Cardiopulmonary Baroreflex Control of Renal Sympathetic Nerve Activity Is Impaired in Dogs With Left Ventricular Dysfunction

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ABSTRACT

Background: Activation of neurohormonal systems contributes to the progression of heart failure (HF). The mechanism(s) whereby these systems become activated is(are) not fully explained. We determined whether vagal cardiopulmonary baroreflex control of renal sympathetic nerve activity is abnormal in dogs with left ventricular (LV) dysfunction in the absence of clinical HF, and the relationship of abnormalities in baroreflexes to the development of the neurohumoral excitatory state.

Methods: LV end-systolic and end-diastolic dimensions (echocardiography), arterial baroreflex sensitivity (slope of ΔRR/Δsystolic BP during phenylephrine or nitroglycerin bolus), and neurohumoral profiles (plasma norepinephrine, renin activity, and arginine vasopressin) were measured serially in conscious dogs (n=24) with progressive LV dysfunction due to rapid ventricular pacing. LV dimensions were used to define groups with mild, moderate, and marked LV dilatation (LVD; increase in LV end-diastolic volume $<15\%$, $15-30\%$, and $>30\%$ of control, respectively). Changes in renal nerve activity (RNA) were recorded in response to increases in pulmonary capillary wedge pressure (PCWP) induced by volume infusion in anesthetized, sinoaortic-denervated dogs.

Results: Cardiopulmonary baroreflex sensitivity (slope of %ΔRNA/ΔPCWP) for mild LVD (−17.8%/mmHg) was the same as controls (−17.7%/mmHg). However, the slopes of moderate (−5.8%/mmHg) and severe LVD (−1.9%/mmHg) were decreased significantly compared with controls ($P < .05$). Arterial baroreflex sensitivity was preserved at all stages of LVD. Plasma norepinephrine, renin activity, and arginine vasopressin remained unchanged after 4, 7, and 11 days of pacing.

Conclusions: Vagal cardiopulmonary baroreflex control of renal sympathetic nerve activity is blunted early in the development of LVD. These abnormalities precede neurohumoral excitation and abnormal arterial baroreflexes and become apparent when LV end-diastolic volume starts to increase. (*J Cardiac Fail 2019;25:819−827*)

Key Words: Heart failure, congestive, autonomic control, sympathetic nervous system, parasympathetic nervous system, mechanoreceptors, arterial baroreflex, cardiopulmonary baroreflex.

Acute baroreceptor denervation leads to increases in sympathetic nerve activity, plasma renin activity, and arginine vasopressin. Because of the similarity between this acute sympathetic and humoral excitation and the chronic neurohumoral excitatory state of heart failure (HF), it has been proposed that reduced baroreflex sensitivity in HF may lead to excessive neurohumoral excitation.[1] Arterial baroreflex control of heart rate is abnormal in humans and dogs with HF,[2,3] but this abnormality may not become apparent until clinical manifestations of HF are fully developed.[4,5] Thus, it seems unlikely that abnormalities of arterial baroreflexes contribute to the development of the neurohumoral excitation that precedes clinical HF.

Dogs with fully developed low-output HF exhibit marked attenuation of vagal cardiopulmonary baroreflex control of renal sympathetic nerve activity.[6] Patients with HF have impaired cardiopulmonary baroreflex control of vascular resistance.[7] If abnormalities of cardiopulmonary baroreflexes contribute to the development of neurohumoral excitation, then it would be expected to occur in the setting of left ventricular (LV) dysfunction

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Manuscript received February 20, 2019; revised manuscript received June 3, 2019; revised manuscript accepted August 20, 2019.

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This work was supported by HL 30506 and by funds from the Medical Research Service of the Department of Veterans Affairs. T.K. was the recipient of a Research Fellowship Award from American Heart Association Northeast Ohio Affiliate, Inc.

1071-9164/$ - see front matter

https://doi.org/10.1016/j.cardfail.2019.08.012

prior to the development of the neurohumoral excitatory state and clinical HF.

We tested the hypothesis that vagal cardiopulmonary baroreflex control of renal sympathetic nerve activity is blunted in dogs with LV dysfunction in the absence of clinical HF. We also investigated the relationship between cardiopulmonary baroreflex abnormalities and the development of the neurohumoral excitatory state during progressive LV dysfunction.

Methods

Induction of Left Ventricular Dysfunction

Twenty-four mongrel dogs of either sex weighing 16−22 kg (mean 18.7 ± 0.4 kg) were anesthetized with sodium thiamylal 18 mg/kg, intravenously (IV) followed by isoflurane 2−4%. A pacemaker lead and custom modified generator were implanted as described previously.[8] The catheter of a vascular access port (model GPV, Access technologies, Skokie, IL) was placed in the left femoral artery to allow for repeated arterial access, and the attached port tunneled subcutaneously to the paravertebral region. The dogs were allowed to recover for 7−10 days postoperatively until all wounds showed good healing at which point the pacemaker was programmed to a rate of 250 beats per minute (bpm). Twice weekly electrocardiographic monitoring was performed to ensure continued pacing. An additional 4 dogs without pacemakers or vascular access ports served as normal controls for the acute (terminal) study, and data from 4 additional dogs were included for hemodynamic measurements. Another 3 normal dogs were used as time controls for echocardiographic measurements. A final group of 4 normal dogs served as time controls for the neurohormonal measurements. All procedures were performed according to institutional guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee and the NIH *Guide for the Care and Use of Laboratory Animals*.

Conscious Testing

The dog was placed on a table and ECG leads attached. A catheter was placed in the brachiocephalic vein for the administration of vasoactive drugs. A 22-gauge Huber point needle (Post-Grip Needle, Access technologies, Skokie, IL) was inserted percutaneously into the diaphragm of the previously implanted vascular access port and connected to a pressure transducer. Because pilot data revealed that rapid pacing caused marked hemodynamic and neurohumoral abnormalities, which were independent of the development of LV dysfunction, the dog was allowed to rest quietly for at least 10 minutes following cessation of rapid pacing. Arterial blood was withdrawn for determination of plasma norepinephrine, renin activity, and arginine vasopressin. Arterial blood pressure and electrocardiographic (ECG) signals were stored on a personal computer using an analogue to digital converter.

Echocardiographic images of the non-paced LV were obtained at the mid-papillary muscle level in the short axis cross-sectional view. M-mode recordings were taken at the same level, guided by the two-dimensional views to measure LV end-systolic and end-diastolic dimensions. Estimates of LV end-diastolic and end-systolic volumes were calculated using the Teichholz modification[9] of the D^3 method. After conscious testing was completed, the pacemaker was reprogrammed to pace at 250 bpm.

Neurohumoral Measurements

Blood samples (15 mL) for plasma norepinephrine, renin activity, and arginine vasopressin were collected on days 0, 4, 7, and 11 of pacing. Plasma norepinephrine,[10] plasma renin activity,[11] and plasma arginine vasopressin[12] were determined as described previously. Briefly, venous blood samples (10 mL) were obtained for measurements of plasma norepinephrine and plasma renin activity. The plasma was immediately separated by centrifugation and stored at −70˚C until the time of analysis. Plasma norepinephrine was determined by high-performance liquid chromatography with electrochemical detection using an ESA Coulochem II electrochemical Detector (ESA, Bedford, MA). Plasma renin activity was determined by an in vitro radioimmunoassay that measures the generation of angiotensin I (GammaCoat, Clinical Assays, Cambridge, MA).

General Methods

The timing of the acute (terminal) experiments was determined by serial echocardiographic measurements (see Protocols). Rapid ventricular pacing was terminated before the induction of anesthesia. Dogs were anesthetized with morphine sulfate 1 mg/kg followed by α-chloralose 60−80 mg/kg, IV. Control animals were pretreated with thiamylal sodium 18 mg/kg, IV (rather than morphine) to cause light sedation followed by chloralose. The animal was intubated, placed on a respirator, and ventilated with a mixture of oxygen and room air. Arterial and venous catheters were placed in the femoral vessels. Systemic arterial pressure was measured with a catheter in the femoral artery connected to a pressure transducer. A Swan-Ganz catheter was inserted through the jugular vein into the pulmonary artery for measurement of pulmonary artery and pulmonary capillary wedge pressures (PCWP), and for cardiac output determination (thermodilution method). All physiologic measurements were recorded on an electrostatic chart recorder. Arterial blood gases were drawn at regular intervals and maintained within the physiologic range. Body temperature was maintained between 37˚C and 38˚C by external warming. Supplemental α-chloralose 10 mg/kg was administered hourly.

A midline cervical incision was made and the vagus nerve dissected free from the carotid sheath. Using a binocular operating microscope, the aortic depressor nerve was located and identified by the recording of typical pulse-synchronous discharges.[13] The aortic nerve then was

sectioned and the procedure repeated on the contralateral side. The carotid sinuses were denervated bilaterally as described previously.[6] Carotid sinus denervation was considered complete if arterial pressure rose no more than 5 mmHg with bilateral carotid occlusion, and sinoaortic denervation was confirmed by the absence of an increase in renal sympathetic nerve activity (see below) during nitroglycerin-induced hypotension. The afferent nerves from vagal cardiopulmonary baroreceptors were denervated at the appropriate time in the experiment by ligating and dividing the cervical vagi bilaterally.

The left renal nerves were exposed via a retroperitoneal dissection and renal nerve traffic was recorded and quantitated as described previously.[6]

Protocols

Conscious Testing During the Course of Developing LV Dysfunction. The dogs were acclimated to lie at quiet rest during several practice sessions prior to conscious study. Blood sampling, determination of arterial baroreflex control of heart rate (HR), and echocardiographic measurements were performed with the dog in the sitting position at baseline prior to pacing and repeated twice weekly, until the dog met the criteria for the acute protocol (see below). During conscious testing, the pacemaker was inhibited and all conscious studies were performed with the dog in sinus rhythm. Blood samples were drawn 10 minutes after the cessation of rapid pacing (by which time the acute effects of rapid pacing on hemodynamics have been shown to abate).[3] Bolus injections of phenylephrine (200−1000 μg) or nitroglycerin (200−1000 μg) were administered intravenously (in random order) to raise and lower systolic blood pressure by ~30 mmHg. After at least 10 minutes and return to baseline blood pressure, the other drug was given. Echocardiographic measurements then were made. The pacemaker was reprogrammed to pace the ventricles at 250 bpm after conscious testing was completed.

We divided the dogs with LV dysfunction into 3 groups according to increases in calculated LV end-diastolic volume (EDV) determined prospectively (all measurements represent percentage change from baseline): group I: EDV $< 15\%$; group II: $15\% \leq$ EDV $\leq 30\%$; group III: EDV $> 30\%$. Acute studies were done the day following the last session of conscious testing.

Stimulation of Vagal Cardiopulmonary Baroreceptors in Anesthetized Dogs. Sinoaortic denervation was performed as described. In the dogs with elevated PCWP, hemorrhage (into heparinized syringes) was used to decrease PCWP to near normal levels (~5 mmHg). This blood (with additional dextran in normal saline as needed) was infused to expand plasma volume. One hundred and twenty milliliters of volume were infused over 2.5 minutes followed by 1 minute of data collection for analysis. This infusion was repeated until PCWP was elevated and/or renal nerve activity suppressed. Arterial blood pressure, PCWP, and renal nerve activity were recorded continuously. After volume infusion, hemorrhage again was used to decrease PCWP back to pre-infusion baseline levels. The same volume infusion protocol was repeated ~60 minutes after bilateral vagotomy.

Analytical Methods

Conscious Study. Blood pressure and ECG signals both were sampled at 500 Hz and stored on a personal computer using an analogue to digital converter (DATAQ Instruments, Akron, OH). The sampled data were processed *post hoc* using CODAS software, and RR interval and peak systolic blood pressure computed. Arterial baroreflex sensitivity was calculated as the slope of the regression line relating RR interval to changes in systolic blood pressure.[14] Assessments of arterial baroreflex sensitivity were considered acceptable when the coefficient of the regression line (R value) was >0.70. Bolus injections of phenylephrine or nitroglycerin were performed twice and the baroreflex slope of higher R value was chosen for the arterial baroreflex sensitivity. When respiratory sinus arrhythmia was prominent, only systolic blood pressure-pulse interval data pairs in end expiration were used for data analysis.

Nerve Recordings. Mean arterial blood pressure, PCWP, and integrated renal nerve activity were measured during the 1-minute data collection period at baseline and following each 2.5-minute period of volume infusion by use of a digitizer tablet (model 2210, Jandel Scientific, San Rafael, CA) and a custom-designed computer program (BASIC). For analysis of nerve activity, percentage change from baseline nerve traffic was used to account for differences in basal traffic because of differences in the size of nerves used and the number of active fibers on the recording electrodes. The slope of the linear portion of the plot relating percentage change in renal nerve activity to change in PCWP was taken as an index of cardiopulmonary baroreflex sensitivity.

Statistical Analysis. All statistical analyses were performed using PC SAS software (SAS Institute Inc, Cary, NC). Analysis of echocardiographic data and neurohumoral data obtained during the course of developing LV dysfunction was performed using repeated-measures analysis of variance (ANOVA) for the effect of TIME. The Greenhouse−Geisser adjustment was calculated for each repeated-measures ANOVA, but uncorrected P values are reported because of the absence of any change in significance of the tests. Individual comparisons were made using paired Student t test with the Bonferroni correction when a significant effect was observed. Group comparisons for baseline echocardiographic data, arterial baroreflex sensitivity, neurohumoral data, and hemodynamic parameters were made using one-way ANOVA. When a significant effect was observed, individual comparisons were made using the Duncan multiple range test. For the volume infusion data, analysis of covariance (ANCOVA) was used to determine whether there were significant differences among the groups in the slope of the regression lines relating changes in renal nerve activity to changes in PCWP. Least-squares means procedure was applied to compare the individual slopes of the groups. For the relationship between cardiopulmonary

baroreflex sensitivity and changes in LV end-diastolic volume, a second-order polynomial curve fit was used to generate a curve using SigmaPlot scientific graph system (Jandel Scientific, San Rafael, CA). All data are presented as mean ± SEM. A *P*value < .05 was considered significant.

Results

Serial Measurements During the Course of Developing LV Dysfunction

LV end-diastolic volume was unchanged at 4 days of pacing but elevated significantly by 18 ± 3% at 7 days and by 40 ± 7% at 11 days of pacing (Fig. 1). LV end-systolic volume increased significantly by 55 ± 7% at 4 days of pacing and increased progressively thereafter. LV volume data obtained from 3 time-control dogs (open symbols) revealed that LV end-systolic and end-diastolic volumes were constant in non-paced animals over this time period.

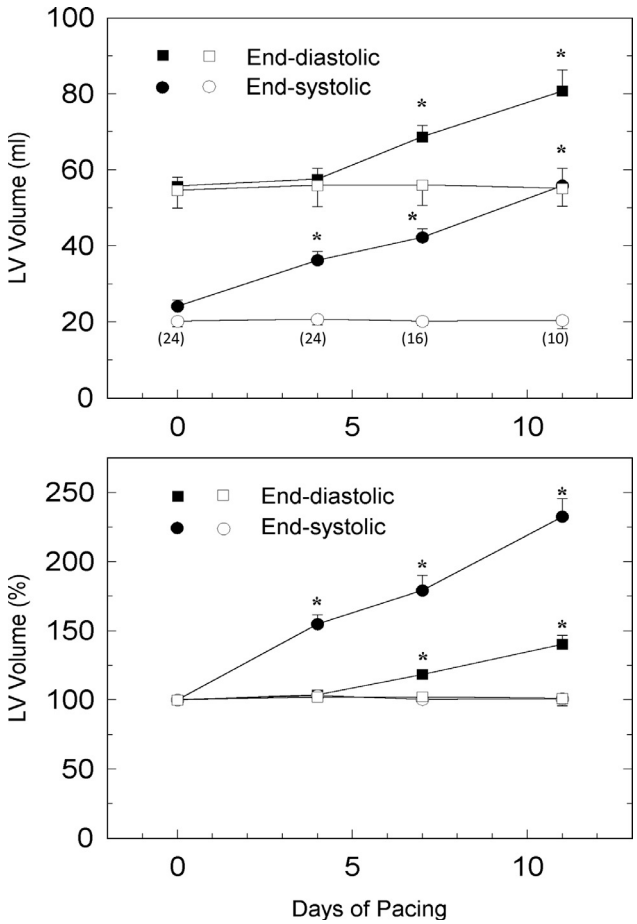

Fig. 1. Serial changes in calculated LV end-systolic (circles) and end-diastolic volumes (squares) in dogs with LV dysfunction because of rapid ventricular pacing (closed symbols) and time control dogs (open symbols). Measurements were made at baseline (day=0 [n=24]), and after 4 (n=24), 7 (n=16), and 11 (n=10) days of rapid ventricular pacing. Top panel shows calculated LV volumes (mL), bottom panel shows LV volumes expressed as percentage of baseline (day=0). *P < .05 versus baseline (day=0).

Baseline values of plasma norepinephrine, renin activity, and arginine vasopressin before pacing were 269 ± 41 pg/mL, 1.77 ± 0.22 ng/mL/h, and 3.52 ± 0.14 pg/mL, respectively, and for the non-paced control dogs were 195 ± 30 pg/mL, 2.5 ± 1.0 ng/mL/h, and 1.6 ± 0.2 pg/mL. Over the course of developing LV dysfunction there were no significant changes in plasma norepinephrine, renin activity, or arginine vasopressin in either group (Table 1 and Fig. 2). Plasma osmolality also did not change significantly over this period in either group.

Baseline Measurements by Groups

Paced dogs were divided into 3 groups (groups I, II, and III) according to the LV end-diastolic volume. Baseline data for each group (Table 1) were collected during the last conscious testing day, which was performed the day before the acute terminal protocol. Baseline echocardiographic parameters, arterial pressure, HR, and arterial baroreflex control of HR for each group are summarized in Table 1. LV end-diastolic volumes increased only in groups II and III. Phenylephrine slopes were similar in all groups. Nitroglycerin slopes tended to be different between groups, although the effect did not reach statistical significance (P = .08). Baroreflex slopes for the combined phenylephrine and nitroglycerin data also did not change. This was due to 2 factors: 1) the changes in HR with phenylephrine were larger than with nitroglycerin; and 2) phenylephrine and nitroglycerin slopes trended in opposite directions. Hemodynamic parameters on the day of acute study (Table 2) revealed that cardiac output was lower in all paced dogs, and pulmonary capillary wedge pressure was higher in groups II and III compared with controls.

Cardiopulmonary Baroreflex Sensitivity

Twenty-one of the 24 paced dogs met the criteria for acute study (ie, no signs of HF). There was little evidence of fluid accumulation even in group II and III dogs with modestly increased PCWP but no weight gain. Cardiopulmonary baroreflex sensitivity was determined in 21 paced dogs with LV dysfunction in the absence of HF (7 dogs in each group) and in 4 normal control (non-paced) dogs. Group data for changes in renal nerve activity as a function of PCWP are illustrated in Fig. 3. In controls, volume infusion raised PCWP from 3.1 ± 1.0 mmHg to 8.6 ± 0.8 mmHg and resulted in an 86.1 ± 3.7 % reduction in renal nerve activity. The responses of group I were virtually superimposable upon those of controls. In group III, PCWP rose to a greater degree (from 5.9 ± 1.2 to 22.6 ± 2.4 mmHg) during volume infusion, but renal nerve activity decreased only by 33.2 ± 14.0 %. Group II showed responses intermediate between groups I and III. In all groups of animals, decreases in renal nerve activity with volume infusion were linearly related to the increases in PCWP. There was an overall difference in slope of the regression lines among the 4 groups (ANCOVA, P = .0001). The slope for group I (−17.8%/mmHg) was not

Table 1. Baseline Echocardiographic Data, Arterial Baroreflex Sensitivity, and Neurohumoral Profiles, and Changes in Body Weight in Control and in 3 Groups (based on LV end-diastolic volume, see text) of Conscious LV Dysfunction Dogs

		LV Dysfunction		
	Control* (n=24)	Group I (n=7)	Group II (n=7)	Group III (n=7)
Paced days	—	4.0 ± 0	7.6 ± 0.9	11.6 ± 1.2
ΔSV (%)	—	38 ± 7	87 ± 16$^{\Delta}$	133 ± 201§
ΔDV (%)	—	1 ± 1	22 ± 2$^{\Delta}$	44 ± 4$^{\Delta}$
LVEF (%)	57 ± 1	41 ± 5†	36 ± 4†	35 ± 3†
HR (beats/min)	104 ± 4	138 ± 1†	121 ± 1	136 ± 6†
MAP (mmHg)	120 ± 3	100 ± 1†	99 ± 4†	95 ± 6†
PE slope (ms/mmHg)	25.1 ± 2.7	27.4 ± 1.5	27.4 ± 6.2	33.7 ± 6.7
NTG slope (ms/mmHg)	9.2 ± 1.0	8.2 ± 2.2	6.1 ± 1.2	4.4 ± 0.9
PNE (pg/mL)	269 ± 41	413 ± 59	364 ± 60	310 ± 17
PRA (ng/mL/h)	1.77 ± 0.22	2.59 ± 0.54	3.54 ± 2.17	2.09 ± 0.91
AVP (pg/mL)	3.52 ± 0.14	4.01 ± 0.39	3.98 ± 0.44	3.97 ± 0.38
Body weight (%)	–	− 4 ± 1	− 3 ± 2	− 3.1

AVP, arginin vasopressin; ΔDV, % change in LV end-diastolic volume; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; PE slope, phenylephrine slope; PNE, plasma norepinephrine; PRA, plasma renin activity; ΔSV, % change in LV end-systolic volume.

*Control values were obtained from baseline (day=0) measurements in paced dogs.

$^{\dagger}P < .05$ vs control,

$^{\Delta}P < .05$ vs group I,

$^{\S}P < .05$ vs group II. Values are mean ± SEM.

different from that of controls (−17.7%/mmHg). However, the slope of group II (−5.8%/mmHg) was significantly reduced compared with controls and group I ($P = .038$). The slope of group III (−1.9%/mmHg) was reduced significantly compared with each of the other groups (all $P \leq .0003$). Bilateral vagotomy resulted in striking increases in baseline sympathetic nerve activity and abolished the renal sympatho-inhibition that resulted from volume expansion pre-vagotomy (data not shown). Cardiopulmonary baroreflex sensitivity correlated well (r=0.83, $P < .01$) with LV end-diastolic volume (Fig. 4). Cardiopulmonary baroreflex control correlated also with pulmonary artery pressure, PCWP, LV end-systolic volumes, and duration of pacing but with lower r values (<0.62 in all cases).

Discussion

The major novel findings of this study are as follows: 1) vagal cardiopulmonary baroreflex control of renal sympathetic nerve activity is blunted in dogs with LV dysfunction in the absence of HF; 2) these abnormalities become apparent when LV end-diastolic volume begins to increase and correlates well with the degree of LV diastolic dilatation; 3) when cardiopulmonary baroreflex abnormalities are first apparent, arterial baroreflex control of HR is preserved; and 4) cardiopulmonary baroreflex abnormalities become apparent even before significant increases in plasma norepinephrine, renin activity, and arginine vasopressin are apparent.

Cardiopulmonary Baroreflex Impairment in LV Dysfunction

Prolonged rapid ventricular pacing (4−6 weeks) in dogs produces low-output HF characterized by biventricular pump dysfunction, high cardiac filling pressures, cardiac dilatation, and signs of neurohumoral activation.[8,15−17] In our model, LV end-systolic volume started to increase very early (4 days), followed by increases in end-diastolic volume, which we detected after 1 week of pacing. These changes are consistent with findings reported by Komamura et al[18] using this model of rapid ventricular pacing, and indicate that progressive LV dilatation precedes the development of HF.

Significant increases in cardiac filling pressure were present in groups II and III, and cardiac output was decreased in all groups of paced dogs. Our data of decreased cardiac output in the anesthetized state are consistent with a previous report.[16] Shannon et al[19] showed no decrease in cardiac output in pacing-induced conscious HF dogs. This might be because of the difference in conscious versus anesthetized state in the 2 studies.

In our model there was no significant neurohumoral activation even though LV dysfunction had developed. Moe et al[17] showed in this model that plasma norepinephrine increased at 1 week of pacing, but plasma renin activity was not increased until severe HF developed (after 3 weeks). Riegger et al[20] reported that there was a trend toward increased arginine vasopressin after 2 weeks of rapid pacing. The differences in neurohumoral profiles, which we observed compared with those observed by other investigators,[17,20] could be because of the fact that we waited 10 minutes after cessation of pacing to ensure that the neurohumoral profiles reflected established neurohumoral excitation related to LV dysfunction rather than the independent effects of pacing alone. Our pilot data show that plasma norepinephrine levels during rapid pacing are higher than 10 minutes after cessation of pacing. Other groups[17,20] apparently drew samples during rapid pacing, although the precise time of collection was not stated explicitly. The lack of a rise in norepinephrine during pacing was not due to a training effect, because, in non-paced animals, plasma norepinephrine values were comparable to paced dogs and did not change over 11 days.

Vagal cardiopulmonary baroreflex sensitivity of group III in the current study was essentially the same as that of dogs

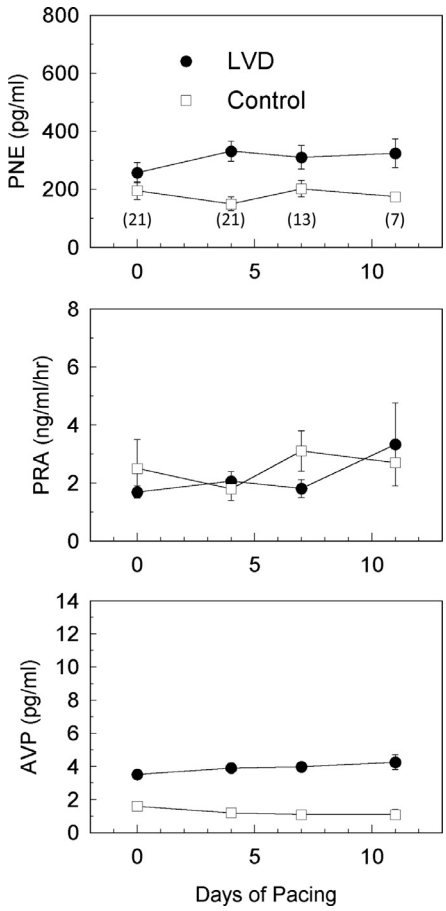

Fig. 2. Serial changes in plasma norepinephrine (PNE; pg/mL), plasma renin activity (PRA; ng/mL/h), and arginine vasopressin (AVP; pg/mL) in dogs with LV dysfunction because of rapid ventricular pacing. Measurements were made at baseline (day=0 [n=21]), and after 4 (n=21), 7 (n=13), and 11 (n=7) days of rapid ventricular pacing. Data shown in this figure are aggregate data, ie, data from all 3 groups (n=21) are included for day 4, data from groups II and III (n=13) are included for day 7, and data from only group III (n=7) are included at day 11. There were no significant changes in neurohumoral factors during the development of LV dysfunction.

with fully developed HF reported earlier from our laboratory (group III vs HF: −1.9%/mmHg vs −2.4%/mmHg).[6] These findings indicate that the vagal cardiopulmonary baroreflex abnormalities are fully manifest before clinical signs of HF develop. These data are in accordance with the

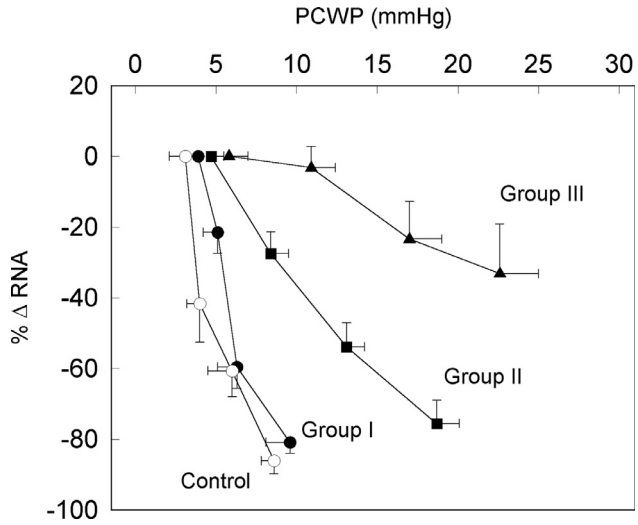

Fig. 3. Effects of volume infusion on PCWP (mmHg) and renal nerve activity (RNA; percentage change) in normal controls and in 3 groups with LV dysfunction. There was an overall difference in slope of regression lines relating percentage change in RNA to changes in PCWP among 4 groups (ANCOVA $P = .0001$). Slope for group I (solid circle) was not different from that of Control (open circle), whereas slopes for group II (solid square) and group III (solid triangle) were significantly less than that of Control (n=4 in Control, n=7 in groups I−III).

findings by Modesti et al[21] showing the impairment of cardiopulmonary baroreflex control of forearm vascular resistance in the early phase of HF in humans.

Possible Mechanisms of Vagal Cardiopulmonary Baroreflex Impairment

The experimental protocols used in this study do not allow us to determine the precise mechanism(s) for the abnormalities in cardiopulmonary baroreflexes in LV dysfunction; however, there are several possibilities. First, it is possible that there are structural and or functional changes of afferent mechanoreceptor sensory endings in cardiopulmonary regions related to chronic stretch. This speculation is consistent with the findings reported by DiBona and Sawin[22] who found diminished gain of the afferent limb of cardiopulmonary baroreflex control of renal sympathetic

Table 2. Baseline Hemodynamic Parameters in Normal Controls and in 3 Groups (based on LV end-diastolic volume, see text) of LV Dysfunction Dogs

		LV Dysfunction		
	Control (n=8)	Group I (n=7)	Group II (n=7)	Group III (n=7)
CO (L/min)	1.93 ± 0.23	1.30 ± 0.09*	1.33 ± 0.14*	1.41 ± 0.06*
PCWP (mmHg)	3.4 ± 0.7	4.7 ± 1.1	10.1 ± 2.0*	13.1 ± 2.2*Δ
MPA (mmHg)	11.9 ± 0.9	12.4 ± 1.0	17.9 ± 2.4	26.0 ± 2.3*Δ§
RAP (mmHg)	0.6 ± 0.7	2.0 ± 0.9	4.3 ± 0.7*	6.5 ± 1.8*Δ
MAP (mmHg)	135 ± 5	110 ± 4*	102 ± 9*	101 ± 8*

CO, cardiac output; MAP, mean arterial pressure; MPA, mean pulmonary artery pressure; RAP, right atrial pressure. All measurements were made in sinus rhythms.

*$P < .05$ vs control,

Δ$P < .05$ vs group I,

§$P < .05$ vs group II. Values are mean ± SEM.

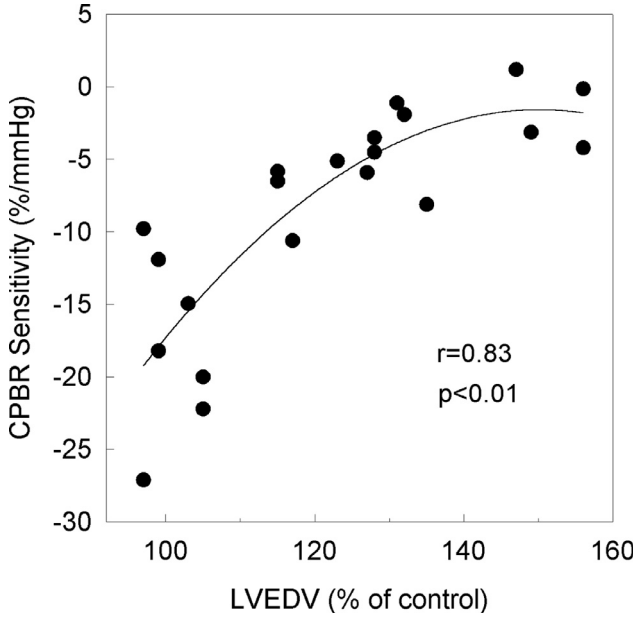

Fig. 4. Graph showing the relationship between cardiopulmonary baroreflex (CPBR) sensitivity (%/mmHg) and LV end-diastolic volume (LVEDV; percentage of control). Each estimation of cardiopulmonary baroreflex sensitivity of all LV dysfunction dogs (n=21) is plotted against LVEDV. A second-order polynomial curve fit was used to obtain curve fitting. Cardiopulmonary baroreflex sensitivity correlated well with LVEDV.

nerve activity in rat model of HF. Second, it is possible that there are alterations in the compliance of the cardiac walls that contain these mechanoreceptors. The fact that volume infusion raised PCWP of dogs with LV dysfunction (groups II and III) to more than twice that of the control group supports this possibility. Data from Pliquett et al[23] suggest that the probable explanation for the impaired responses is due to alterations at the receptor level and not because of changes in cardiac compliance. Our data do not exclude a role for central neural abnormalities controlling the efferent pathways of the reflex arc. Another possible explanation for impaired vagal cardiopulmonary baroreflexes during volume expansion is augmented activation of cardiopulmonary sympathetic afferents in HF, which could result in sympatho-excitation. Although our data do not exclude this possibility, we have previously reported[6] that in dogs with pacing-induced HF, neither volume expansion nor inflation of balloons at the junctions of the pulmonary veins and left atrium induced sympatho-excitation after vagotomy. We suggest that activation of excitatory mechanisms is unlikely to account for the profound impairment in vagal cardiopulmonary baroreflex control of renal sympathetic nerve activity that we observed in the current study.

Abnormal Vagal Cardiopulmonary Baroreflexes Precede Impairment of Arterial Baroreflex Control of Heart Rate

Arterial baroreflex control of HR is impaired both in humans with HF[2,24] and in experimental models of HF.[3,15] Baroreflex control of HR in our model assessed by the phenylephrine bolus technique is preserved until late in the course of developing HF.[4] Data from Grima et al[25] showed that the baroreflex-mediated HR responses to nitroprusside-induced hypotension were impaired after 1 week of rapid ventricular pacing in the course of developing HF. In our study, nitroglycerin slope tended to be depressed during this period though was not statistically significant ($P = .08$). Our findings are thus consistent with the results of other studies[4,5,25] in that the bradycardia response is preserved until signs of HF are fully developed, whereas the tachycardia response has a tendency to be depressed early in the course of developing HF. In this study of dogs with LV dysfunction, we observed preservation of baroreflex-mediated bradycardia in response to phenylephrine, but a tendency for the baroreflex-mediated tachycardia in response to nitroglycerin (NTG) to be reduced. These observations are consistent with those reported by Brandle et al.[26] The impairment in the responses to NTG was statistically significant in their study, but their data were obtained in dogs that had progressed from LV dysfunction (our study) to frank HF (their study). Reflex tachycardia in response to NTG is normally due to withdrawal of vagal parasympathetic influence plus an increase in sympathetic influence.[27] Kinugawa and Dunlap[4] and Ishise et al[28] have demonstrated that basal parasympathetic influence is already reduced after only 4 days of pacing, resulting in less parasympathetic influence to be withdrawn in response to NTG. This is reflected in the higher resting HR of paced dogs in the basal state. In our dogs, reduced resting parasympathetic influence probably accounted for most of the increase in resting HR in the paced dogs. The absence of an increase in plasma norepinephrine in the paced group would suggest a more modest contribution of sympathetic activity to the higher resting HRs observed in these dogs. Bradycardia in response to phenylephrine is mainly because of vagal parasympathetic activation plus sympathetic withdrawal. Our data (and those of Brandle et al[26]) support the view that recruitment of parasympathetic influences is preserved during the development of LV dysfunction (our study) and even after the development of frank clinical HF (data of Brandle et al[26]), even though resting parasympathetic influence is markedly decreased.

Abnormal Cardiopulmonary Baroreflexes Precede Neurohumoral Excitation in LV Dysfunction

Abnormalities in vagal cardiopulmonary baroreflexes *preceded* the development of the neurohumoral excitatory state in the course of developing LV dysfunction, thus supporting the hypothesis that abnormalities in cardiopulmonary baroreflexes could contribute to the subsequent development of the neurohumoral excitatory state seen in later stages of LV dysfunction and HF. Note that the weights of our paced dogs did not increase during pacing (Table 1) and there was no evidence of ascites or shortness of breath in these dogs during this brief period of pacing.

If cardiopulmonary baroreflex abnormalities contribute to the development of neurohumoral excitation, then why

were there no increases in neurohumoral factors even though cardiopulmonary baroreflexes were already impaired in our model? This could be because of the known redundancy in the control of sympathetic nerve activity by arterial and cardiopulmonary baroreflexes,[29] and we found that arterial baroreflex control of HR was preserved in dogs with LV dysfunction. We have also reported previously[13] that arterial baroreflex control of renal sympathetic nerve activity is preserved in fully developed HF in the pacing model. Thus, neurohumoral excitation may not become apparent until both arterial and vagal cardiopulmonary baroreflexes become markedly abnormal. We do acknowledge that neither our findings nor those of others establishes a causal role for arterial or vagal cardiopulmonary baroreflexes in the neurohumoral excitatory state of HF.

Brandle et al[30] examined the responses of plasma norepinephrine to rapid ventricular pacing in neurally intact dogs and in dogs with sinoaortic denervation, ie, with reduction or elimination of redundancy of sympathetic control. They measured plasma norepinephrine levels pre-pacing and after 1, 2, 3, and 4 weeks of pacing. They found increases in plasma norepinephrine in both groups, and thus concluded that the arterial baroreflex is not the sole mechanism for the increase in sympathetic drive in HF. Our findings support this view and suggest that vagal cardiopulmonary reflexes may play a key role in mediating the neurohumoral excitation associated with HF. We are unable to fully account for the differences between our findings and theirs in terms of the increase in plasma norepinephrine in their dogs with intact sinoaortic baroreflexes during the first 2 weeks of pacing. It should be noted, however, that in their intact dogs, plasma norepinephrine was nearly the same as controls 10 minutes after cessation of pacing after 2 weeks of pacing and are consistent with our findings.

Although our findings regarding arterial baroreflex control of HR were similar to other groups using this model of LV dysfunction, our studies were unique in that we correlated those findings to alterations in cardiopulmonary baroreflexes at early stages of LV dysfunction, experiments that were technically more difficult to perform than others because of their invasive (and terminal) nature.

Limitations

We acknowledge several limitations in this study. First, although the rapid pacing model of LV dysfunction has features representative of dilated cardiomyopathy in humans, tachycardia-induced cardiomyopathy represents only a small subset of patients with dilated cardiomyopathy, and it is possible that some of these abnormalities could be model-dependent. Second, our data suggest that as LV dysfunction progresses, increases in filling pressure are necessary to maintain cardiac output, but we could not determine specifically if vagal cardiopulmonary baroreflexes modify this relationship and how it was affected by LV dysfunction and altered baroreflex sensitivity.

Conclusion

The principal finding of this study is that cardiopulmonary baroreflex control of renal sympathetic nerve activity is abnormal in dogs with LV dysfunction in the absence of HF. Our study documents the time course of the development of this abnormality, and its relationship to the development of left ventricular dysfunction. This abnormality became apparent when LV end-diastolic volume started to increase and correlated well with the degree of LV diastolic dilatation. Cardiopulmonary baroreflex abnormalities precede the development of the neurohumoral excitatory state and abnormal arterial baroreflexes, and may contribute to the subsequent development of the neurohumoral excitatory state and to the progression from LV dysfunction to clinical HF. Our findings may have special significance in light of recent observations that HF symptoms may often be the result of splanchnic sympatho-excitation and shifts of volume from the splanchnic venous capacitance into the cardiopulmonary region.[31] In normal subjects, vagal cardiopulmonary baroreflexes would be expected to detect this shift, inhibit this sympathetic activation, and restore venous compliance and increase splanchnic venous capacitance. Our data fit with the concept that abnormal vagal cardiopulmonary baroreflexes, which develop as LV function deteriorates, may be unable to "protect" the central circulation from these sympathetically mediated volume shifts, potentially leading to elevated filling pressures at early stages of LV dysfunction and prior to reductions in cardiac output.

Acknowledgements

The authors thank Janis Malycky, Will Austin, and Itaf Kakhry for their technical assistance.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2019.08.012.

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