Temporal Evolution of Endothelial Dysfunction in a Rat Model of Chronic Heart Failure

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Objectives. The goal of this study was to investigate the evolution of endothelial dysfunction and plasma renin activity in a rat model of heart failure.

Background. Endothelial dysfunction has been demonstrated in heart failure and may play a significant role in this pathophysiologic process. Studies have also suggested a physiologic interaction between the renin-angiotensin system and endothelium-derived relaxing factor. However, the evolution of endothelial dysfunction and plasma renin activity in heart failure has not been studied to date.

Methods. Endothelium-dependent and -independent relaxations were studied at 1, 4 and 16 weeks after coronary artery ligation in a rat model of heart failure. Thoracic aortic rings were placed in isolated organ baths and acetylcholine and sodium nitroprusside concentration response curves were generated. Plasma renin activity was assessed at each time point.

Decreased endothelium-dependent relaxation of the systemic vasculature in response to acetylcholine has been demonstrated in patients with severe heart failure (1-3). However, the confounding effects of other diseases known to affect endothelium-dependent relaxation could not be entirely controlled in these investigations. In the canine pacing model of heart failure, studies have shown conflicting results (4,5), whereas in the rat coronary artery ligation model, significantly decreased endothelium-dependent relaxation in the pulmonary (6) and systemic (7,8) vasculature has been demonstrated. The evolution of these changes in the systemic vasculature over time has not been evaluated to date.

The importance of time in the pathogenesis of heart failure has been established in many studies, especially those investigating ventricular enlargement (9), and has affected current approaches to the treatment and prevention of congestive heart failure (10). Endothelial dysfunction may represent another pathophysiologic state that evolves over time and may contribute to the maintenance and progression of heart failure. An analysis of the temporal evolution of endothelial dysfunction in patients would be extremely difficult to perform and consequently we used the coronary artery ligation model to investigate the evolution of endothelial dysfunction in the thoracic aorta of rats with heart failure. In addition, a secondary goal of our study was to assess the evolution of plasma renin activity, a marker of renin-angiotensin system stimulation, and its possible relation to endothelial dysfunction.

Results. Aortic rings from rats with heart failure demonstrated no evidence of endothelial dysfunction at week 1, although progressive rightward shifts in the acetylcholine curves and decreasing maximal relaxation over time compared with findings in sham-operated control rats were evident at weeks 4 and 16. The sodium nitroprusside curves were not different between rats with heart failure and sham-operated rats. Plasma renin activity was elevated at week 1 and progressively increased through week 16, even though it correlated poorly with endothelial dysfunction.

Conclusions. This study suggests that endothelial dysfunction in heart failure is a progressive time-dependent process that probably plays a minor role early in heart failure. Although plasma renin activity increased significantly in rats with heart failure, it was poorly predictive of endothelial dysfunction.

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the thorax was closed. Within the 1st 48 h after this procedure, 60% of the ligated and 99% of the sham-operated animals survived. The rats were housed in clear plastic cages with free access to normal rat chow and water and were handled according to the "Position of the American Heart Association on Research Animal Use" adopted November 11, 1984 by the American Heart Association.

At least 3 days after the coronary ligation or sham procedure and 3 days before isolated organ bath studies, the rats underwent catheterization. Under ether anesthesia, a microtipped pressure transducing catheter (2F, model SPC 320, Millar Instruments) was introduced into the thoracic aorta through the right carotid artery where arterial blood pressure and heart rate tracings were obtained. The catheter was advanced into the left ventricle and simultaneous tracings of left ventricular pressure, expanded-scale left ventricular end-diastolic pressure, maximal rate of rise in left ventricular pressure (+dP/dt) and heart rate were also recorded on a linear chart recorder (Linearcorder Mark VII, model WR3101, Graphtec Corp.). Sham-operated rats were randomly selected to enter the study, whereas only rats that underwent ligation with a left ventricular end-diastolic pressure ≥15 mm Hg were considered to have congestive heart failure and completed the study protocol.

Organ bath studies. At 1, 4 and 16 weeks after myocardial infarction, the rats were anesthetized with ether, 8 ml of blood was withdrawn for biochemical analysis and the thoracic aorta and heart were immediately excised. The heart was placed in Krebs-Henseleit solution until preparation for pathologic studies could be performed. The thoracic aorta was dissected and cut into two 5-mm rings, the first of which was left intact, whereas the second was denuded of endothelium by gentle rubbing of the intimal surface. In each experiment, an aortic segment from a sham-operated control rat was studied in parallel with that from a rat with heart failure. Each ring was suspended in a 10-ml isolated organ bath filled with Krebs-Henseleit solution in mmol/liter: sodium chloride 2.5, glucose 11.1 kept at 37°C and gassed with a mixture of 95% oxygen/5% carbon dioxide. The rings were connected to force transducers and isometric tension was recorded (Linearcorder Mark VII, Graphtec Corp.).

The rings were stretched to a tension of 3 g at rest and, after an equilibration period, all rings were contracted with 10^-7 mol/liter norepinephrine. Subsequently, 10^-5 mol/liter of acetylcholine was administered to check for the presence of functioning endothelium on rings with intact endothelium and for the absence of endothelium on the rings that were rubbed. After the rings had been washed and returned to a stable baseline state, norepinephrine (3 × 10^-8 to 1 × 10^-7 mol/liter) was administered to the rings in a concentration so as to obtain a stable contraction and to approximate the developed tension of the matching control ring. In rings with intact endothelium, acetylcholine concentration-response curves were then generated by adding cumulative doses of acetylcholine (10^-9 to 10^-4 mol/liter). The concentration of acetylcholine inducing 50% maximal relaxation and the maximal relaxation were measured. On rings where the endothelium had been mechanically removed, cumulative concentrations of sodium nitroprusside from 10^-10 to 10^-7 mol/liter were given and the concentration inducing 50% of the maximal relaxation and the maximal relaxation were measured.

Biochemical and pathologic studies. At the time of induced death, the blood was withdrawn into a syringe containing 50 µliter of 5% ethylenediaminetetraacetic acid per ml of blood. These blood samples were immediately centrifuged for 5 min at 13,000 rpm. Subsequently, the plasma was placed in dry ice and then stored at −20°C until time of assay. Plasma renin activity was assessed by a standard technique at pH 7.4 (13), using a commercial radioimmunoassay kit (Baxter Healthcare Corp.) to quantify the amount of angiotensin I generated from angiotensinogen. The great vessels and adherent tissues were trimmed from the heart and the right ventricular free wall was dissected away from the left ventricle. Ventricular weights were obtained to provide a rough estimate of ventricular mass and then the ventricles were immediately placed in a 10% formalin solution. The size of the infarct was estimated by a method previously described (14), where 10 10-µm thin slices of the left ventricle were stained with Masson trichrome. The endocardial and epicardial circumferences of the infarcted portion and the total left ventricle were determined with a planimeter digital image analyzer (Sony) and values were averaged to yield a percent of ventricular infarction for each rat.

Study design and statistical analysis. The overall design of this investigation consisted of studying the two groups of rats at three time periods (1, 4 and 16 weeks) after myocardial infarction. All statistical comparisons were performed with commercially available general linear model statistical packages for the Macintosh personal computer (SuperANOVA, Version 1.11; Statview, Version 4.0; Abacus Concepts, Inc.). Comparison of all variables was done with a two-way analysis of variance with time (1, 4 and 16 weeks) and group as main effects, and three prospectively planned means comparison contrasts between rats with heart failure and sham-operated rats at each time point. One-way analysis of variance with Duncan's multiple comparison tests was used to analyze differences within a group across time. Stepwise multivariate linear regression and simple linear regression were also performed to investigate the relation between different variables. Differences were considered significant at a level of p < 0.05 and results are expressed as mean value ± SEM.

Results

Hemodynamic and baseline characteristics (Table 1). At least 10 rats in each study group completed the protocol. Rats with heart failure weighed less than the time-matched control rats at each period (p < 0.01 for all comparisons), though both groups gained weight at a similar rate. The mean
arterial blood pressure, left ventricular systolic pressure and heart rate were significantly lower in rats with heart failure than in control rats, but did not change significantly with time. The maximal rate of rise in the left ventricular pressure (+dP/dt) was also lower in rats with heart failure than in sham-operated rats, though it decreased with age in a similar fashion in both groups of rats. The left ventricular end-diastolic pressure was markedly elevated in the rats with heart failure, as would be expected given the selection criteria. The left ventricular mass indexed for body weight remained stable in the sham-operated rats (overall mean 1.84 ± 0.02 g/kg), but increased through time in the rats with heart failure at 16 weeks. Log = logarithmic; M = milliter; RV = right ventricular; Shane = sham-operated rats.

### Results

#### Table 1. Physical Characteristics, Hemodynamic Variables and Plasma Renin Activity Over Time

<table>
<thead>
<tr>
<th>Time</th>
<th>Body Weight (g)</th>
<th>LV Mass (g/kg)</th>
<th>RV Mass (g/kg)</th>
<th>HR (beats/min)</th>
<th>MAP (mm Hg)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>LV + dP/dt (×10³ mm Hg/s)</th>
<th>PRA (pg Al/ml per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 1</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Sham</td>
<td>21</td>
<td>362 ± 4</td>
<td>1.88 ± 0.03</td>
<td>0.51 ± 0.02</td>
<td>384 ± 7</td>
<td>121 ± 2</td>
<td>137 ± 2</td>
<td>0.9 ± 0.2</td>
<td>15.5 ± 0.6</td>
</tr>
<tr>
<td>CHF</td>
<td>18</td>
<td>321 ± 7</td>
<td>2.00 ± 0.05</td>
<td>0.84 ± 0.05</td>
<td>362 ± 6</td>
<td>88 ± 2</td>
<td>101 ± 2</td>
<td>19.1 ± 0.8</td>
<td>6.9 ± 0.3</td>
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<tr>
<td><strong>Week 4</strong></td>
<td></td>
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<tr>
<td>Sham</td>
<td>23</td>
<td>375 ± 7</td>
<td>1.83 ± 0.02</td>
<td>0.50 ± 0.02</td>
<td>375 ± 8</td>
<td>118 ± 2</td>
<td>135 ± 2</td>
<td>0.7 ± 0.3</td>
<td>13.4 ± 0.6</td>
</tr>
<tr>
<td>CHF</td>
<td>24</td>
<td>352 ± 4</td>
<td>2.14 ± 0.04</td>
<td>1.10 ± 0.05</td>
<td>356 ± 5</td>
<td>93 ± 2</td>
<td>103 ± 2</td>
<td>23.1 ± 1.1</td>
<td>6.0 ± 2.2</td>
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<tr>
<td><strong>Week 16</strong></td>
<td></td>
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</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>426 ± 7</td>
<td>1.82 ± 0.03</td>
<td>0.48 ± 0.02</td>
<td>370 ± 11</td>
<td>120 ± 3</td>
<td>135 ± 3</td>
<td>1.4 ± 0.5</td>
<td>12.8 ± 0.6</td>
</tr>
<tr>
<td>CHF</td>
<td>10</td>
<td>402 ± 9</td>
<td>2.30 ± 0.09</td>
<td>1.24 ± 0.12</td>
<td>363 ± 8</td>
<td>95 ± 4</td>
<td>106 ± 3</td>
<td>22.7 ± 2.0</td>
<td>5.8 ± 0.5</td>
</tr>
</tbody>
</table>

Interaction effect (p value) 0.2254 0.0012 0.0001 0.6342 0.1127 0.3863 0.0565 0.2793 0.0500

Time effect (p value) 0.0001 0.0597 0.0091 0.5031 0.0001 0.0031 0.0456 0.7199 0.0005

Group effect (p value) 0.0001 0.0001 0.0001 0.0104 0.0001 0.0001 0.0041 0.0001 0.0001

Results are expressed as mean value ± SEM. CHF = rats with congestive heart failure; +dP/dt = maximal rate of rise in left ventricular pressure; HR = heart rate; LV = left ventricular; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; PRA = plasma renin activity; RV = right ventricular; Shane = sham-operated rats.

#### Figure 1. Acetylcholine concentration-response curves in aortic rings with endothelium from rats with heart failure. The shaded area represents the 95% confidence range for the sham-operated control rats. Notice the progressive rightward shift over time in the rats with heart failure and the significant decrease in maximal relaxation in the rats with heart failure at 16 weeks. Log = logarithmic; M = milliter; NE = norepinephrine; ? = p < 0.01 for rats with heart failure at that time period versus control rats.
failure (overall mean in sham-operated rats 0.87 ± 0.04 g vs. overall mean in rats with heart failure 1.17 ± 0.04 g, p < 0.01), but there were no significant differences within the heart failure or sham groups with respect to time. Thus, any changes in these curves with respect to time were not due to different initial contractions.

Sodium nitroprusside concentration response curves. Aortic rings without endothelium from rats with heart failure and sham-operated rats were exposed to cumulative concentrations of sodium nitroprusside after precontraction with norepinephrine to investigate endothelium-independent relaxation. The concentration-response curves for the rats with heart failure were not different from the control curves (Fig. 3), nor was the sodium nitroprusside concentration that produced 50% of maximal relaxation or maximal relaxation response different between the two rat groups. The starting tension in the sham rings did not change through time (overall mean 2.74 ± 0.06 g). The initial contractions in vessels from rats with heart failure at weeks 1 and 4 were significantly less than the corresponding control contractions (week 1, 2.18 ± 0.10 g; week 4, 2.19 ± 0.11 g; p < 0.05), though at week 16 there was no difference between the heart failure and sham rings (week 16, 2.88 ± 0.20 g).

Plasma renin activity in chronic heart failure. Plasma renin activity was significantly increased at every time point in the heart failure group (p < 0.0001). Absolute values of plasma renin activity changed significantly with time in both the control and heart failure groups (Table 1). However, when the plasma renin activity was expressed as a percent of time- and assay-matched control values (Fig. 4), plasma renin activity clearly increased through time in the rats with heart failure. Multiple stepwise linear regression analysis was used to determine which of the hemodynamic and vascular responsiveness variables were most closely associated with the plasma renin activity in rats with heart failure and three variables were moderately correlated with plasma renin activity, namely, left ventricular and right ventricular mass and the acetylcholine concentration that produced 50% of maximal relaxation (Plasma renin activity = 212 Left ventricular mass + 142 Right ventricular mass - 0.265 Acetylcholine concentration that produced 50% of maximal relaxation - 272; r = 0.607, r² = 0.368). To address the question of whether plasma renin activity could be predictive of the endothelial dysfunction, linear regression was performed with plasma renin activity as the dependent variable versus either the acetylcholine concentration that produced 50% of maximal relaxation or maximal acetylcholine-induced relaxation. Plasma renin activity was poorly predictive of the acetylcholine concentration that produced 50% of maximal relaxation or maximal acetylcholine-induced relaxation.
duced 50% of maximal relaxation ($r = 0.317, p = 0.0043$) and was not predictive of maximal relaxation.

**Discussion**

The present study using the coronary artery ligation rat model shows that endothelial dysfunction is a delayed phenomenon that evolves over time. Despite marked hemodynamic abnormalities present at week 1, endothelial dysfunction in response to acetylcholine emerged only after 4 weeks of heart failure and progressed through week 16. This worsening dysfunction occurred in the presence of a preserved vascular smooth muscle response to sodium nitroprusside, confirming the finding that this dysfunction is endothelium-dependent. Plasma renin activity was increased in the rats with heart failure at week 1 and continued to be elevated above control values through week 16, though it was a poor predictor of the endothelial dysfunction.

Coronary artery ligation in the rat has been one of the most useful models in elucidating the pathophysiology of heart failure and was instrumental in evaluating the effectiveness of angiotensin-converting enzyme inhibitors in prolonging survival (15,16). The presence of heart failure as demonstrated by the increased left ventricular end-diastolic pressure was associated with increases in left and right ventricular mass, which corresponds to findings of previous studies (11). The absence of any confounding diseases in this model, such as atherosclerosis (17), hypercholesterolemia (18), hypertension (19), insulin-dependent diabetes mellitus (20) or other diseases known to effect endothelium-dependent relaxation, provides an opportunity to investigate independently the evolution of endothelial dysfunction in heart failure.

**Role of progressive endothelial dysfunction in heart failure**

In this study, decreased endothelium-dependent relaxation in the systemic circulation was evident only at 4 weeks after myocardial infarction and progressively worsened through week 16. Another study (21) of the time course of the hemodynamic changes in rats with similar infarct sizes demonstrated that the systemic vascular resistance followed the same pattern, with nonsignificant increases evident at 10 weeks and marked increases present 20 weeks after infarction. The findings from our study suggest that progressive endothelial dysfunction over time could contribute to the inexorable hemodynamic deterioration evident in severe heart failure. Furthermore, because the hemodynamic compromise was present at week 1 at a time when endothelial dysfunction was absent, our findings suggest that endothelial dysfunction in heart failure is not merely the direct result of hemodynamic insufficiency.

Since the discovery of endothelium-derived relaxing factors (22), one of which is now considered to be nitric oxide (23), and other endothelium-derived factors, the endothelium has been recognized as a dynamic regulator of vascular tone. In the coronary artery ligation rat model of heart failure, decreased relaxation in response to acetylcholine has also been shown in isolated aortic (8) and pulmonary (6) artery ring segments and in the isolated hindquarter (7) of rats with 10 to 12 weeks of heart failure. Other studies in the ventricular overdrive pacing canine model of heart failure have found both decreased (4) and normal (5) endothelium-dependent vasorelaxation. Similarly, the basal release of nitric oxide has been reported to be normal in the coronary artery ligation rat model (7,24) but significantly decreased in the dog model (25) of heart failure. Studies in humans with severe heart failure have consistently demonstrated abnormalities in endothelium-mediated relaxation as assessed by changes in the coronary blood flow (1), forearm blood flow (2) and superficial femoral artery blood flow velocity (3). Thus, endothelial dysfunction as demonstrated by decreased relaxation to acetylcholine appears to be present in established severe heart failure. However, our study is the first to demonstrate the time course of these pathophysiologically important changes in the coronary artery ligation rat model of heart failure, where effects of concomitant diseases can be eliminated.

The acetylcholine-induced release of nitric oxide causes vasodilation through a soluble guanylate cyclase in the smooth muscle cell that can also be stimulated independently of the endothelium by organic nitrates, such as sodium nitroprusside (26). In our study, endothelium-independent relaxation was intact from week 1 to 16, as has been shown in other studies at 10 to 12 weeks after coronary artery ligation (7,8). Studies (2) in humans with heart failure have likewise demonstrated no difference in vasodilator response to nitroprusside, though one study suggested that there was decreased responsiveness to nitroglycerin in patients with heart failure (3). These findings suggest that the decreased relaxation response to acetylcholine is not due to diminished vascular smooth muscle responsiveness. Other possible mechanisms include alterations in acetylcholine receptor number of affinity, dysfunction of the nitric oxide synthase pathway and abnormal diffusion of the relaxation factor in heart failure.

**Plasma renin activity and endothelial dysfunction**

Neurohumoral activation plays a prominent role in the pathophysiology of heart failure; in studies (27,28) of patients with heart failure, plasma renin activity was significantly increased and was shown to correlate with mortality by univariate analysis. In the ventricular overdrive pacing dog model of heart failure (29), increased plasma renin was noted, though studies in the rat coronary artery ligation model have found both no increase (30–32) and variable increases (33–35) in plasma renin activity. In our study, the plasma renin activity of rats with heart failure was clearly elevated at week 1 and progressively increased through week 16. Unlike the studies that found no increase in renin, this study had specific selection criteria to establish the presence of heart failure (left ventricular end-diastolic pressure ≥ 15 mm Hg), which may account for our results.

Endothelial dysfunction may play an important role in the pathogenesis of heart failure, though no noninvasive markers for this abnormality have been discovered. In the rat coronary artery ligation model, administration of an
angiotensin-converting enzyme inhibitor, captopril, prevented the development of endothelial dysfunction in the pulmonary artery (36), suggesting a possible relation between stimulation of the renin-angiotensin system and endothelial dysfunction in heart failure. A physiologic relation between endothelium-derived relaxing factor and renin release has also been suggested (37–39). However, in our study, plasma renin activity was poorly predictive of endothelial dysfunction and thus is not a useful noninvasive marker for endothelial dysfunction in heart failure.

**Conclusions.** The present study shows that systemic endothelial dysfunction is a delayed phenomenon that evolves over time in heart failure. Ventricular remodeling is also a slowly evolving process that has been shown to be prevented by chronic angiotensin-converting enzyme inhibition. Thus, further studies are needed to determine if therapeutic intervention can prevent the systemic endothelial dysfunction evident in heart failure.

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### References


