocardiography session. Rats in the left ventricular remodeling study protocol were subjected to one survival surgery and four echocardiographic sessions. After each procedure, animals recovered from anesthesia under observation in the laboratory before being returned to their cages. All surgical procedures were performed using sterile technique. Animals were treated for postoperative pain with buprenorphine (0.03 mg sc) immediately after surgery, 12 h later, and then as needed.

Jugular catheterization. The procedure for jugular catheterization has been described before (7). Under ketamine anesthesia (100 mg/kg ip), a midline cervical incision was made and the jugular vein was isolated by blunt dissection. A Silastic catheter (ID 0.025 in.; OD 0.047 in.; Dow Corning, Midland, MI) was inserted into the vein and held in position by sutures. The free end of the catheter was externalized at the base of the neck. The catheter was flushed every other day for the first 2 wk and then every day with a mixture of polyvinylpyrrolidone, bacteriostatic 0.9% saline, and heparin (100 U/ml) and sealed with a piece of blunt steel tubing to prevent clogging.

Induction of CHF or sham operation. Heart failure was induced using the coronary occlusion technique, which is widely described in the literature (for example, see Ref. 11). The day after collecting baseline samples, rats were anesthetized with ketamine (100 mg/kg ip), endotracheally intubated, and mechanically ventilated with room air, respiratory rate 50–55 breaths/min, tidal volume 2.5 ml. Under sterile conditions, a left thoracotomy was performed to expose the heart. The pericardium was opened and the heart was exteriorized. The left anterior descending coronary artery was ligated between the pulmonary outflow tract and the left atrium with a 6-0 silk suture that was passed through the superficial layers of myocardium. The heart was returned to the chest cavity, the lungs were reinflated, and the chest incision was closed. Sham-operated rats were prepared in the same manner but did not undergo coronary artery ligation. After completion of the surgical procedures, rats were removed from the ventilator and the endotracheal tube was removed. Postoperative care included postoperative anesthetics, perioperative antibiotics (benzathine penicillin [30,000 U im] and lidocaine [2 mg im every 4 h for 2 doses]).

Preparation for sympathetic nerve recording and baroreflex testing. Under pentobarbital anesthesia (50 mg/kg ip), the left femoral artery and vein were cannulated and the catheters were tunneled to the back of the neck. The left kidney was exposed via a flank incision. One of the nerves to the left kidney was dissected free from surrounding tissue and placed on bipolar silver wire recording electrodes. When an optimal signal-to-noise ratio was achieved, the electrode and the renal nerve were covered with President light dental acrylic (Celtene, Alstalten, Switzerland), and the electrodes were sutured to back muscles and then tunneled to the back of the neck.

Echocardiography. Light general anesthesia was induced with ketamine (25 mg/kg ip). This resulted in maintenance of some postural tone but lack of aversive response to confronation. The anterior chest was shaved and acoustic coupling gel was applied. The animal was positioned in the left lateral recumbent position to optimize the windows for echocardiography. Imaging was performed with an Acuson (Mountain-view, CA) Sequoia model 256 clinical echocardiograph fitted with an 8-MHz sector-scanning probe. The unit provides very high-resolution two-dimensional cardiac images at a frame rate of 100/s. Left ventricular short-axis images were acquired at the level of the chordae tendineae and were deemed acceptable when the left ventricular epicardial silhouette best approximated the arc of a circle. Long-axis images were acquired perpendicular to the short axis and were deemed appropriate when left ventricular length was longest and both mitral and aortic valves were contained in the image. Images were stored digitally without analog conversion and were replayed in single-frame mode. End diastole was defined as the frame in which ventricular volume was largest, and end systole was defined as the frame in which ventricular volume was lowest. Endo- and epicardial borders were
identified at end diastole and end systole using the leading-edge convention. Ventricular volumes and mass were calculated using the area-length method, which has been validated in rodents (10) and humans (26). Infarct size was roughly estimated by planimetric measurement of the percentage of the left ventricle that demonstrated diastolic wall thinning and systolic akinesia or dyskinesia. After completion of two-dimensional imaging, pulse-wave Doppler interrogation of mitral inflow was performed for the purpose of determination of heart rate (HR).

Metabolic cage assessment of fluid balance. Ingestion of food, water, and 1.8% NaCl, body weight, urine volume, and urinary sodium content were measured twice weekly over two consecutive 24-h periods, and an average value for each variable was reported for that time point. Urine sodium was measured using a NOVA sodium and potassium analyzer (NOVA Biomedical, Waltham, MA).

Peptide measurements. Blood (3 ml) was collected every week for a period of 6 wk, 0.5 ml into chilled EDTA tubes for the measurement of PRA and 2.5 ml into EDTA tubes containing aprotinin to inhibit the activity of proteinases for the measurements of ANF and AVP. Blood samples were centrifuged at 4°C, and the plasma samples were separated and stored at −70°C until assayed for these peptides using radioimmunoassay. After each collection, blood cells were reconstituted with the same volume of bacteriostatic heparin in 0.9% saline and reinfused.

Peptides were extracted from plasma using trifluoroacetic acid and acetonitrile in a pretreated C18 SEP column. Using this method of extraction, we determined the recovery of AVP to be 91% and of ANP to be 82%, using radiolabeled AVP and ANP.

PRA. ANG I was measured and expressed as PRA using NEN Life Science Products (Boston, MA) ANG I radioimmunoassay kit. Under the conditions of this assay, the accumulation of angiotensin is favored by allowing the endogenous substrate to react in the presence of reagents that inhibit both plasma converting enzymes and proteolysis by angiotensinases. The sensitivity of the assay is 6.0 pg/ml, and the intra- and interassay coefficients of variation are 8 and 10%, respectively.

ANF. Plasma ANF was measured using a rat ANF radioimmunoassay kit from Phoenix Pharmaceuticals (Belmont, CA). The minimum detectable limit using this kit is 0.02–0.04 ng/ml, and the range of detection is 0–100 ng/ml. The intra- and interassay coefficients of variation were 5 and 10%, respectively.

AVP. Plasma AVP levels were measured using a rat AVP radioimmunoassay kit from Assay Design (Ann Arbor, MI). The sensitivity of the assay was 10 pg/ml. The intra- and interassay coefficients of variation were found to be 6.8 and 11.4%, respectively.

Sympathetic nerve recording and baroreflex testing. Sympathetic nerve recordings were made in the conscious, freely mobile state 4 h after recovery from anesthesia to implant bipolar electrodes on the renal nerve. The externalized recording electrodes were connected to a Grass P511 AC amplifier to record RSNA. The externalized femoral artery catheter was connected to a strain-gauge transducer to record arterial pressure (AP, mmHg). HR (beats/min) was derived by computer analysis of the AP pulse frequency. After 20 min of stabilization, baseline AP, HR, and RSNA were recorded for 15 min. Baroreflex testing was performed using bolus intravenous injections of phenylephrine (PE, 2–10 μg/kg) and sodium nitroprusside (SNP, 5–20 μg/kg). AP and RSNA returned to baseline between interventions.

RSNA was passed to a Paynter filter (20-ms time constant, Bak Electronics, Germantown, MD) to rectify and integrate the raw signal. AP, rectified and integrated RSNA (mV), and windowed RSNA (spikes/s, selected from the raw RSNA tracing by a window discriminator, model DIS-1, Bak Electronics) were passed to a Cambridge Electronic Design (CED, Cambridge, UK) 1401 Laboratory Interface linked to a PC. The raw RSNA signal and AP were also recorded on video-cassette recorder tape using a PCM Recording Adaptor (A.R. Vetter, Rebersberg, PA) for analysis offline.

HR, mean AP, windowed RSNA, and rectified and integrated RSNA were determined for five sequential 2-min intervals, and those values were averaged to obtain a single baseline value for each variable. HR, mean AP (MAP), and windowed RSNA data from baroreflex testing were analyzed in 1-s time bins, constructing baroreflex curves comparing responses to PE and SNP with steady-state control values over the prior 20 s.

Using CED software, two further quantitative measures of the rectified and integrated RSNA voltage at baseline were obtained. 1) At the nadir (user defined) of the major bursts of RSNA was identified, so that the frequency of bursting activity (bursts/s) could be estimated; and 2) a waveform average of the RSNA voltage signal, triggered by the peak of the systolic pressure wave, was obtained over a 2-min interval of baseline recording to determine the relationship between RSNA and HR. The triggered waveform average was quantitated as the area under the curve.

Measurement of left ventricular end-diastolic pressure. After completion of the sympathetic recording, the animals were anesthetized with pentobarbital (50 mg/kg ip), the right carotid artery was exposed, and a PE-50 cannula attached to a pressure transducer was advanced through the carotid artery across the aortic valve into the left ventricular chamber. Pressure was recorded continuously while the cannula was being positioned at a site within the left ventricle at which left ventricular pressure could be accurately recorded (i.e., the onset of the rapid rise in left ventricular pressure after the atrial “kick” could be observed) and left ventricular systolic pressure was not higher than aortic pressure on entering the left ventricle (i.e., there was no evidence of ventricular outflow obstruction). Left ventricular pressure was then recorded for an interval of 2 min. A single left ventricular end-diastolic pressure (LVEDP) measurement was obtained by applying a horizontal cursor across the visually estimated end-diastolic pressure of five sequential left ventricular pressure waveforms.

Anatomic assessment of heart failure. At the conclusion of the protocol, the hearts were arrested under anesthesia, and the heart and lungs were removed for examination. Because left ventricular size and function were assessed echocardiographically in vivo, the presence of myocardial scar was confirmed only qualitatively. The heart-to-body weight and lung-to-body weight ratios were determined.

Statistics. Differences in food intake, body weight, sodium intake, urine sodium, urine volume, and hormonal changes (PRA, AVP, and ANF) between the sham-operated and CHF groups were analyzed using repeated-measures ANOVA followed by post hoc Fischer’s least significant difference tests. Baroreflex data were analyzed using a standard sigmoid curve-fitting program (Sigma Plot, Jandel Scientific) and a nonlinear regression program for analysis of components of the baroreflex curve. Baroreflex data from each animal were analyzed individually using this program, and the values determining the individual curves were then averaged to generate the mean values describing a curve for each group. Baseline values of RSNA, MAP, and HR, and a maximal gain...
hypertrophy commonly reported with heart failure in infarct zone, likely secondary to the right ventricular ejection fraction (LVEF) and the neurohumoral variables (PRA, AVP, ANF, and RSNA). Significance levels were determined using Pearson's r correlation coefficient.

RESULTS

Anatomy

In the CHF rats, gross examination typically revealed a dense scar in the anterior and lateral left ventricular wall. Figure 1, top, shows a transverse section of a CHF heart, indicating the location of the CL and the extensive scarring and thinning of the myocardium beyond. Figure 1, bottom, shows the normal left ventricular wall thickness in a sham-operated animal.

Figure 2 illustrates the ratios of heart weight to body weight and lung weight to body weight for CHF and sham-operated rats in the neurohumoral study group. The body weight was quite similar (CHF 357.6 g; sham operated 377.50 g) in the two groups. Heart weight (CHF 2.22 ± 0.11 g; sham operated 1.79 ± 0.11 g) and lung weight (CHF 5.04 ± 0.50 g; sham operated 2.27 ± 0.13 g) were both higher in CHF rats. Heart-to-body weight ratio increased by 34.7 ± 2.8% in the CHF rats, despite the loss of muscle mass in the infarct zone, likely secondary to the right ventricular hypertrophy commonly reported with heart failure in this model. Lung-to-body weight ratio increased more than twofold in the CHF rats compared with sham-operated rats, suggesting increased fluid volume in the pulmonary vasculature. Pleural fluid was found in six CHF rats and ascitic fluid in five CHF rats.

Echocardiography

Figure 3 shows representative long-axis echocardiographic images of the left ventricle from a CHF and a sham-operated rat. The images from sham-operated rats in Fig. 3, top, show normal left ventricular muscle thickness during systole and diastole, with typical nearly complete left ventricular emptying in systole. The CHF images (Fig. 3, bottom) show a dilated left ventricular chamber with thinning of the anterior and apical muscle and minimal systolic thickening of the remaining myocardium in the posterior wall.

Left Ventricular Remodeling Study

Figure 4 summarizes the quantitative echocardiographic data from the left ventricular remodeling study, showing the progression of changes in left ventricular function over the 6-wk interval after CL. The initial ischemic injury in these rats (n = 6) involved 48.1 ± 4.6% of the left ventricular wall. LVEF after CL was 0.31 ± 0.02 and did not change over the 6-wk period. In contrast, left ventricular end-diastolic volume (LVEDV) had increased as early as 24 h after CL (0.70 ± 0.1 ml) and increased progressively over the 6-wk study interval. Stroke volume also increased initially (0.21 ± 0.03 ml), matching the increase in LVEDV, but reached a plateau at week 4. Because HR (initially 385 ± 25 beats/min) did not change over the 6-wk study interval, cardiac output paralleled the changes in stroke volume, increasing initially but leveling off after week 4. The mismatch between LVEDV and the cardiac output late in the protocol is tangible evidence of the failure of compensatory mechanisms. Because left ventricular mass (initially 947 ± 37 mg) did not increase over the 6-wk period, it can be inferred...
that left ventricular wall stress (proportional to chamber size divided by wall thickness) increased progressively. An increased LVEDV/left ventricular mass predicts further decline in left ventricular function.

Neurohumoral Study

Left ventricular function. Figure 5 shows the quantitative echocardiographic measurements for rats in the neurohumoral study, evaluated once, 2–3 wk after CL. The values obtained from the CHF rats in this study closely resembled those obtained at 2 wk in the left ventricular remodeling study. The CHF rats in the neurohumoral study had ischemic injury involving 37.3 ± 11.1% of the left ventricular wall. Overall ejection fraction was 0.36 ± 0.05 in the CHF rats compared with 0.82 ± 0.02 in the sham-operated rats. At 2–3 wk after CL, there was a dramatic increase in left ventricular volume (0.93 ± 0.1 vs. 0.48 ± 0.03 ml) and in left ventricular volume/mass ratio (1.37 ± 0.19 vs. 0.52 ± 0.04), findings pathognomonic of heart failure. Cardiac output was reduced in the CHF rats compared with the sham-operated rats (121 ± 9 vs. 153 ± 10 ml/min, respectively; \( P < 0.05 \)). These differences in cardiovascular dynamics between the CHF and sham-operated rats could not be attributed to differences in HR, which was similar (395 ± 15 vs. 398 ± 12 beats/min, CHF vs. sham operated) under ketamine sedation. Notably, HR under these conditions was only slightly less than that of conscious rats (see Cardiovascular and neural assessment).

Regulatory peptides. Figure 6 shows the humoral measurements over the entire study. Baseline measurements of PRA (3.61 ± 0.21 vs. 3.45 ± 0.28 ng/ml h⁻¹), AVP (1.95 ± 0.50 vs. 2.24 ± 0.44 ng/ml), and ANF (171.47 ± 13.6 vs. 145.92 ± 15.3 pg/ml) were similar in the sham-operated and CHF groups, respec-
tively. In the sham-operated rats, these peptide levels did not change over the duration of the protocol. In contrast, the development of CHF in the CL rats was accompanied by dramatic shifts in PRA and AVP levels. PRA rose early within the first week after CL, peaked at 2 wk with a value two- to threefold above normal, and then adapted to a lower level that was sustained for the remainder of the study. AVP had already risen to pathophysiological levels by week 1 but peaked by week 3 with a value 10-fold higher than normal and remained high for the remainder of the study. Unlike PRA and AVP, ANF reached a steady state early, in the first week after coronary occlusion. There was a tendency toward a slight increase at the end of the study protocol.

Metabolic cage measurements. Before coronary occlusion, measurements of food intake (22.79 ± 0.26 vs. 23.60 ± 0.32 g), water intake (25.27 ± 0.98 vs. 24.45 ± 1.44 ml/day), and 1.8% NaCl intake (3.06 ± 0.31 vs. 3.1 ± 0.2 ml/day) did not differ between rats assigned to the sham-operated and the CHF groups. In the sham-operated group, intake of rat chow remained stable over the 6-wk study protocol; in the CHF group, food intake was diminished early after CL and then again later in the course of the study. CHF rats exhibited a stronger preference for ingestion of the sodium chloride solution (Fig. 8) within the first week (5.11 ± 0.35 ml/day) after CL, and this preference continued for the 5-wk period of observation. In the CHF rats, water intake peaked 2 wk after CL (29.55 ± 1.47 ml/day). Water and sodium chloride intake did not change in the sham-operated animals over the course of the study.

Before CL, rats assigned to the sham-operated and CHF groups had similar values for urine volume (12.03 ± 0.89 vs. 12.03 ± 0.44 ml/day) and urine sodium (2.48 ± 0.34 vs. 2.66 ± 0.13 meq/day). After CL, the CHF rats had significant decreases from baseline in urine sodium and urine volume at all time points. The sham-operated rats experienced no changes in urine sodium and urine volume over the course of the study. The differences between the CHF and sham-operated animals were significant at all time points.

Cardiovascular and neural assessment. Recordings of RSNA and AP were obtained from conscious CHF (n = 5) and sham-operated (n = 6) animals at the conclusion of the metabolic study. LVEFs in these animals, measured at protocol weeks 2–3, were 0.81 ± 0.0004 (sham operated) and 0.34 ± 0.0009 (CHF). Thus, although RSNA and AP recordings were not obtained from all animals in the neurohumoral study group, the animals studied were representative of the group as a whole.

Figure 9 shows group data demonstrating an increase in RSNA (mV and bursts/s) in the CHF group. The HR was higher in the CHF group, and the MAP tended to be lower but this change did not achieve statistical significance. The HRs in both groups were higher than we observed (unpublished data) in a group of conscious freely mobile rats monitored by telemetry for 6 wk after CL or sham CL without further intervention (HR at week 6: CHF vs. sham operated, 368 ± 15 vs. 360 ± 8 beats/min; P = NS). The higher HRs in
the present study may reflect increased sympathetic drive in these rats, studied 4 h after recovery from a brief anesthesia to implant the recording electrodes. Figure 10, top, shows representative tracings from a CHF and a sham-operated animal, demonstrating the general finding of increased renal sympathetic discharge at rest. Note the loss of HR variability in the CHF rat, a finding widely reported in clinical and experimental heart failure. Figure 10, bottom, also illustrates the finding that integrated RSNA/heart-beat, averaged over 2 min, was higher in the CHF vs. the sham-operated rat. Mean values for the area under the curve of the averaged waveform (insets) were significantly larger ($P < 0.01$) in the CHF (2.04 ± 0.12 mV·s) than sham-operated (1.49 ± 0.07 mV·s) rats, indicating that the increase in integrated RSNA was independent of HR. As shown in the faster time base record in Fig. 10, bottom, there is a loss of the beat-to-beat modulation of RSNA likely respiratory related although not further examined in these experiments.

Figure 11 shows the baroreflex responses of HR and RSNA to raising and lowering AP with PE and SNP, respectively. Compared with the baroreflex curves of the sham-operated group, the curves of the CHF group were blunted, with a diminished ($P < 0.05$) maximum gain (HR $1.67 ± 0.15$ vs. $2.83 ± 0.25$ beats·min$^{-1}$·mmHg$^{-1}$; RSNA $1.89 ± 0.19$ vs. $3.24 ± 0.27$ %/mmHg) and range (HR $113.4 ± 10.3$ vs. $170.0 ± 5.9$ beats/min; RSNA $112.8 ± 3.7$ vs. $159.0 ± 5.1$%), consistent with a blunted baroreflex response. The calculated MAP midpoints for the HR ($105.9 ± 5.2$ vs. $117.5 ± 11.5$ mmHg) and the RSNA ($107.0 ± 4.0$ vs. $115.7 ± 3.0$ mmHg) curves closely resembled the resting MAP in the two groups of animals (CHF 105.7 ± 3.3 mmHg; sham operated 115.3 ± 5.4 mmHg).

LVEDP measured under anesthesia at completion of the study was increased in the CHF (18.3 ± 2.8 mmHg, $n = 5$) vs. the sham-operated (8.2 ± 3.7 mmHg, $n = 5$) rats ($P < 0.05$).

Cardiovascular and neurohumoral interactions. In CHF rats, neurohumoral activation and the severity of left ventricular dysfunction both appeared to be continuous variables. Using linear regression analysis, we sought to determine the relationships between two common measures of left ventricular function (LVEF and LVEDP) and the neurohumoral indicators of CHF. Using data from the neurohumoral study group (Fig. 12), in which nearly simultaneous measurements of left ventricular function and humoral responses were available in the same animals at 2–3 wk after CL, there was a strong correlation between LVEF and PRA, an indicator of RAAS activation. There was also a strong correlation between LVEF at 2–3 wk and RSNA recorded at the conclusion of the neurohumoral protocol (6 wk), the only time point at which RSNA was measured. LVEDP, the indicator of diastolic filling measured at the conclusion of the protocol (6 wk), was...
linearly related to PRA and RSNA measured at about that same time, but the correlations were less striking. Factors related to precision in the measurement of LVEDP may contribute to its less definitive relationship to these variables.

**DISCUSSION**

The unique feature of this study is the sequential measurement of indexes of volume regulation and left ventricular function during the progression to heart failure after CL. Over a 6-wk interval after CL, we observed dramatic fluctuations in the humoral factors that regulate intravascular volume and vascular tone and a progressive dilatation of the left ventricle that ultimately exceeded its capacity to generate an increase in stroke volume. However, the activity of the two key compensatory systems, RAAS and sympathetic drive, was linearly related to left ventricular systolic function, which remained relatively constant over the duration of the study.

The plasticity of left ventricular size and function over the first 6 wk after MI may be an important factor in the early dynamic shifts in the humoral milieu. The remodeling data indicate that LVEF is reduced to heart failure levels immediately (day 1) after CL, with insignificant change (a small decrease) thereafter. In contrast, LVEDV is increased immediately (day 1) after CL but increases progressively thereafter. Within limits imposed by the detrimental effects of increased wall stress, the increasing ventricular volume has the beneficial hemodynamic effect of improving left ventricular stroke volume despite the persistently low ejection fraction. Early activation of the RAAS likely contributes to this process by increasing filling pressures within the dilated left ventricle. When assessed 2–3 wk after CL, there is a tight reciprocal relationship between LVEF and activation of the RAAS. The later stabilization of PRA at a lower level of activity likely reflects improved renal perfusion, resulting from this
early dynamic interplay between cardiac function and humoral systems.

In our model, this compensatory mechanism reaches its limit at 4 wk, when stroke volume has peaked but LVEDV continues to increase. Progressive ventricular dilatation, in the absence of further increase in stroke volume, indicates exhaustion of the compensatory gain produced by ventricular remodeling and signals the advent of a grossly maladaptive response. In this setting, continued neurohumoral drive, with associated volume retention and peripheral vasoconstriction, assumes a malignant character.

In this context, the dissociation between left ventricular dynamics and neurohumoral indexes in the later phase of CHF is of particular interest. For example, PRA appears to play a major role in the initial compensatory response to CL, rising early and peaking at 2 wk. However, in the later phase of CHF, when the gap between LVEDV and stroke volume is widening, PRA does not respond. Similarly, plasma AVP peaks at 3 wk and remains above baseline, but does not respond with a further increase as left ventricular dilatation proceeds beyond the point at which stroke volume stabilizes. Thus, while persistent higher than normal levels of PRA and AVP contribute to the volume retention and vasoconstriction that characterize established CHF, the RAAS does not signal the transition from the initial compensatory response to left ventricular dysfunction to the decompensated heart failure state. On the contrary, circulating PRA in particular remains stable at a lower level. The only late increase in humoral activity we observed, which was not statistically significant, was in ANF, which is released in proportion to left ventricular distension (4) and counteracts the effects of circulating ANG II.

Despite the dynamic shifts in left ventricular volume and in the release of humoral factors, the outcome measures of extracellular volume regulation remain relatively constant over the 6 wk after CL. With the exception of an early (week 1) dramatic decline and transient partial recovery in sodium excretion, there is little indication that these behavioral and physiological responses are closely correlated with any of the humoral indexes we measured. For example, sodium appetite is a definitive manifestation of forebrain activation by circulating ANG II and aldosterone (14), the downstream humoral products of the increased PRA. Yet sodium intake increased early and persisted at about the same level over the 6-wk study interval despite wide swings in PRA. Urine volume, which reflects (among other factors) the peripheral influences of circulating AVP, decreased early and remained at about the same level despite the fluctuations in circulating AVP.

These observations suggest a relative insensitivity to effects of humoral mediators on extracellular fluid balance in heart failure. With respect to the peripheral mechanisms of sodium reabsorption and urine volume, other mechanisms such as renal vascular responses to low cardiac output and increased sympathetic drive may well override the effects of humoral changes. In the brain, however, different possibilities must be considered. Thirst and sodium appetite are well-recognized behavioral effects of ANG II and aldosterone actions on AT1 and mineralocorticoid receptors, respectively, in the forebrain (14). It may be that the circulating levels of neuropeptides in CHF exceed those required to saturate receptors in central volume-regulatory sites. There is some evidence that the intrinsic forebrain renin-angiotensin system may be upregulated in heart failure (25), so that an alternative source of ANG II production may be contributing. In addition, counterregulatory influences of ANF on the RAAS (17) may modulate the influences of circulating ANG II at its central sites of action.

ANG II and aldosterone can both act on forebrain receptors to increase sympathetic drive (8, 15) and AVP release (13, 24) in normal rats. Our linear regression analysis indicates that RSNA correlates tightly with LVEF, suggesting a rather precise signaling mechanism may be informing the brain of the extent of myocardial injury and driving the sympathetic response. The possibility that RAAS activity might be

![Fig. 11. HR (beats/min (bpm)) (A) and RSNA responses (B) to raising and lowering arterial pressure with phenylephrine and nitroprusside in CHF (n = 5) and sham-operated (n = 6) rats. Baroreflex responses were present in both groups but were blunted in the CHF rats. Insets: gain of the baroreflex. Curves were generated by averaging variables describing the curve fits for the data from the individual animals comprising each group. Dots indicate midpoint values.](http://ajpregu.physiology.org/article(SqlArticleID) http://ajpregu.physiology.org/article(SqlArticleID) http://ajpregu.physiology.org/article(SqlArticleID)
that signaling mechanism is indirectly supported by our finding of a tight correlation between LVEF and the peak circulating values of PRA. However, our data cannot establish a causal relationship between circulating RAAS and sympathetic drive, which could be independent responses to another driving mechanism.

The relative importance of the effects of circulating RAAS vs. altered peripheral afferent inputs in determining the state of sympathetic neural activity in CHF is an important and unresolved issue in heart failure research. The metabolic activity of the paraventricular nucleus of the hypothalamus, a critical outflow center for the forebrain influences of circulating RAAS, is increased in CHF (23). However, this region may be activated either by signals descending from the forebrain (2, 19) or by signals ascending from the brain stem (18), where the cardiovascular afferent nerves terminate. Numerous studies have now demonstrated that the cardiovascular reflex mechanisms that inhibit sympathetic drive at the brain stem level are blunted in CHF (28, 30), and more recently there is compelling evidence that the cardiovascular reflex mechanisms driving the sympathetic nervous system (e.g., arterial chemoreceptor, cardiac sympathetic afferent) may be augmented (27, 29).

Whether hypothalamic activation in heart failure arises from altered cardiovascular reflexes or from circulating RAAS acting on forebrain structures, a cogent argument can be mustered to support the hypothesis that the forebrain contributes to the volume retention and augmented sympathetic drive in CHF. However, the magnitude of the forebrain influence on the development and the progression of CHF remains to be determined. This is fertile ground for future research. If central nervous system mechanisms contribute significantly to the CHF syndrome, new and potentially more effective modalities targeting critical central sites may ultimately be developed.

Several limitations must be considered in the interpretation of our results. First, the renal nerve recordings and hemodynamic measurements were obtained just 4 h after recovery from a brief anesthesia to implant the renal recording electrodes and vascular catheters. The high HRs of these rats (compared with unstressed rats on telemetry) suggest that the sympathetic nerve activity and baroreflex responses may have been affected by the stress of the experimental conditions. Although both sham-operated and CHF rats were subjected to the same procedure, we cannot exclude the possibility that the CHF rats had a greater or more prolonged sympathetic response to the stress of anesthesia and surgery. Second, we have only a single measurement of sympathetic drive, taken at the completion of the metabolic study. Thus we cannot assess the progression of changes in RSNA or the relationship of RSNA with the other variables over the course of the study. Finally, the serial echocardiographic assessments of left ventricular function were obtained from groups of rats different from those undergoing metabolic, humoral, and sympathetic recording studies, so that the correlations between left ven-

Fig. 12. Linear regression curves showing correlations between left ventricular end-diastolic pressure (LVEDP; A) and LV ejection fraction (LVEF; B), respectively, and the neurohumoral indicators of heart failure: RSNA, PRA (ng/ml), AVP (ng/ml), and ANF (ng/ml × 10, values adapted to fit graph). A: the 6-wk values of PRA, AVP, and ANF are plotted as a function of LVEDP, measured after the completion of the protocol. B: the 2-wk values of PRA, AVP, and ANF are plotted as a function of LVEF, obtained by echocardiography at about the same time. RSNA, which was obtained after the completion of the metabolic protocol, is plotted in A and B using the integrated voltage values. LVEF is more closely correlated with all humoral indexes of CHF. Both LVEF and LVEDP correlate well with RSNA. $R^2$ values are shown. *$P < 0.05$, **$P < 0.001$. 

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tricular function and those values are qualitative approximations.

In summary, we have examined the relationships between neuroregulatory mechanisms and left ventricular dynamics leading to CHF early after MI in the rat. These studies have provided new insights into the sequence of events leading to cardiac decompensation early after ischemic injury and have supported earlier suggestions (22) that the central nervous system plays an important role in this process. These data demonstrating the progression of heart failure after MI in untreated animals provide a strong foundation for further investigations of the complex interplay among neurohumoral mechanisms in ischemia-induced heart failure and a basis for examining the physiological impact of therapeutic interventions.

Perspectives

MI is the leading cause of CHF in this country. Because MI is usually a recognized event, an opportunity exists to intervene early to modify factors that promote the development of irreversible changes in heart function. Early intervention with β-blockers and angiotensin-converting enzyme inhibitors has already improved the long-term prognosis after MI, acting at least in part to prevent further myocardial damage and deleterious left ventricular remodeling. A careful analysis of the neurohumoral events that foster the development of CHF after MI may identify additional therapeutic targets. However, the aggressive medical and surgical management of patients presenting with acute MI precludes acquisition of natural history data from humans; human studies rely on comparisons across groups of patients with already established heart failure of varying severity (e.g., see Ref. 9).

The rat model of ischemia-induced CHF, which closely resembles human heart failure (see Refs. 20 and 21 for reviews), offers an alternative means of gaining new insights into the factors contributing to the development of CHF after MI. An example from our study is the finding that RAAS activity increases dramatically early after MI, but subsequently adapts to a lower level despite progressive left ventricular dilatation. Thus factors other than the circulating components of the RAAS must signal the transition from the adaptive (left ventricular dilation with increased cardiac output) to the maladaptive (further left ventricular dilation without increase in cardiac output) state. Cytokines (6) and reactive oxygen species (5), both activated in myocardial ischemia and heart failure, are among the likely candidates.

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