Diminished Endothelium-Derived Relaxing Factor Activity in an Experimental Model of Chronic Heart Failure

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Abnormalities in vasomotor tone, including enhanced vasoconstriction at rest and diminished vasodilation in response to various stimuli, develop as a consequence of chronic heart failure. This study was undertaken to evaluate whether a specific local mechanism, namely endothelium-derived relaxing factor (EDRF) activity, might be impaired in an experimental model of chronic heart failure. Segments of thoracic aorta (TA) and pulmonary artery (PA) were isolated from a group of rats that had hemodynamic evidence of heart failure 10 weeks after ligation of the left coronary artery \( (n=25) \) and from a group of sham-operated control rats \( (n=18) \). Both endothelium-dependent and endothelium-independent vascular responses were assessed by exposing arterial segments to increasing concentrations of agonists. All studies were performed in the presence of \( 10 \mu M \) indomethacin to avoid the influence of vasoactive prostanoids. The dose–response curve for EDRF-mediated relaxation to acetylcholine was shifted rightward in rats with heart failure, and the concentrations of acetylcholine required to achieve 50% maximal relaxation \( (EC_{50}) \) were increased compared with those of control rats in both TA and PA segments. Additionally, the dose–response curve for relaxation to ADP was shifted rightward with significantly increased \( EC_{50} \) in PA segments from rats with heart failure. In contrast, EDRF-mediated relaxation to the calcium ionophore A23187 was similar in the groups. Endothelium-independent relaxation to nitroglycerin was slightly increased in TA but not PA segments in the heart-failure group. Basal EDRF activity, as assessed by the increase in force after exposure to hemoglobin, was diminished in PA segments from rats with heart failure. These results provide evidence that EDRF activity is impaired in an experimental model of chronic heart failure. Since relaxation to A23187 was not significantly affected and the response to nitroglycerin was somewhat enhanced in arteries from rats with heart failure, it appears that neither impaired EDRF diffusion through the arterial intima nor diminished vascular smooth muscle responsiveness is involved. The results suggest that endothelial cell receptor–related properties or a subsequent step(s) in the production or release of EDRF may be altered in chronic heart failure. A defect in EDRF activity may contribute to the abnormalities in vasomotor tone that have been noted in chronic heart failure. \( (\text{Circulation Research } 1991;69:1088-1096) \)

Chronic heart failure is a clinical syndrome characterized by the inability of the heart to provide adequate nutrient supply to metabolically active tissue. A reduction in cardiac output is often accompanied by an increase in ventricular filling pressures and by alterations in systemic and pulmonary arterial pressures. Abnormalities in vasomotor tone are a well-known component of chronic heart failure. Both enhanced vasoconstriction at rest and impaired responsiveness to vasodilatory stimuli such as exercise and ischemia have been described.\(^1\)–\(^9\) These changes appear to play an important role in the development of many of the signs and symptoms of chronic heart failure.

The abnormalities in vasomotor tone in chronic heart failure are believed to develop on the basis of changes in systemic neurohumoral factors, local tissue metabolism, and blood vessel structure.\(^1\)–\(^10\) Although systemic factors have been extensively described, there is little available information regarding local vasomotor regulatory processes in this setting. Endothelium-derived relaxing factor (EDRF) is a

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nonprostanoid activity produced by endothelial cells in response to various chemical and physical stimuli. It has both vasodilatory and platelet antiaggregatory effects.

Based on its short half-life and rapid inactivation by hemoglobin, EDRF effects are almost certainly local. Impaired EDRF vasodilatory activity has been reported in arteries from experimental animal models and human patients with a variety of diseases including hypertension, diabetes, and atherosclerosis. Although the changes in vasomotor tone that are seen in chronic heart failure raise the possibility that vasodilatory influences might be diminished, information regarding this issue is limited. Therefore, we evaluated vascular responsiveness in an experimental model of chronic heart failure to test the hypothesis that EDRF activity is impaired in this syndrome.

Materials and Methods

Coronary Artery Ligature

Myocardial infarction was produced in male Sprague-Dawley rats (175–225 g) by using techniques similar to those described previously. Briefly, rats were anesthetized with methoxyflurane, and a left thoracotomy was performed. The proximal left coronary artery was ligated, and the thorax was closed. In some rats, the operative procedure was performed in an identical manner but without ligation of the artery. The rats were then maintained on standard rat chow with water ad libitum for a 10-week period.

Hemodynamic Measurements

At termination, the rats were anesthetized with 1 ml/kg of a 1:1 volumetric solution of ketamine HCl (100 mg/ml) and xylazine (20 mg/ml) injected intramuscularly. The right carotid artery was cannulated with a solid-state micromanometer-tipped catheter (Millar Instruments, Houston, Tex.). The catheter was advanced into the aorta and then into the left ventricle (LV). The right external jugular vein was cannulated with polyethylene tubing (0.58 mm i.d.), and the fluid-filled system was connected to a solid-state pressure transducer (Millar Instruments). Body temperature was maintained at 36–38°C by heat lamps. With the rat breathing spontaneously, LV pressures and LV dp/dt were recorded. The left heart catheter was pulled into the ascending aorta from the LV, and systemic arterial and venous pressures were recorded with the mean values determined by electronic averaging on a physiological recorder (model 2200, Gould Instrument Co., Cleveland, Ohio). Heart rate was determined from the arterial pressure recording.

Tissue Preparation

After completion of hemodynamic measurements, the heart and lungs were removed en bloc along with a section of the thoracic aorta (TA) and placed in an aerated physiological solution (described below) at 4°C. Hilar pulmonary artery (PA) segments were gently dissected free of adjacent tissue. Ring segments measuring 1.5–2.0 mm in length were cut using a fine surgical blade. TA ring segments 2.0–3.0 mm in length were taken from the portion of the vessel just distal to the aortic arch. All arteries were gently cleaned of adherent tissue; care was taken to preserve the endothelial cell lining. In some experiments, the endothelium was deliberately removed by gently rubbing the lumen with the tips of a pair of fine forceps as described previously. Removal of endothelium was confirmed in organ bath studies by the absence of a relaxation response to 0.3 μM acetylcholine (ACh).

Infarct Size Determination

The heart was dissected free of adjacent tissues and lung. The ventricles were separated from the atria, and the right ventricular free wall was dissected free of the septum. The LV was opened with an incision along the septum from base to apex. Both ventricles were rinsed, blotted dry, and then immediately weighed. Myocardial infarct size was measured using techniques previously described by Chien et al. Incisions were made in the LV so that the tissue could be pressed flat. The circumferences of the LV and the region of infarction were outlined on a clear plastic sheet for both the endocardial and epicardial surfaces. Infarct size was calculated and expressed as a percentage of LV surface area, based on the weight of the areas marked on the plastic sheet. An average of endocardial and epicardial surface areas is reported.

Heart Failure and Control Groups

Rats were considered to have heart failure if the LV end-diastolic pressure was >15 mm Hg and there was evidence of myocardial infarction extending over >25% of the LV on gross pathological examination. The control group was comprised of 18 rats that had undergone the surgical procedure but without ligation of the coronary artery. Rats in this group did not develop any gross pathological evidence of myocardial infarction or hemodynamic evidence of heart failure.

Pharmacological Studies

These studies were carried out in individual organ bath chambers filled with 10 ml modified Krebs-Henseleit (KH) solution maintained at 37°C by an outer water jacket and circulating heat pump. The KH solution consisted of (mM) NaCl 118, KCl 5.9, CaCl2 2.5, MgSO4 1.2, NaH2PO4 1.2, NaHCO3 25.0, edetate calcium disodium 0.026, and glucose 5.6. The KH was continuously aerated with a mixture of 95% O2–5% CO2. Arterial ring segments were placed on stainless-steel wire stirrups and connected to force transducers (model FT.03, Grass Instrument Co., Quincy, Mass.). Changes in isometric tension were recorded on a multichannel device (model 7-D, Grass). The rings were stretched in a stepwise fashion to their optimal length for isometric contraction by repeated exposure to 60 mmol KCl. The arteries...
were then allowed to equilibrate for 60 minutes with frequent flushing of the chambers with KH.

All pharmacological studies were performed in the presence of 10 μM indomethacin to avoid the influence of vasoactive prostanoids. Since the arterial segments used in these experiments generate little or no resting force, relaxation studies were performed after they had been precontracted to ~50% of their maximal level with prostaglandin (PG) F2α. In assessing both contraction and relaxation, the arterial segments were exposed to increasing amounts of agonist, and the response to the cumulative concentration in the bath was assessed. Some arterial segments were exposed to more than one agonist. However, no more than three successive dose–response curves were performed on an individual ring, and the order of drugs was kept constant throughout the study. Preliminary studies in our laboratory using rat TA and PA demonstrate that endothelium-dependent and endothelium-independent responses to the drugs used in this study do not change significantly over the course of three dose–response experiments.

Basal EDRF activity was assessed by adding 10 μM hemoglobin to arterial rings that had been precontracted with PGF2α to ~50% of their maximal level. Since this concentration of hemoglobin will virtually abolish EDRF vasodilatory activity, the increase in force on exposure to the drug was considered to represent the influence of basal EDRF release.22,23

Drugs and Reagents

PGF2α, dinoprost tromethamine (5 mg/ml, The Upjohn Co., Kalamazoo, Mich.), ACh and ADP (Sigma Chemical Co., St. Louis, Mo.), and KCl (molar solution) were dissolved in distilled water. The calcium ionophore A23187 (Sigma) was initially dissolved in dimethyl sulfoxide (Mallinckrodt Inc., Paris, Ky.), and subsequent dilutions were made in distilled water. Bovine hemoglobin Type 1 (Sigma) was prepared as previously described by Martin et al.22 Nitroglycerin in 30% alcohol (5 mg/ml, Dupont Pharmaceuticals, Wilmington, Del.) was prepared in distilled water just before use and protected from light. Indomethacin (Sigma) was dissolved in sodium carbonate monohydrate and sonicated in distilled water for subsequent use. All drugs were prepared fresh daily and stored at 4°C.

Statistical Methods

Results are expressed as mean±SEM. In rings precontracted with PGF2α, relaxation responses to various agonists are expressed as percentage of change from the contracted levels. In quiescent rings, responses are expressed as percentage of changes of the maximal contraction to PGF2α. EC50 is defined as the concentration needed to reach 50% of the maximal response. EC50 values for contraction and relaxation and the maximal responses were compared between heart-failure rats and control group rats by means of the unpaired t test. Comparison of responses at submaximal concentrations of agonist was performed only when

| Table 1. Cardiac and Hemodynamic Variables in Rats With Heart Failure and Sham-Operated Rats |
|-------------------------------------------------|-----------------|-----------------|
| CHF (n=25)                                      | Sham (n=18)     |
| Body weight (g)                                 | 416.5±7.5       | 441.9±11.5      |
| Heart wt/body wt (mg/g)                         | 3.00±0.06       | 2.47±0.05*      |
| RV wt/body wt (mg/g)                            | 0.91±0.04       | 0.37±0.02*      |
| LV wt/body wt (mg/g)                            | 2.09±0.04       | 2.11±0.05       |
| Heart rate (beats/min)                          | 267±5           | 261±8           |
| MAP (mm Hg)                                     | 90±2.9          | 121±3.2*        |
| RAP (mm Hg)                                     | 2.6±0.48        | 0.06±0.06*      |
| LVEDP (mm Hg)                                   | 25.0±1.0        | 4.0±0.4*        |
| LVESPD (mm Hg)                                  | 108±3           | 126±4*          |
| LV dP/dt (mm Hg/seg)                            | 4,744±235       | 7,639±364*      |
| Infarct size (% of LV area)                     | 34±1            | 0±0*            |

Values are mean±SEM. CHF, rats with chronic heart failure; Sham, sham-operated control rats; RV, right ventricular; LV, left ventricular; MAP, mean arterial pressure; RAP, right atrial pressure; LVEDP, end-diastolic pressure; LVESPD, LV end-systolic pressure; LV dP/dt, first derivative of the change in LV systolic pressure over time.

*p<0.001 vs. CHF.

EC50 values for the groups differed with a value of p=0.10. Values of p≤0.05 for individual comparisons were considered to be significant.

Basal EDRF activity was assessed as the increase in force after exposure to hemoglobin in arteries that had been preconstricted with PGF2α. This value is expressed in grams tension and, since maximal tension was variable between segments, as a percentage of maximal contraction to PGF2α.

Results

Cardiac and Hemodynamic Findings

As summarized in Table 1, the heart-failure rats had a slightly lower body weight than the sham-operated control rats, but the difference between the groups was not significant. Heart weight, however, was increased in rats with heart failure. This was due to a greater than twofold increase in right ventricular weight, since LV weight tended to be slightly lower in the heart-failure group.

Hemodynamic variables in the study groups are also summarized in Table 1. There was no significant difference in heart rate between the groups. However, right atrial pressure and LV end-diastolic pressure were significantly higher in the heart-failure rats. Systemic arterial and LV systolic pressures and LV dP/dt were significantly reduced in the heart-failure rats compared with the control rats. Additional evidence of heart failure, including pleural effusions and both pulmonary and hepatic congestion, was noted consistently at the time of organ harvest in the heart-failure rats but not in the sham-operated control rats.

The infarct size averaged 34±1% of the LV in rats with heart failure.
Diminished EDRF Activity in Chronic Heart Failure

5.83±0.22 for heart-failure rats; \( p<0.001 \) and by nearly twofold in the TA (6.64±0.13 for sham-operated rats compared with 6.36±0.09 for heart-failure rats; \( p<0.08 \)). Maximal relaxation to ACh was significantly less in PA segments from rats in the heart-failure group.

The EC\(_{50}\) value for relaxation in response to ADP was also significantly less in PA segments from rats with heart failure compared with the sham-operated control rats. Relaxation to the calcium ionophore, however, was similar in both PA and TA segments in the heart-failure and control groups (Figure 2).

Endothelium-independent relaxation to nitroglycerin in the PA and TA is also summarized in Table 2. Maximal relaxation and EC\(_{50}\) values were significantly greater in TA segments obtained from rats with heart failure. No significant differences were seen, however, in the dose–response curves in the PA for relaxation to nitroglycerin between the groups.

As shown in Figure 3 and summarized in Table 3, contraction in response to PGF\(_{2\alpha}\) was enhanced in PA segments from rats with chronic heart failure. In the PA, there was both a shift of the dose–response curve leftward, as indicated by a significantly lower EC\(_{50}\) value, and an increase in the maximal force developed. Enhanced constriction to PGF\(_{2\alpha}\) was not seen in TA segments from the heart-failure group.

The increase in force after exposure to 10 \( \mu \)M hemoglobin was used as a measure of basal EDRF activity. As shown in Figure 4, when the response in arterial segments from sham-operated control rats was measured, a significantly greater increase in force in PA compared with TA (76.8±7.1% versus 45.6±11.4%, respectively; \( p<0.05 \)) was noted. This difference was not seen when arterial segments from heart-failure rats were compared. Figure 5 compares the increase in force between heart-failure and control rat arteries both as an absolute value (panel A) and as a percentage of the maximal agonist-induced constriction (panel B). Basal EDRF activity appeared to be diminished in the PA of heart-failure rats compared with the PA of controls when expressed either as an absolute change in force (0.27±0.06 versus 0.43±0.05 g, respectively; \( p=0.065 \)) or as percentage of the maximal response to PGF\(_{2\alpha}\) (37±6.8% versus 76.8±7.1%, respectively; \( p=0.001 \)). Significant differences between groups were not seen in the TA segments.

Discussion

Peripheral vasoconstriction is a prominent feature of congestive heart failure. Although it has been recognized that factors in the vessel wall can influence vasomotor tone, the possibility that abnormalities in local pathways may contribute to the enhanced vasoconstriction seen in heart failure has not been fully evaluated. The present study was undertaken to determine whether a specific local mechanism, namely, EDRF vasodilatory activity, is diminished in this setting. The results show that both basal and
agonist-stimulated EDRF activity is impaired in an animal model of chronic heart failure.

Relaxation of rat arteries to ACh and the calcium ionophore A23187 can be ascribed to EDRF activity, since it can be inhibited by removal of the endothelium and by preincubating the arteries with hemoglobin.12,13,22,23,34 The same is true for ADP, although at high concentrations of agonist, endothelium-independent relaxation is seen. Involvement of vasodilatory prostaglandins in the relaxation response to those agonists is unlikely, since all experiments were performed in the presence of indomethacin in concentrations known to inhibit cyclooxygenase activity.

In arterial segments from rats with chronic heart failure, EDRF-mediated relaxation to ACh and ADP was diminished in comparison to the response measured in sham-operated control rats. This defect in EDRF activity could be due to diminished EDRF production/release, impaired diffusion to the underlying smooth muscle, or alterations in the responsiveness of the smooth muscle itself. Both EDRF and nitroglycerin are known to initiate relaxation of vascular smooth muscle through activation of soluble guanylate cyclase with subsequent cGMP-dependent protein phosphorylation.35,36 The observation that relaxation to nitroglycerin was, if anything, enhanced in the heart-failure group suggests that an abnormality in this pathway or the ability of smooth muscle to respond is not involved.

In contrast to the results with ACh and ADP, relaxation in response to the calcium ionophore A23187 was similar in arteries from heart-failure and sham-operated control rats. The fact that both ACh and ADP stimulate EDRF production by initially binding with specific endothelial cell surface receptors (A23187 does not require this step) raises the possibility that the defect in EDRF seen in this study is related to an alteration in receptor properties or in postreceptor signal transduction in chronic heart failure. Alternatively, stimulation of EDRF by the calcium ionophore may not be a sensitive enough method to detect changes in EDRF activity of the magnitude seen in this study. The observation that relaxation to A23187 was not impaired in arterial segments from rats with heart failure, however, provides evidence that impaired diffusion or enhanced breakdown of EDRF is an unlikely cause for the impairment in EDRF activity.

Basal EDRF release has been described, and it is possible that this activity plays an important role in modifying the response to vasoconstrictor influences and perhaps in the regulation of blood pressure.37,38 In the present study, basal EDRF activity was assessed by measuring the increase in force that occurred when arterial segments were exposed to hemoglobin. Since the increase in force in response to hemoglobin should be proportional to the amount of EDRF activity, the fact that the response was significantly greater in PA segments from the sham-operated control rats than in those segments from CHF rats is consistent with the finding that EDRF activity is diminished in vessels from heart-failure rats. From these studies, it appears that the impairment in EDRF production/release is significant in heart-failure rats.

**Table 2.** Endothelium-Dependent and Endothelium-Independent Relaxation of Vessels From Heart-Failure Rats Versus Sham Control Rats

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Sham</th>
<th>CHF</th>
<th>p</th>
<th>Maximal relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>6.87±0.14</td>
<td>5.83±0.22</td>
<td>&lt;0.001</td>
<td>97±2</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>6.64±0.13</td>
<td>6.36±0.09</td>
<td>&lt;0.08</td>
<td>85±4</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>5.35±0.19</td>
<td>4.44±0.31</td>
<td>0.019</td>
<td>77±4</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>4.46±0.23</td>
<td>4.57±0.27</td>
<td>NS</td>
<td>87±5</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A23187</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>6.35±0.11</td>
<td>6.35±0.17</td>
<td>NS</td>
<td>89±3</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>6.38±0.22</td>
<td>6.76±0.13</td>
<td>NS</td>
<td>81±5</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>10</td>
<td></td>
<td></td>
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<tr>
<td>NTG</td>
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<tr>
<td>PA</td>
<td>6.74±0.17</td>
<td>6.55±0.18</td>
<td>NS</td>
<td>87±4</td>
</tr>
<tr>
<td>n</td>
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<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>6.50±0.19</td>
<td>7.07±0.16</td>
<td>0.03</td>
<td>81±5</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>14</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are mean±SEM. EC50 concentration of agonist causing 50% of response; Sham, sham-operated control rats; CHF, rats with chronic heart failure; ACh, acetylcholine; PA, pulmonary artery; n, number of experiments in each subgroup; TA, thoracic aorta; A23187, calcium ionophore; NTG, nitroglycerin; NS, not significant.
ventricular pacing has been described by Kaiser et al.29 In that study, however, administration of indomethacin significantly enhanced relaxation in response to ACh in the heart-failure group. Rather than identifying a defect in EDRF activity, it appears that these data are most consistent with the hypothesis that production of a cyclooxygenase-dependent endothelium-derived constriction factor is the cause for the abnormal response to ACh in the canine heart-failure model. The reason(s) why the results of the present study differ from those reported by Kaiser et al. is probably related to species differences and to the methods used to induce heart failure.

Table 3. Contraction in Sham-Operated Rats and Rats With Chronic Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CHF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF2α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal force (g)</td>
<td>0.63±0.04</td>
<td>0.95±0.09</td>
<td>0.009</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>E50 (-log10 M)</td>
<td>5.11±0.06</td>
<td>5.61±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF2α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal force (g)</td>
<td>2.08±0.12</td>
<td>2.08±0.10</td>
<td>NS</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>E50 (-log10 M)</td>
<td>5.18±0.06</td>
<td>5.32±0.10</td>
<td>NS</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. Sham, sham-operated control rats; CHF, rats with chronic heart failure; PA, pulmonary artery; PGF2α, prostaglandin F2α; E50, concentration of PGF2α that would result in 50% of maximal force development; TA, thoracic aorta; NS, not significant.
The mechanism(s) responsible for impaired EDRF activity in chronic heart failure is uncertain. Necropsy findings from rats undergoing successful coronary ligation demonstrated the presence of extensive myocardial infarction involving 34±1% of the LV muscle mass. Although cardiac output was not measured directly in the present study, the significant reductions in LV dP/dt, systolic pressure, and mean arterial pressure in association with a marked increase in LV end-diastolic pressure strongly suggest that it was reduced. Previous studies performed in infarcted rats have shown that these hemodynamic measurements in the presence of a large myocardial infarction are associated with a significant reduction in cardiac output.32,40 Miller et al.37 have shown in a canine model that endothelial-dependent relaxation is increased in response to sustained increases in flow produced by an arteriovenous shunt. It is possible that chronic decreases in blood flow that develop as a consequence of myocardial damage alter endothelial cell function so that the production of EDRF in response to certain stimuli is reduced.41 Such a change would alter the balance of vasoregulatory factors in favor of enhanced constriction. If this were indeed the case, it would have the effect of maintaining shear stress closer to the normal range, since a reduction in blood flow would then be matched by a reduction in vessel diameter. However, the syndrome of chronic heart failure eventually involves multiple physiological and pathophysiological pathways throughout the organism that could affect EDRF activity. For instance, increased plasma levels of catecholamines, renin, arginine vasopressin, and atrial natriuretic peptide have been shown to occur at an early stage of heart failure.1 Although the long-term effects of these substances on endothelial cell function are unknown, it is possible that one of these factors may be involved in the impairment in EDRF activity.

The abnormalities in EDRF activity demonstrated in this study were of greater magnitude in the PA than in the TA. For instance, the EC50 for the response to ACh indicated that this agonist was nearly 10-fold less potent in the PA of heart-failure rats than in the PA of control rats. The differences in response to ACh between heart-failure and control rats, however, was somewhat less in TA segments. Furthermore, a significant impairment in the re-
response to ADP was seen in PA but not in TA segments from rats with heart failure. There are several possible reasons for this. As evidenced by the increase in LV end-diastolic pressure and the greater than twofold increase in right ventricular weight, pulmonary artery pressures must have been increased in rats with heart failure. Arterial pressure in the aorta, on the other hand, was shown to be significantly reduced. Previous studies have shown that acute increases in arterial pressure can diminish EDRF activity, presumably on the basis of endothelial cell injury. Our findings raise the possibility that more modest but sustained increases in pressure (particularly if they occur in a relatively low pressure bed such as the pulmonary artery) can also result in endothelial cell dysfunction with diminished EDRF activity. In addition, when arteries from control rats were exposed to hemoglobin, constrictor was greater in PA than in TA segments, suggesting higher basal EDRF activity in the PA. These findings are consistent with the PA being a relatively low pressure circuit. However, greater basal EDRF activity may have rendered the mechanisms involved in EDRF production more susceptible to impairment as heart failure developed.

Vasomotor tone is determined by the interplay of several factors, including circulating and locally released neurohumoral factors, local tissue metabolites, and structural changes in the vessel wall. The net effect of changes that occur in chronic heart failure is enhanced vasoconstriction at rest and diminished vasodilation in response to stimuli, including exercise and ischemia. When heart failure occurs acutely, these changes are of benefit to the organism in that they help maintain arterial perfusion pressure at a time when cardiac output is reduced. In chronic heart failure, however, the effects of a sustained increase in vascular tone on the ability of the impaired LV to empty during systole and on blood flow to peripheral and pulmonary tissue tend to be deleterious. Although cardiac dysfunction and the resultant hemodynamic abnormalities that are seen in chronic heart failure can be improved acutely by pharmacological means or by heart transplantation, abnormalities in peripheral blood flow often persist. This paradox may account for the failure of patients to derive clinical benefits from therapy despite the demonstration of substantial hemodynamic improvement. The findings in the present study demonstrate that a defect in EDRF production may contribute to the abnormalities in vascular tone and tissue perfusion that occur in chronic heart failure. It is possible that persistence of this specific local defect could help explain the dissociation between the acute hemodynamic and clinical effects of therapy. It is also important to note that, in addition to impairing the ability of an artery to dilate to various stimuli, a defect in basal EDRF activity could enhance the response to stimuli that cause vasoconstriction.

Finally, it is possible that physiological responses that depend on suppression of EDRF activity may be adversely affected by the impairment in EDRF activity that develops in chronic heart failure.

In this series of experiments, responses in large conduit arteries were evaluated. However, resistance to flow is, to a large extent, a property of the microvasculature, and the effects of chronic heart failure in these vessels were not evaluated. Nevertheless, the changes demonstrated provide a useful model for studying the pathophysiology of chronic heart failure and for testing the effects of various therapeutic modalities.

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