Functional Effects of Endogenous Bradykinin in Congestive Heart Failure

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Objectives. The purpose of this study was to determine the level and functional effects of endogenous bradykinin in congestive heart failure (CHF).

Background. There is experimental evidence that bradykinin is increased in several cardiac disease states. However, it is unknown whether plasma levels of bradykinin are elevated in CHF. Further, the cardiac and vascular responses to bradykinin in CHF are unclear.

Methods. The circulating levels of bradykinin and the effects of endogenous bradykinin were assessed in eight instrumented, conscious dogs both before and after pacing-induced CHF.

Results. Before CHF, the plasma bradykinin level was 53.1 ± 12.4 pg/ml. Blocking endogenous bradykinin with HOE-140 (0.3 mg/kg), a specific bradykinin B_2-receptor antagonist, produced no significant alterations in heart rate, left ventricular (LV) end-systolic pressure (P_{es}), total systemic resistance (TSR), the time constant of LV relaxation (tau) or the maximal rate of LV filling (dV/dt_{max}). However, coronary blood flow was significantly reduced ($p < 0.05$). LV contractile performance measured by the slopes of pressure–volume relations was unaffected. After induction of CHF, the plasma bradykinin level increased to 234.2 ± 19.4 pg/ml ($p < 0.05$). Blocking endogenous bradykinin with HOE-140 reduced coronary blood flow and produced significant increases in P_{es} and TSR, prolonged tau, decreased dV/dt_{max} and elevated minimal LV pressure and mean left atrial pressure. Furthermore, the slopes of pressure–volume relations ($p < 0.05$) were decreased, indicating depressed contractility with HOE-140 after CHF.

Conclusions. Before CHF, endogenous bradykinin results in coronary dilation but has no effect on systemic arterial vasodilation or cardiac performance. After CHF, endogenous bradykinin is significantly increased and, acting through B_2-receptors, produces coronary and arterial vasodilation and improves LV relaxation and contractile performance. Thus, endogenous bradykinin may play an important role in preserving cardiovascular function in CHF.

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There is neurohormonal activation in congestive heart failure (CHF) (1,2). This stimulation of the renin–angiotensin system, norepinephrine, vasopressin and endothelin may help acutely compensate for impaired cardiac function. However, the long-term activation of these systems has adverse consequences, including vasoconstriction (1,3–6), sodium retention and cardiac remodeling (7,8). In addition, angiotensin II may also produce direct depression of left ventricular (LV) contraction and relaxation in CHF (9). In contrast, activation of the kallikrein–kinin system, if it occurs in CHF, may be beneficial. Bradykinin reduces both systemic and coronary resistance (10–13), has positive inotropic and lusitropic effects (14–16) and is a cardioprotective agent that may decrease myocardial oxygen consumption and ischemia (17,18). If bradykinin is increased in CHF, it may help counteract some of the detrimental effects of sympathetic and renin–angiotensin system activation. There is experimental evidence that bradykinin (11,18–22) is increased in cardiac ischemia (23,24), myocardial infarction (25,26), inflammation, hypotension (27) and shock (28). However, it is unknown whether plasma levels of bradykinin are elevated in CHF. Further, the cardiac and vascular responses to bradykinin have not been assessed in CHF.

Thus, we hypothesized that circulating bradykinin is increased in CHF and has a beneficial effect on cardiac function. We tested this hypothesis by measuring bradykinin levels and determining the consequences of blocking the cardiovascular effects of endogenous bradykinin in conscious dogs before and after pacing-induced CHF.

Abbreviations and Acronyms

CHF = congestive heart failure
dP/dt = rate of rise of left ventricular pressure
dV/dt_{max} = maximal rate of left ventricular filling
E_a = arterial elastance
LA = left atrial or atrium
LV = left ventricular or ventricle
P_{ed} = left ventricular end-diastolic pressure
P_{es} = left ventricular end-systolic pressure
SV = stroke volume
TSR = total systemic resistance

Methods

Instrumentation. This investigation conformed with the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH publication no. 86-25, revised 1985).

Eight healthy, adult, heart-worm negative, mongrel dogs (weight 25 to 34 kg) were instrumented under anesthesia induced with xylazine (2 mg/kg body weight intramuscularly) and sodium thiopental (6 mg/kg intravenously) and maintained with halothane (0.5% to 2%). They were intubated and ventilated with oxygen-enriched room air to maintain an arterial oxygen tension >100 mm Hg and pH between 7.38 and 7.42. A left lateral thoracotomy was performed aseptically. Micromanometer pressure transducers (Konigsberg Instruments) and polyvinyl catheters for transducer calibration (internal diameter 1.1 mm) were inserted into the LV and left atrium (LA). Three pairs of ultrasonic crystals (5 MHz) (Custom Transducers) were implanted in the endocardium of the LV to measure the anterior-posterior, septal-lateral and base-apex (long-axis) dimensions, using previously described methods (29). Hydraulic occluded cuffs were placed around the superior and inferior venae cavae. Two 54-cm sutureless myocardial leads (model 4312, Cardiac Pacemakers, Inc.) were implanted in the myocardium of the right ventricle and right atrium, and the leads were attached to an unipolar multiprogrammable pacemaker (model 8329, Medtronics, Inc.) positioned under the skin of the chest. Two ultrasonic time-transit flow probes (model 2R or 3R Transonic Systems, Inc.) were placed on the proximal left circumflex and anterior descending coronary arteries. The wires and tubing were tunneled subcutaneously and brought out through the skin of the neck.

Data collection and calculation. Studies were performed after full recovery from instrumentation (~2 weeks after the initial operation), with the unsedated dogs lying on their right sides and in a sling. The LV and LA catheters were connected to pressure transducers (Statham P23DB) calibrated with a mercury manometer. The signal from the micromanometer was adjusted to match that of the catheters. The transit time of 5-MHz sound between the crystal pairs was determined and converted to distance, assuming a constant velocity of sound in blood of 1.55 m/ms. The analog signals were digitized with an on-line analog-to-digital converter (Data Translation Devices) at 200 Hz, using the SPECTRUM program (Wake Forest University School of Medicine). Each data acquisition lasted for 15 to 20 s, spanning several respiratory cycles. The derivatives of LV pressure and volume were calculated using the five-point Langrangian method (30).

Experimental protocol. *Studies before CHF: effect of HOE-140.* We determined the effects of endogenous bradykinin by using HOE-140. This agent is a potent, stable and specific antagonist of the kinin beta$_2$-receptor (11,31–33). We confirmed the adequacy of bradykinin blockade produced by the dose of HOE-140 used in this study (0.3 mg/kg intravenously) in a separate group of six conscious, instrumented animals (14). Before HOE-140, the infusion of bradykinin (6 µg/kg plus 6 to 18 µg/kg per min intravenously) produced a decrease in LV end-systolic pressure (P_{es}) (100 ± 14 to 81 ± 1 mm Hg, p < 0.05) and significant (p < 0.05) increases in heart rate, stroke volume (SV) and rate of LV relaxation, as well as improved LV contractile performance. All of these hemodynamic effects of bradykinin were completely blocked by pretreatment with HOE-140 (0.3 mg/kg intravenously).

To obtain baseline values, data were initially recorded with the study animals lying quietly on their sides without medication. Three sets of variably loaded pressure–volume loops were generated by sudden, transient occlusion of the cavae. This caused a progressive fall in LV end-systolic pressure and volume over a 15-s recording period. Immediately after the recording period, the caval occlusion was released and hemodynamic variables were allowed to restabilize. After all variables returned to their baseline level, 0.3 mg/kg of HOE-140 was infused intravenously. The steady-state and transient caval occlusion data were collected 5 to 10 min after the infusion when a stable effect was present.

Studies during development of CHF. After completion of the baseline studies, the pacing rate was adjusted, using the external magnetic control unit, to 200 to 250 beats/min. Three times per week the pacemaker rate was deactivated. The animals were allowed to equilibrate for 30 min and then data were collected. The pacing rate was returned to 200 to 250 beats/min. The pacing period lasted for 4 to 5 weeks. When the LV end-diastolic pressure (P_{ed}) during the nonpaced period had increased by >15 mm Hg over the prepacing control level and the animals had begun to show clear evidence of CHF (anorexia, ascites and pulmonary congestion), the pacing was discontinued and CHF data were obtained.

Studies after onset of CHF. The pacemaker was turned off and the animals were allowed to stabilize for at least 30 min. Data were recorded with the animals lying quietly on their sides. Then the aforementioned protocols were repeated as before CHF.

Data processing and analysis. To account for respiratory changes in intrathoracic pressure, steady-state measurements were averaged over a 15-s recording period that spanned multiple respiratory cycles (29). The stored digitized data were analyzed using the SPECTRUM program.

The LV volume was calculated as a modified general ellipsoid using the equation $V_{LV} = (\pi/6)D_{AP}D_{SL}D_{LA}$, where

V_{LV} = volume of LV; D_{AP} = anterior-posterior LV dimension; D_{SL} = septal-lateral LV dimension; and D_{LA} = long-axis LV dimension. This method of volume calculation gives a consistent measure of LV volume despite changes in LV loading conditions, chamber configuration and inotropic state (34).

Stroke work was calculated by point-by-point integration of the LV pressure–volume loop for each beat, as described by Glower et al. (35). SV was calculated as LV end-diastolic volume minus end-systolic volume. The rate of LV relaxation was analyzed by determining the time constant of the isovolumic fall of LV pressure. LV pressure, from the time of peak (−) rate of rise in LV pressure (dP/dt) until mitral valve opening, was fit to an exponential equation $P = P_A \exp(-t/\tau), + P_B$, where P = LV pressure; t = time; and P_A, P_B and τ are constants determined by the data. Although the fall in isovolumic pressure is not exactly exponential (36), the time constant, derived from the exponential approximation, provides an index of the rate of LV relaxation (37). The total systemic resistance (TSR) was estimated as P_{es}/cardiac output, as suggested by Sunagawa et al. (38). The arterial elastance (E_a) was calculated as P_{es} divided by SV.

Only caval occlusions that produced a fall in P_{es} of at least 30 mm Hg were analyzed. Premature beats and subsequent beats were excluded from the analysis. The LV end-systolic pressure and volume data during the fall in LV pressure, produced by each caval occlusion, were fit using the least-squares technique to

$$P_{es} = E_{es} (V_{es} - V_{0,es}),$$

where E_{es} = slope of the linear end-systolic pressure–volume relation, representing the LV end-systolic elastance; and $V_{0,es}$ = intercept with the volume axis. The volume (V_{100}) associated with a P_{es} of 100 mm Hg was calculated as follows:

$$V_{100,es} = V_{0,es} + 100/E_{es}.$$

The dP/dt_{max}–end-diastolic volume (V_{ed}) and stroke work–end-diastolic volume (SW − V_{ed}) relations were quantitated by fitting the data from the same beats from each caval occlusion used to evaluate the P_{es}–V_{es} relation to

$$dP/dt_{max} = dE/dt_{max}(V_{ed} - V_{0,dP/dt}),$$

where dE/dt_{max} = slope of the dP/dt_{max}–V_{ed} relation, M_{SW} = slope of the SW–V_{ed} relation, and

$$SW = M_{SW}(V_{ed} - V_{0,SW}).$$

The positions of the dP/dt_{max}–V_{ed} and SW–V_{ed} relations were calculated by determining the V_{ed} associated with a dP/dt of 1,000 mm Hg/s and SW of 1,000 mm Hg/ml:

$$V_{1,000,dP/dt} = V_{0,dP/dt} + 1,000/(dE/dt_{max}),$$

$$V_{1,000,SW} = V_{0,SW} + 1,000/M_{SW}$$

Plasma bradykinin determinations. The plasma bradykinin levels were determined from arterial blood samples before and after CHF. Ten milliliters of blood was withdrawn from the LA catheter into a chilled preservative tube containing 5 ml

of an inhibitor mixture of aprotinin (10,000 kallikrein inhibitor units per milliliter), phenanthroline (10 mg/ml), EDTA (20 mg/ml), trypsin inhibitor (0.8 mg/ml) and hexadimethrine bromide (0.4 mg/ml). The samples were mixed with acetone and immediately separated by centrifugation at 3,000 rpm for 10 min. Then a 5-ml plasma extract was transferred into a tightly sealed, screw-capped polypropylene vial and stored upright in dry ice. After labeling the vials, the specimens, frozen in dry ice, were shipped to the Inter Science Institute.

Bradykinin was measured by the Inter Science Institute using a radioimmunoassay kit according to the procedures described by Jauch et al. (31) and Vavrek and Stewart (40), with modifications made by the Inter Science Institute (41), using a specific rabbit antibody directed against bradykinin-bovine serum albumin. The specificity of the antibody precludes the necessity of further purification before the radioimmunoassay test of specimens. The sensitivity of the assay, determined as the least amount of bradykinin that can be distinguished from zero, is 10 pg/ml. There is <1% cross reactivity with angiotensin I and II, arginine vasopressin and endothelin. The interassay variabilities are 13%, 7% and 7.9% at bradykinin levels of 38, 210 and 430 pg/ml, respectively. The interassay variabilities are 16%, 8% and 9% at bradykinin levels of 40, 220 and 420 pg/ml, respectively.

Statistical analysis. Variables of LV function were compared before and after CHF, before and after drug use, using the Student t test for paired observations and analysis of variance with the Bonferroni correction for multiple-paired comparisons, as appropriate. The slopes, volume–axis intercepts and positions in the physiologic range of each of the three relations for each condition were evaluated as the mean values of the two or three caval occlusions performed under each condition. Significance was accepted at $p < 0.05$. Steady-state data were expressed as the mean value ± SD; values for LV pressure–volume relations were expressed as the mean value ± SE.

Postmortem evaluation. At the conclusion of the studies, the animals were killed by lethal injection of sodium pentobarbital (100 mg/kg intravenously), and the hearts were examined to confirm the proper instrumentation.

Results

Effects of pacing-induced CHF. The plasma bradykinin concentration was 53.1 ± 12.4 pg/ml before CHF and increased to 234.2 ± 19.4 pg/ml ($p < 0.05$) after CHF. Steady-state measurements before and after CHF are summarized in Table 1. LV pressure–volume analyses before and after CHF are summarized in Table 2. After 4 to 5 weeks of rapid pacing, P_{ed}, LV end-systolic and end-diastolic volume, minimal LV pressure, mean LA pressure, the time constant of LV relaxation (tau), TSR and E_a all significantly increased, whereas SV, the peak rate of mitral flow (dV/dt_{max}), minimal LV pressure and cardiac output were all significantly decreased.

After 4 to 5 weeks of rapid pacing, the slopes of the LV end-systolic pressure–volume relation, the dP/dt_{max}–V_{ed} rela-

Table 1. Effect of HOE-140 on Steady-State Hemodynamic Data Before and After Pacing-Induced Congestive Heart Failure in Eight Dogs

	Before CHF		After CHF	
	Control	HOE-140	Control	HOE-140
Heart rate (beats/min)	108 ± 6	106 ± 8	115 ± 9	120 ± 18
Peak $(+)$ dP/dt (mm Hg/s)	$2,737 \pm 223$	$2,735 \pm 210$	$1,699 \pm 157$†	$1,678 \pm 189$
Peak $(-)$ dP/dt (mm Hg/s)	$-2,118 \pm 139$	$-2,093 \pm 114$	$-1,519 \pm 122$†	$-1,501 \pm 129$
LV end-diastolic pressure (mm Hg)	9.0 ± 1.7	9.1 ± 1.6	24.2 ± 1.7†	29.1 ± 2.7‡
LV end-systolic pressure (mm Hg)	102 ± 12	103 ± 13	98 ± 8	104 ± 7‡
Minimal LV pressure (mm Hg)	0.5 ± 3.4	0.6 ± 3.6	10.6 ± 4.5†	12.9 ± 2.8‡
Mean LA pressure (mm Hg)	5.5 ± 3.4	5.7 ± 3.3	17.9 ± 5.1†	19.4 ± 5.6‡
End-diastolic volume (ml)	40.4 ± 7.0	40.7 ± 6.8	50.2 ± 5.1†	50.6 ± 5.7
End-systolic volume (ml)	26.7 ± 4.9	26.9 ± 5.0	39.4 ± 3.9†	40.8 ± 3.7‡
Stroke volume (ml)	13.7 ± 3.2	13.8 ± 3.1	10.8 ± 1.9†	9.8 ± 2.0‡
E_a (mm Hg/ml)	7.5 ± 1.1	7.6 ± 1.6	10.5 ± 2.2†	12.2 ± 2.9‡
TSR (mm Hg/ml per min)	0.068 ± 0.013	0.070 ± 0.013	0.096 ± 0.024†	0.110 ± 0.032‡
Tau (ms)	25.5 ± 3.0	26.1 ± 4.1	43.5 ± 3.1†	47.2 ± 3.1‡
Maximal dV/dt (ml/s)	222 ± 35	219 ± 47	184 ± 42	146 ± 39
Mean coronary blood flow (ml/beat)	0.30 ± 0.07	0.26 ± 0.06*	0.31 ± 0.16	0.27 ± 0.14‡

*$p < 0.05$ compared with control value before CHF. †$p < 0.05$ compared with control value before CHF. ‡$p < 0.05$ compared with control value after CHF. Data are presented as mean value $\pm$ SD. CHF = congestive heart failure; dP/dt = rate of rise of left ventricular pressure; dV/dt$_{max}$ = peak rate of mitral flow; E_a = arterial elastance; LA = left atrial; LV = left ventricular; tau = time constant of LV relaxation; TSR = total systemic resistance.

tion and the SW–V_{ed} relation all significantly decreased and were shifted toward the right, indicating a depression of LV contractile performance. These observations are consistent with our previous reports (9,42) demonstrating that long-term, rapid pacing produces cardiac dilation, impairment of LV systolic and diastolic function with clinical evidence of pulmonary congestion and ascites.

Effect of HOE-140. The steady-state hemodynamic data during control and HOE-140 infusion before and after CHF are summarized in Table 1 and displayed in Figure 1. Before CHF, HOE-140 caused no significant alterations in heart rate (108 ± 6 vs. 106 ± 8 beats/min), P_{es} (102 ± 12 vs. 103 ± 13 mm Hg), TSR (0.068 ± 0.013 vs. 0.070 ± 0.013 mm Hg/ml per min), mean LA pressure (5.5 ± 3.4 vs. 5.7 ± 3.3 mm Hg),

minimal LV pressure (0.5 ± 3.4 vs. 0.6 ± 3.6 mm Hg), tau (25.5 ± 3.0 vs. 26.1 ± 4.1 ms) and dV/dt$_{max}$ (222 ± 35 vs. 219 ± 47 ml/s). As shown in Figure 2, the contractile performance measured by the slopes of the P_{es}–V_{es} (E_{es}) (6.8 ± 0.6 vs. 6.7 ± 0.6 mm Hg/ml, p = NS), dP/dt$_{max}$–end-diastolic volume (dE/dt$_{max}$) (80.5 ± 3.9 vs. 80.1 ± 4.7 mm Hg/s per ml, p = NS) and stroke work–V_{ed} (M_{SW}) (80.8 ± 5.3 vs. 80.3 ± 4.9 mm Hg, p = NS) relations were also unaffected. However, with HOE-140, coronary blood flow was significantly reduced (0.30 ± 0.07 vs. 0.26 ± 0.06 ml/beat, $p < 0.05$) due to the significant increase in coronary vascular resistance.

After CHF (Tables 1 and 2, Fig. 1 and 3), HOE-140 exacerbated both the LV systolic and diastolic dysfunction present with CHF. After CHF, HOE-140 produced a signifi-

Table 2. Effect of HOE-140 on the P_{es}–V_{es}, dP/dt$_{max}$–V_{ed} and SW–V_{ed} Relations Before and After Pacing-Induced Congestive Heart Failure in Eight Dogs

	P_{es}–V_{es} Relation				dP/dt$_{max}$–V_{ed} Relation			SW–V_{ed} Relation		
	E_{es}	$V_{0,es}$	r	$V_{100,es}$	dE/dt$_{max}$	$V_{0,dP/dt}$	$V_{1,000,dP/dt}$	M_{SW}	$V_{0,SW}$	$V_{1,000,SW}$
					Before CHF					
Control	6.8 ± 0.6	10.2 ± 1.9	0.994 ± 0.01	25.4 ± 2.0	80.5 ± 3.9	6.4 ± 0.8	19.0 ± 1.3	80.8 ± 5.3	20.1 ± 1.6	32.8 ± 1.6
HOE-140	6.7 ± 0.6	10.1 ± 2.3	0.981 ± 0.01	25.6 ± 2.0	80.1 ± 4.7	6.2 ± 3.4	18.9 ± 3.6	80.3 ± 4.9	20.0 ± 1.8	32.6 ± 1.6
					After CHF					
Control	5.3 ± 0.7†	9.1 ± 2.0	0.956 ± 0.02	29.9 ± 3.5*	51.2 ± 2.7*	8.2 ± 1.9	28.0 ± 2.5*	59.6 ± 2.6*	19.0 ± 1.5	36.5 ± 1.8*
HOE-140	4.6 ± 0.5‡	7.9 ± 1.1‡	0.975 ± 0.01	32.6 ± 3.0†	40.2 ± 3.9†	5.5 ± 2.4†	31.9 ± 2.1†	47.5 ± 2.5†	17.1 ± 1.8†	38.8 ± 2.8†

*$p < 0.05$ compared with control value before CHF. †$p < 0.05$ compared with control value after CHF. Data are presented as mean value $\pm$ SE. dE/dt$_{max}$ = slope of dP/dt$_{max}$–V_{ed} relation; E_{es} = slope of linear P_{es}–V_{es} relation; M_{SW} = slope of stroke work–end-diastolic volume relation; P_{es} = end-systolic pressure; SW = stroke work; V_{ed} = end-diastolic volume, V_{es} = end-systolic volume; $V_{0,es}$ = intercept with volume axis; $V_{100,es}$ = volume associated with P_{es} of 100 mm Hg; $V_{0,dP/dt}$ = intercept with volume axis; $V_{1,000,dP/dt}$ = volume associated with dP/dt of 1,000 mm Hg/s; $V_{0,SW}$ = intercept with volume axis; $V_{1,000,SW}$ = volume associated with SW of 1,000 mm Hg/ml.
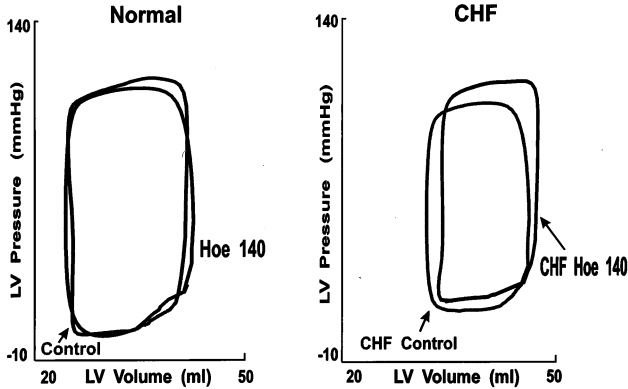
Figure 1. An example of the effect of HOE-140 on LV pressure–volume loops. Each loop was generated by averaging the data obtained during a 15-s recording period, spanning several respiratory cycles. Before CHF (**left**), the LV pressure–volume loops were relatively unchanged after HOE-140. In contrast, after CHF (**right**), HOE-140 caused an increase in P_{es} and the pressure–volume loops were markedly shifted upward.

cant increase in P_{es} (98 ± 8 vs. 104 ± 7 mm Hg, p < 0.05), with associated increases in TSR (0.096 ± 0.024 vs. 0.110 ± 0.032 mm Hg/ml per min, p < 0.05) and Ea (10.5 ± 2.2 vs. 12.2 ± 2.9 mm Hg/ml, p < 0.05), whereas the heart rate was relatively unchanged. Tau was markedly prolonged (43.5 ± 3.1 vs. 47.2 ± 3.1 ms, p < 0.05), and dV/dt_{max} (184 ± 42 vs. 146 ± 39 ml/s, p < 0.05) was reduced due to an elevated minimal LV pressure, despite an increase in mean LA pressure (17.9 ± 5.1 vs. 19.4 ± 5.6 mm Hg, p < 0.05). Furthermore, as demonstrated in Figure 3, the slopes of LV pressure–volume relations decreased: E_{es} (5.3 ± 0.7 vs. 4.6 ± 0.5 mm Hg/ml, p < 0.05), dE/dt_{max} (51.2 ± 2.7 vs. 40.2 ± 3.9 mm Hg/s per ml, p < 0.05)

Figure 2. Left ventricular pressure–volume relations determined from one conscious dog before CHF. Before CHF, HOE-140 produced a slight parallel rightward shift of the end-systolic pressure–volume relation (**top graph**). Both the slopes and positions of dP/dt_{max}–end-diastolic volume (**bottom left graph**) and stroke work–end-diastolic volume (**bottom right graph**) relations were unchanged with HOE-140, indicating that before CHF, blocking endogenous bradykinin with HOE-140 had no inotropic effect on LV contractile performance.
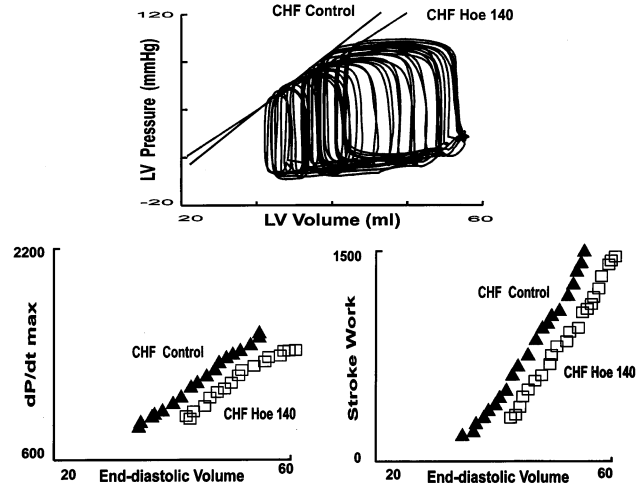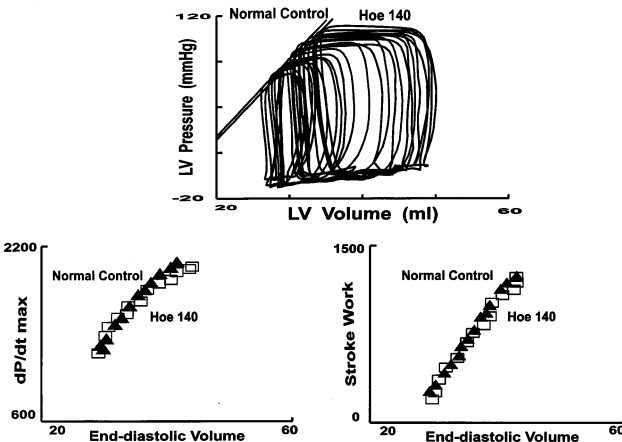

Figure 3. Left ventricular pressure–volume relations determined from the same conscious animal as in Figure 2 after CHF. After CHF, HOE-140 produced rightward shifts of the end-systolic pressure–volume, dP/dt_{max}–end-diastolic volume and stroke work–end-diastolic volume relations, with decreased slopes. This indicates that blocking endogenous bradykinin in CHF with HOE-140 caused decreased LV contractile performance, which is in contrast to the effect of HOE-140 before CHF.

and M_{SW} (59.6 ± 2.6 vs. 47.5 ± 2.5 mm Hg, p < 0.05). This indicates depressed contractility.

Discussion

We found that plasma levels of bradykinin are elevated fourfold in conscious animals with pacing-induced CHF. This elevation of endogenous bradykinin stimulates B_2-receptors, producing both coronary and systemic arterial vasodilation and improving LV relaxation, LV filling and LV contractile performance. These data suggest that endogenous bradykinin may help blunt the detrimental functional consequences of the activation of other neurohormonal systems in CHF.

Measurements of normal plasma bradykinin levels have varied owing to the short half-life of bradykinin in the blood and its easy destruction during extraction procedures (23,43). In the current study, we used the procedures described by Jauch et al. (39), with modifications made by the Inter Science Institute (41). Both destruction and activation of kinins were arrested immediately in samples of blood, and the extraction procedure allowed the active kinins to be concentrated. We found plasma bradykinin levels of 53.1 ± 12.4 pg/ml in normal conscious dogs, similar to the measurements in normal humans (39,44,45). After CHF, the plasma bradykinin levels increased more than fourfold (234.2 ± 19.4 pg/ml). The increase in bradykinin with CHF was far higher than the intrasample and intersample variability of the bradykinin assay. Furthermore, the increase in bradykinin was not due to cross reactivity of the assay with other neurohormones that are elevated in CHF. The elevated plasma bradykinin in CHF may be due to cytokine stimulation with CHF. In addition, aldosterone also triggers

kallikrein activation (20). Thus, the result of elevated circulating bradykinin in CHF may at least partially be stimulated by activation of the renin-angiotensin system in CHF. It is also possible that a decrease in bradykinin breakdown may also contribute to the elevated bradykinin levels we observed in CHF.

Bradykinin is a potent vasodilator. Consistent with previous observations in normotensive rats (46) and normal humans (11), we found that blocking endogenous bradykinin with HOE-140 produced a reduction in coronary blood flow, with a corresponding increase in coronary vascular resistance. We also observed a similar response to HOE-140 in dogs with CHF, suggesting that endogenous bradykinin plays a role in the regulation of coronary circulation in both normal and CHF states.

Although bradykinin has a potent vasodilatory effect, we found that blocking the effect of the low level of bradykinin (53.1 ± 12.4 pg/ml) in normal animals had no detectable effect on systolic pressure or systemic arterial resistance. This is consistent with the observation in normal humans that the threshold concentration of bradykinin to reduce arterial pressure is at least 100 pg/ml (45). After CHF, the bradykinin level in our animals was 234.2 ± 19.4 pg/ml, far exceeding this threshold. Blocking bradykinin B_2-receptors after CHF increased arterial pressure and resistance. These data imply that endogenous bradykinin does not normally play a role in the regulation of rest arterial blood pressure; however, after CHF, the elevated bradykinin levels produced a vasodilatory effect that partially offsets the vasoconstriction produced by activation of other neurohormonal systems.

The administration of exogenous bradykinin produces positive inotropic and lusitropic effects in the normal circulation (14–16). Consistent with the lack of effect of the low levels of bradykinin on systemic arterial resistance, we found that bradykinin B_2-receptor blockade had no effect on LV systolic or diastolic performance before CHF. However, after CHF, when bradykinin levels were elevated, HOE-140 produced a further depression of LV contractile performance, a further slowing of LV relaxation and a further increase in LV diastolic and LA pressures. This indicates that the elevated levels of bradykinin present in CHF help counteract the depressed LV systolic and diastolic performance in CHF. The use of load-insensitive measures of LV contractile performance demonstrates that the decreased contractile performance demonstrates that the decreased contractile function, when bradykinin effects are blocked in CHF, are not just due to increased arterial resistance.

Our study did not address the mechanism of endogenous bradykinin's actions in CHF. The vasodilatory actions of bradykinin are mediated largely through the activation of bradykinin B_2-receptors that stimulated release of endothelium-derived nitric oxide and prostacyclin (15,17,47–49). It is therefore likely that the action of HOE-140 was to reduce the endogenous bradykinin stimulated release of one or more of these endothelium-derived vasodilators. The effect of bradykinin on LV contractile performance may be partially mediated through the B_2-receptor–mediated inositol pathway that changes the mobilization and reuptake of cytosolic Ca^{2+} and alters myofibrillar Ca^{2+} sensitivity (15,50–52). Bradykinin also activates phospholipase adenyl cyclase and promotes intracellular alkalization by enhancing $Na^{+}-H^{+}$ exchange through activation of protein kinase C (52–54). Bradykinin may also potentiate the release of catecholamines (16,55).

Study limitations. There are several methodologic issues that should be considered in interpreting our data. First is the experimental model of CHF. Although rapid pacing produces an animal model of CHF that closely mimics the clinical picture of congestive cardiomyopathy, we cannot be certain our results apply to CHF that is due to other causes. Incessant tachycardia does lead to clinical CHF in patients.

We studied the effect of acutely blocking the action of bradykinin. It is possible that some of the biologic actions of bradykinin may take weeks or months to reverse. These effects, if any, cannot be determined from our study. However, our study does demonstrate that blocking the effect of endogenous bradykinin produces an acute hemodynamic response in CHF that is different from the effect before CHF.

A third limitation is that we measured bradykinin levels in arterial blood. Because bradykinin is inactivated by angiotensin-converting enzyme, which exists in high levels in the pulmonary circulation, venous levels of bradykinin may be higher than the levels we measured in the arterial circulation. Further, local tissue levels of bradykinin may also be higher than the circulating arterial bradykinin level. However, these limitations did not interfere with our assessment of the functional effects of endogenous bradykinin by blocking the bradykinin B_2-receptor.

Angiotensin-converting enzyme inhibitors are beneficial in patients with CHF (56). These effects have been attributed to a reduction of neurohormonal activation by interfering with the formation of angiotensin II. However, bradykinin inactivation is also blocked by angiotensin-converting enzyme inhibition (20). Our study suggests that the elevated endogenous bradykinin level in CHF has a beneficial effect that partially counterbalances the detrimental effects of angiotensin II in CHF. Thus, some of the beneficial effects of angiotensin-converting enzyme inhibition may be achieved by favorably shifting the balance by both decreasing angiotensin II and increasing bradykinin (17,57–59).

Conclusions. Levels of bradykinin found in normal conscious animals mildly increase coronary blood flow but do not influence systemic arterial resistance or LV performance. After CHF, the elevated endogenous bradykinin levels decrease systemic and coronary arterial resistance and enhance LV contraction and relaxation. Thus, increased bradykinin may partially offset the detrimental effects of activation of other neurohormonal systems in CHF.

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