Increased Vascular Responsiveness to Norepinephrine in Rats With Heart Failure Is Endothelium Dependent

Dissociation of Basal and Stimulated Nitric Oxide Release

John R. Teerlink, MD; Gillian A. Gray, PhD; Martine Clozel, MD; Jean-Paul Clozel, MD

Background  Endothelial dysfunction and abnormal vascular responsiveness to vasoconstrictors may play an important role in chronic heart failure (CHF). The purpose of our study was to (1) evaluate whether the vascular response to norepinephrine is abnormal in a rat model of heart failure; (2) investigate the role of α₁- and α₂-adrenergic receptors; and (3) assess the contribution of the endothelium, and specifically endothelium-derived nitric oxide, to this response.

Methods and Results  Concentration-response curves of rat thoracic aortic rings were studied in isolated organ baths at 1 week after coronary artery ligation. In CHF rats, norepinephrine-induced contractions were increased in intact rings compared with rings from sham rats, despite decreased contraction in denuded rings. Decreased α₁-receptor sensitivity was demonstrated by the increased EC₅₀ of methoxamine in endothelium-denuded rings from CHF rats, although maximal responses to KCl contraction were also decreased in CHF. There was no difference in the vascular response to clonidine, and acetylcholine-mediated relaxations were preserved in CHF rats, suggesting normal stimulated nitric oxide release. However, nitric oxide synthase inhibition with N⁴-nitro-L-arginine methyl ester, as well as measurements of basal cGMP, demonstrated that basal nitric oxide release was decreased in CHF rats.

Conclusions  This study demonstrates that the increased vascular responsiveness to norepinephrine in intact vessels from rats with heart failure is the result of decreased basal nitric oxide release and suggests that the dissociation of basal and stimulated nitric oxide release may play a pathophysiological role at an early stage of heart failure. (Circulation. 1994; 89:393-401.)

Key Words  • endothelium-derived factors • myocardial infarction • receptors, adrenergic, alpha • methoxamine served responses to the endothelium-independent relaxing agent nitroprusside. Acetylcholine-induced relaxation was normal at 1 week after myocardial infarction, but severe dysfunction was evident at 4 weeks. Preliminary experiments suggested that increased vasoconstriction to norepinephrine in vascular rings with endothelium was present in this rat model of heart failure at 1 week after myocardial infarction, despite decreased vasoconstriction in rings without endothelium. Therefore, the purpose of our study was to (1) evaluate whether the vascular response to norepinephrine is abnormal in this rat model of heart failure using vascular ring segments from the thoracic aorta in isolated organ bath experiments; (2) investigate the role of the vascular α₁- and α₂-adrenergic receptors; and (3) assess the contribution of the endothelium, and specifically endothelium-derived nitric oxide, to this response through the use of the specific nitric oxide synthase inhibitor N⁴-nitro-L-arginine methyl ester (L-NAME) in functional studies and through measurement of vascular intracellular cGMP.

Methods  Myocardial Infarction and Selection Procedure

Thirteen-week-old, male Wistar rats were randomly selected to undergo coronary artery ligation or sham operation by a technique previously described. In brief, the rats were anesthetized with ether, and a left thoracotomy was performed. Through gentle pressure applied to the right hemithorax, the heart was exteriorized, and a ligature was placed around the proximal left coronary artery. This silk suture was tied securely

Received June 25, 1993; revision accepted September 15, 1993.
From the Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, Basel, Switzerland.
Correspondence to Jean-Paul Clozel, MD, c/o F. Hoffmann-La Roche Ltd, Pharma Division, Preclinical Research, Bldg 70/411, CH-4002 Basel, Switzerland.
in the rats randomized to coronary artery ligation but was pulled through in the sham-operated animals. The heart was rapidly replaced, and the thorax was closed. More than 100 rats underwent coronary artery ligation, and within the first 48 hours after this procedure, 62% of the ligated and 100% of the sham-operated animals survived. The rats were housed in clear plastic cages with free access to normal rat chow and water and 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 microtip pressure-transducing catheter (2F, model SPC 320, Millar Instruments Inc, Houston, Tex) was introduced into the thoracic aorta via the right carotid artery, in which 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, the maximal rate of rise in left ventricular pressure, and heart rate were also recorded on a chart recorder (Linearorder Mark VII, model WR3101, Graphtec Corp., Tokyo, Japan). Sham-operated rats were randomly selected to enter the study, whereas only rats that underwent ligation with left ventricular end-diastolic pressures of ≥15 mm Hg were considered to have CHF and completed the study protocol.

Organ Bath Studies

At 1 week after myocardial infarction, the rats were anesthetized with ether, and the thoracic aorta and heart were immediately excised. The heart was prepared for pathologic studies (see below), and the thoracic aorta was dissectioned and cut into two 5-mm rings, the first of which was left intact and the second denuded of endothelium by gentle rubbing of the intimal surface. Each ring was suspended in a 10-μL isolated organ bath filled with Krebs-Henseleit solution (in mmol/L: NaCl 115, KCl 4.7, MgSO4 1.2, KH2PO4 0.5, NaHCO3 25, CaCl2 2.5, glucose 11.1; all compounds analytic grade; Fluka Chemical, Buchs, Switzerland) kept at 37°C and gassed with a 95% O2/5% CO2 mixture. The rings were connected to force transducers, and isometric tension was recorded (Linearorder Mark VII, Graphtec Corp.). The vascular rings were stretched to a resting tension of 3 g, and after an equilibration period, all rings were contracted with 10^{-7} mol/L l-norepinephrine hydrochloride (Fluka Chemical). Subsequently, 10^{-3} mol/L acetylcholine hydrochloride (Sigma Chemical Co, St Louis, Mo) was administered to check for the presence of functioning endothelium on intact rings and for the absence of endothelium on the denuded rings. After the rings were thoroughly washed and the resting tension returned to a stable baseline, the effects of different agonists and antagonists were evaluated in series (at least 5 rats per group). A maximum of five concentration-response curves were performed on each ring, with at least 60 minutes of washing between each curve. Aortic segments from heart failure rats and sham-operated controls were matched for the same series of concentration-response curves for all experiments.

The constricting effect of nonselective a-adrenergic receptor stimulation was evaluated by adding cumulative doses of norepinephrine from 10^{-10} to 10^{-4} mol/L. Concentration-response curves were constructed, and the concentration of norepinephrine exhibiting 50% maximal contraction (EC_{50}), as determined by logarithmic curve-fitting equations, and the maximal tension were calculated. The modulating effect of the endothelium on the vascular response to norepinephrine was assessed by calculating the ratio of the maximal tension in a ring with endothelium to the maximal contraction in the matching ring without endothelium (norepinephrine ratio) for each rat. At the end of one series of experiments, the a-adrenergic receptor antagonist yohimbine hydrochloride (Sigma; 10^{-5} mol/L) was also administered before the generation of norepinephrine concentration-response curves. The effects of specific a1-adrenergic receptor stimulation with methoxamine hydrochloride (Sigma) were also investigated at concentrations ranging from 3 × 10^{-8} to 10^{-3} mol/L. EC_{50} and the methoxamine ratio (the ratio of the maximal tension in a ring with endothelium to the maximal contraction in the matching ring without endothelium) were calculated. In some rings, potassium chloride (Fluka Chemical; 1 to 60 mmol/L) concentration-response curves were also obtained to directly assess the contractile apparatus.

Clonidine hydrochloride (Sigma; 10^{-6} to 10^{-4} mol/L) was administered after precontraction with methoxamine to assess the response of rings with and without endothelium to a-adrenergic receptor stimulation. The precontraction with methoxamine was maximal in some rings, whereas in others, the contraction-induced tension was matched between rings from heart failure and sham-operated rats. Concentration-response curves, the maximal relaxation (expressed as a percentage of the initial contraction), and IC_{50} (the concentration producing 50% of the maximal relaxation) was calculated.

To verify the findings from our previous study, norepinephrine (3 × 10^{-8} to 1 × 10^{-7} mol/L) was administered to the rings in a concentration that would obtain a stable contraction and approximate the developed tension of the matching control. In rings with intact endothelium, acetylcholine concentration-response curves were then generated by adding cumulative doses of acetylcholine (10^{-5} to 10^{-2} mol/L). The EC_{50} of acetylcholine and the maximal relaxation were measured. On rings in which the endothelium had been mechanically removed, cumulative concentrations of sodium nitroprusside from 10^{-10} to 10^{-3} mol/L were given, and the EC_{50} and the maximal relaxation were measured.

At the end of one series of experiments, the effect of nitric oxide synthase inhibition was assessed by adding L-NAME (synthesized at F. Hoffmann-La Roche Ltd, Basel, Switzerland; 3 × 10^{-3} mol/L) to the aortic rings at rest and incubating for at least 10 minutes. Subsequently, concentration-response curves with norepinephrine (10^{-10} to 10^{-4} mol/L) and methoxamine (3 × 10^{-8} to 10^{-2} mol/L) were obtained; EC_{50} and the ratio of the maximal tension in a ring with endothelium to the maximal contraction in the matching ring without endothelium were calculated and compared with the responses in the absence of L-NAME.

cGMP Assays

Fresh thoracic aortic segments (=5 to 10 mg) with and without endothelium were incubated for at least 60 minutes in isolated organ baths with Krebs-Henseleit solution and gassed with a 95% O2/5% CO2 mixture. The aortas were then removed from the organ baths and immediately frozen in liquid nitrogen and stored at −80°C until the time of assay. Aortas were homogenized for 60 seconds (Potter S Homogenizer, B Braun, Melsungen AG, Germany) in 1 mL 6% trichloroacetic acid and then sonicated for 10 seconds. All samples were then centrifuged at 2500g for 15 minutes at 4°C. The protein pellet was saved for protein determinations, and the supernatant was extracted with 3 volumes of water-saturated diethyl ether. The ether phase was discarded, and the samples were evaporated to dryness at 50°C under a vacuum with a Vortex-Evaporator (Haake Buchler Instruments, Fairfield, NJ) for subsequent use in the cGMP assay.

The cGMP content of the vessels with and without endothelium was determined with a commercially available kit (Cyclic GMP [125I] RIA Kit NEX-133, Biotechnology Systems, NEN Research Products, DuPont, Dreieich, Germany). The residue samples were dissolved in 1 mL of 0.5 mol/L sodium acetate buffer, pH 6.2, and acetylated with 5 μL of acetic anhydride and triethylamine (1:2 vol/vol). Standard curve samples were prepared, and 50-μL aliquots of the samples were added to 150 μL of buffer in each assay tube (duplicates).
Succinyl-cGMP-tyrosine methyl ester-[125I] with normal rabbit serum (100 μL) and cGMP antiserum complex (100 μL) was added to all tubes and incubated for 18 hours. Cold sodium acetate buffer (1 mL) was added to each tube, mixed, and then centrifuged for 15 minutes at 4°C and 2000g. The supernatant was discarded, and the residual radioactivity of the tubes was counted in a gamma counter (Kontron Instruments, Switzerland). Values for the cGMP content of the samples were obtained from the standard curve by use of a data analysis program for the Macintosh personal computer (ASSAYZAP, version 2.0; Biosoft, Cambridge, UK). The protein content was measured by the Folin phenol reagent method with bovine serum albumin standards, and cGMP values are presented as femtomoles per microgram of protein.

Pathological Studies

The hearts were trimmed, and the right ventricular free wall was dissected away from the left ventricle. Ventricular weights were obtained to provide a rough estimate of ventricular masses and then immediately placed in a 10% formalin solution. The size of the infarct was estimated by a method previously described, in which ten 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, Tokyo, Japan), and values were averaged to yield a percentage of ventricular infarction for each rat.

Study Design and Statistical Analysis

The design of this investigation consisted of studying two groups of rats (sham and CHF) at 1 week after myocardial infarction. All statistical comparisons were performed with a commercially available statistical package for the Macintosh personal computer (STATVIEW, version 4.0, Abacus Concepts, Inc, Berkeley, Calif). Unpaired two-tailed t tests comparisons were made between the CHF and sham groups. Paired two-tailed t tests were performed to evaluate the effects of yohimbine on the norepinephrine responses and L-NAME on norepinephrine and methoxamine responses. Differences were considered significant at a level of P<.05, and results are expressed as the mean±SEM of at least 6 rats.

Results

Hemodynamic and Baseline Characteristics

Twenty-seven CHF rats were included in this study and were compared with 27 age-matched sham-operated control rats. The hemodynamics and physical characteristics of these two groups are presented in Table 1. Although the rats selected to undergo the coronary artery ligation and sham operation had identical weights, the heart failure group weighed significantly less than the sham-operated group at the time of the study. Absolute left ventricular mass was greater in sham-operated rats (638±11 mg) than in those with heart failure (588±17 mg), but when indexed for body weight, left ventricular mass was greater in the heart failure group. Right ventricular mass was markedly increased in the heart failure group when expressed as an absolute mass (sham, 159±3 mg; CHF, 278±19 mg; P<.0001) and when indexed for body weight. The mean arterial blood pressure, left ventricular systolic pressure, and the maximal rate of rise in the left ventricular pressure were significantly lower in heart failure rats than in controls, whereas the left ventricular end-diastolic pressure was markedly elevated in the heart failure group (Table 1), as would be expected given the selection criteria. The size of the myocardial infarction

<table>
<thead>
<tr>
<th>Table 1. Physical Characteristics and Hemodynamic Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Body weight, g</td>
</tr>
<tr>
<td>LV mass, g/kg</td>
</tr>
<tr>
<td>RV mass, g/kg</td>
</tr>
<tr>
<td>HR, bpm</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
</tr>
<tr>
<td>LV +dP/dt, ×10^5 mm Hg/s</td>
</tr>
</tbody>
</table>

CHF indicates chronic heart failure; LV, left ventricle; RV, right ventricle; HR, heart rate; bpm, beats per minute; MAP, mean arterial blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; and LV +dP/dt, maximal first derivative of left ventricular systolic pressure.

*P<.01.

in the heart failure rats was 42±1%, and there was no evidence of infarction in the sham-operated animals.

Vascular Responses to Norepinephrine

The contractile response of the vascular smooth muscle to norepinephrine and the modulating effect of the endothelium were investigated in heart failure and sham-operated control rats (Fig 1A). In rings without endothelium, the maximal contraction to norepinephrine was decreased by 32%, whereas the EC50 was markedly higher (Table 2) in the heart failure group than in controls. In rings with endothelium, the maximal contraction to norepinephrine was increased by 42% in heart failure rats compared with controls, but the EC50 was not significantly different (Table 2). The relative contribution of the endothelium in this abnormal vascular response to norepinephrine was assessed in each rat by calculating the ratio of the maximal contraction in a ring with endothelium to the maximal contraction in a ring without endothelium, the norepinephrine ratio. In control rats, the norepinephrine ratio was <0.4, demonstrating the normal modulating effect of the endothelium on the contraction to norepinephrine. The ratio was significantly higher in heart failure rats than in controls (sham, 0.39±0.05; CHF, 0.83±0.05; P=.0001), suggesting a decreased modulating effect of the endothelium in the CHF rats.

Role of α1-Adrenergic Receptors

The role of α1-adrenergic receptors in the abnormal contractile response to norepinephrine was investigated with methoxamine, an α1-adrenergic receptor agonist (Fig 2A). In rings without endothelium, the maximal contraction to methoxamine was decreased in heart failure rats, and the EC50 was significantly greater in the heart failure group (Table 2) than in controls. Despite this marked impairment in vascular smooth muscle responsiveness to α1-adrenergic receptor stimulation, the methoxamine concentration-response curves of heart failure and sham-operated control rings with endothelium were nearly identical. The maximal contraction to methoxamine was similar in heart failure rats and controls, and the EC50s were also similar (Table 2). The relative contribution of the endothelium in this
endothelium were increased, but the norepinephrine ratio was not significantly changed by yohimbine. In the heart failure group, maximal contractile responses were decreased, whereas the EC50s were increased, in rings both with and without endothelium. The norepinephrine ratio was significantly decreased in heart failure rats by yohimbine. Comparisons between the two groups of rats were also performed with an unpaired t test, which demonstrated a trend toward increased contraction in rings with endothelium (P = .08) but no difference in rings without endothelium (P = .77) from the heart failure group compared with the sham-operated rats. The EC50s in rings with endothelium were identical, although the heart failure group had significantly lower EC50s in denuded rings than controls (P < .001). The norepinephrine ratio continued to be increased in the heart failure group as well (P = .048).

The role of α1-adrenergic receptors in the abnormal vascular response to norepinephrine was also assessed by relaxation concentration-response curves to the α1-adrenergic receptor agonist clonidine. Clonidine was administered in rings with and without endothelium precontracted with methoxamine to matching levels of tension. Clonidine caused no significant relaxation in rings with or without endothelium and was not different between heart failure and sham-operated groups.

Status of Contractile Apparatus

To assess the status of the contractile apparatus independent of receptor-mediated stimulation, cumulative doses of potassium chloride were administered (Fig 3). Significantly decreased maximal contractions were evident in rings both with and without endothelium in heart failure rats compared with sham-operated controls (Table 2). There were no significant differences between the EC50s of rings from heart failure and sham-operated rats.

Role of Nitric Oxide

Concentration-response curves obtained in aortic rings with endothelium demonstrated no difference in acetylcholine-induced relaxation; the EC50 and the maximal relaxation expressed as a percentage of the initial contraction were not significantly different in the heart failure compared with the control group (Table 2). Aortic rings without endothelium from heart failure and sham rats were exposed to cumulative concentrations of sodium nitroprusside to investigate endothelium-independent relaxation. The concentration-response curves for the heart failure rats were not different from the control curves, nor was the sodium nitroprusside EC50 or maximal relaxation response different between heart failure and sham rats (Table 2).

The nitric oxide synthase inhibitor L-NAME was added to the organ baths of rings with and without endothelium from heart failure and sham-operated rats, and norepinephrine (Fig 1B) and methoxamine (Fig 2B) concentration-response curves were generated. L-NAME did not affect the response to norepinephrine in rings without endothelium. However, in intact rings, the attenuating effect of the endothelium on norepinephrine-induced contraction was almost completely eliminated by L-NAME. Maximal contractions from the sham-operated and heart failure groups approached those in the respective rings without endothelium, such that the norepinephrine ratio

Role of α1-Adrenergic Receptors

The role of α1-adrenergic receptors in the abnormal vascular response to norepinephrine was first assessed by norepinephrine concentration-response curves in aortic rings pretreated with the α1-adrenergic receptor antagonist yohimbine (Table 3). Paired comparisons of norepinephrine responses in the presence and the absence of yohimbine within the same rings were performed for both the heart failure and sham-operated groups. In the sham-operated rats, yohimbine had a minimal effect on the maximal contraction of rings with endothelium, although it significantly decreased the maximal contraction induced by norepinephrine in denuded rings to a level similar to that observed in the heart failure group. The EC50 in rings with and without abnormal vascular response to methoxamine was also assessed in each rat by calculating the methoxamine ratio, the ratio of the maximal contraction in a ring with endothelium to the maximal contraction in a ring without endothelium. This ratio was significantly higher in heart failure rats than in controls (sham, 0.43 ± 0.03; CHF, 0.62 ± 0.05; P = .001), suggesting a decreased modulating effect of the endothelium on the methoxamine-induced contraction in CHF rats.
TABLE 2. Selected Concentration-Response Curve Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Sham</th>
<th>CHF</th>
<th>P, Unpaired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine contraction, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>9</td>
<td>3.42±0.15</td>
<td>2.31±0.18</td>
<td>.0003</td>
</tr>
<tr>
<td>+E</td>
<td>9</td>
<td>1.34±0.19</td>
<td>1.90±0.18</td>
<td>.048</td>
</tr>
<tr>
<td>Norepinephrine EC₅₀, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>9</td>
<td>10±1</td>
<td>3.3±0.4</td>
<td>.0004</td>
</tr>
<tr>
<td>+E</td>
<td>9</td>
<td>49±8</td>
<td>31±7</td>
<td>.11</td>
</tr>
<tr>
<td>Methoxamine contraction, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>9</td>
<td>3.58±0.19</td>
<td>2.37±0.20</td>
<td>.0003</td>
</tr>
<tr>
<td>+E</td>
<td>9</td>
<td>1.50±0.09</td>
<td>1.48±0.16</td>
<td>.87</td>
</tr>
<tr>
<td>Methoxamine EC₅₀, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>9</td>
<td>47±6</td>
<td>78±5</td>
<td>.002</td>
</tr>
<tr>
<td>+E</td>
<td>9</td>
<td>1.4±0.5</td>
<td>1.4±0.2</td>
<td>.92</td>
</tr>
<tr>
<td>KCl contraction, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>6</td>
<td>2.48±0.14</td>
<td>1.52±0.08</td>
<td>.0002</td>
</tr>
<tr>
<td>+E</td>
<td>6</td>
<td>2.35±0.13</td>
<td>1.64±0.07</td>
<td>.0009</td>
</tr>
<tr>
<td>SNP Relax, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>6</td>
<td>100±1.0</td>
<td>100±1.0</td>
<td>.98</td>
</tr>
<tr>
<td>+E</td>
<td>6</td>
<td>95±3</td>
<td>92±5</td>
<td>.40</td>
</tr>
<tr>
<td>SNP EC₅₀, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>6</td>
<td>13±3</td>
<td>15±6</td>
<td>.76</td>
</tr>
<tr>
<td>ACh EC₅₀, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-E</td>
<td>6</td>
<td>103±23</td>
<td>75±23</td>
<td>.42</td>
</tr>
</tbody>
</table>

CHF indicates chronic heart failure; Contraction, maximal contraction in response to given agonist; -E, without endothelium; +E, with endothelium; EC₅₀, concentration at which 50% maximal effect was obtained; SNP, sodium nitroprusside; ACh, acetylcholine; and Relax, maximal relaxation, expressed as percentage of maximal contraction.

in the presence of L-NAME was 0.98±0.05 in the sham group and 1.01±0.02 in the heart failure group (P=.52). The responses to methoxamine in the presence of L-NAME were similar; there was no effect on the contraction in rings without endothelium. In rings with endothelium, the maximal contraction was almost identical to that in denuded vessels in each group, and the methoxamine response in the presence of L-NAME was 0.99±0.03 in the sham group and 0.98±0.02 in the heart failure group (P=.93). Thus, L-NAME eliminated the attenuating effect of the endothelium on maximal contractions in both norepinephrine and methoxamine, although there was still a mild rightward shift in the EC₅₀ in intact rings from sham-operated rats that was not inhibited by L-NAME.

The basal release of nitric oxide was also assessed biochemically by determination of cGMP content in aortic segments with and without endothelium from heart failure and sham-operated rats (Fig 4). In segments without endothelium, there was no significant difference between the cGMP content of the vessels from the two groups. However, there was a marked decrease in cGMP content of aortas with endothelium from rats with heart failure compared with those from sham-operated rats. The amount of additional cGMP stimulation induced by the endothelium in heart failure rats was only 32% of the controls (sham, 145±29 versus CHF, 47±18; P=.019), providing further evidence for a marked decrease in the basal release of nitric oxide in heart failure.

**Discussion**

The present study in rats with heart failure shows that there is a marked increase in vascular responsiveness to norepinephrine in intact vessels and that this increased vasoconstriction is endothelium dependent. Abnormal function of the contractile apparatus, as assessed by maximal contraction to potassium chloride, and decreased sensitivity of α₁-receptors contribute to reduced vasoconstriction in vascular rings without endothelium. In rings with endothelium, however, abnormal endothelial modulation of the vascular response to norepinephrine is evident at 1 week after myocardial infarction, with increased maximal tension in intact rings. This abnormal responsiveness in rats with heart failure is the result of decreased basal release of nitric oxide, despite normal relaxation to acetylcholine and sodium nitroprusside.

CHF secondary to 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. The predictive power of this model may be because it produces pathophysiological alterations similar to those seen in the most common contemporary cause of heart failure in humans, namely, ischemic heart disease. The presence of heart failure, "the pathophysiologic state in which an abnormality of cardiac function is responsible for failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues, or to do so only from an elevated filling pressure," was demonstrated by the markedly increased left ventricular end-diastolic pressure during selection of the rats and was supported by the decreased blood pressure and myocardial contractility. These hemodynamic abnormalities were associated with increases in left and right ventricular masses, which corresponds to findings of previous studies. The absence of any confounding diseases, such as atherosclerosis, hypercholesterolemia, diabetes mellitus, or hypertension, in this model of segmental myocardial damage provides an opportunity to investigate the vascular response to heart failure independently. The dramatic alterations in neurohumoral factors in heart failure have received increasing attention as their relation to the severity of heart failure and prognosis has been recognized. The interaction between these neurohumoral alterations and myocardial adrenergic receptors has been extensively studied, although alterations in the peripheral adrenergic receptors have been less well characterized.

In our study, the vascular smooth muscle from rats with heart failure had markedly decreased maximal responses to norepinephrine. It is known that vascular smooth muscle cells have both α₁ and α₂ postjunctional

![Methoxamine concentration (Conc.)-response curves in aortic rings without (A) and with (B) pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME). The curves are labeled by group (Sham [dashed line, open symbols] and chronic heart failure [CHF, solid line, closed symbols]) and the presence (+E, squares) or absence (−E, circles) of endothelium. In B, the administration of L-NAME resulted in elimination of the attenuating effect of the endothelium on the maximal contraction in both the sham and CHF groups.](image)

### Table 3. Effects of Yohimbine on Responses to Norepinephrine

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without Yohimbine</th>
<th>With Yohimbine</th>
<th>P, Paired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal contraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−E</td>
<td>3.42±0.15</td>
<td>2.29±0.18</td>
<td>.0001</td>
</tr>
<tr>
<td>+E</td>
<td>1.34±0.19</td>
<td>0.97±0.16</td>
<td>.07</td>
</tr>
<tr>
<td>CHF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−E</td>
<td>2.31±0.18</td>
<td>2.20±0.25</td>
<td>.02</td>
</tr>
<tr>
<td>+E</td>
<td>1.90±0.18</td>
<td>1.56±0.28</td>
<td>.009</td>
</tr>
<tr>
<td>EC₅₀, nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−E</td>
<td>10±1</td>
<td>195±25</td>
<td>.0006</td>
</tr>
<tr>
<td>+E</td>
<td>49±8</td>
<td>248±34</td>
<td>.002</td>
</tr>
<tr>
<td>CHF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−E</td>
<td>3.3±0.4</td>
<td>63±8</td>
<td>.0005</td>
</tr>
<tr>
<td>+E</td>
<td>31±7</td>
<td>247±51</td>
<td>.004</td>
</tr>
<tr>
<td>Norepinephrine ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.39±0.05</td>
<td>0.44±0.08</td>
<td>.57</td>
</tr>
<tr>
<td>CHF</td>
<td>0.83±0.04</td>
<td>0.69±0.08</td>
<td>.03</td>
</tr>
</tbody>
</table>

CHF indicates chronic heart failure; −E, without endothelium; and +E, with endothelium.
adrenergic receptors\textsuperscript{35-38} that mediate vasoconstriction,\textsuperscript{39} and one study in the dog ventricular pacing model\textsuperscript{16} suggests that increased \( \alpha \)-adrenergic receptor mechanisms are involved in the vascular smooth muscle response to heart failure. In the present study, however, there was not only a marked decrease in the norepinephrine-induced contraction in rings from rats with heart failure but also decreased contraction in response to both methoxamine and potassium chloride. The increased EC\textsubscript{50} for the selective agonist methoxamine suggests a reduced sensitivity of the \( \alpha \)-adrenergic receptors in rats with heart failure. Although the maximal contraction was decreased in response to both norepinephrine and methoxamine, we cannot conclude that there are a reduced number of receptors, since the decreased responsiveness to potassium chloride is suggestive of an abnormality in the contractile apparatus itself and makes evaluation of receptor number on the basis of maximal contraction unreliable.

In intact aortic rings from rats with heart failure, marked increases in the maximal contraction to norepinephrine were demonstrated despite the decreased responsiveness of endothelium-denuded vessels in our study, although there were no changes in the EC\textsubscript{50}. In studies of the dorsal pedal artery from dogs with pacing-induced heart failure,\textsuperscript{15-17} the maximal response to norepinephrine in intact rings was also markedly increased over controls, but in contrast to this study, lower EC\textsubscript{50}s were also observed. However, in coronary arteries in the same dog model,\textsuperscript{40} the maximal tension induced by norepinephrine was markedly reduced.

These findings suggest that there may be regional differences in the vascular response to heart failure, although the exact mechanism of this differential response is unknown.

Our study demonstrates that this increased responsiveness to norepinephrine in heart failure is endothelium dependent. The ratio of the maximal contractile response in intact to denuded rings was markedly increased in response to norepinephrine, suggesting that the increased maximal tension of the rings with endothelium was caused by either pronounced decreases in the normal attenuating effect of the endothelium or by secretion of vasoconstricting factors by the endothelium, or both.

There are many possible mechanisms by which norepinephrine responses may be increased in vessels with endothelium from rats with heart failure, including (1) increased overall vasoconstrictor effect of norepinephrine, probably via an \( \alpha \)-adrenergic receptor pathway; (2) downregulation of vasoconstricting endothelium \( \alpha \)-adrenergic receptors; (3) a generalized defect in either the stimulated or basal EDRF pathway; or (4) endothelial secretion of a vasoconstricting factor.

The first possible mechanism, an increase in vascular smooth muscle sensitivity to the vasoconstricting effects of norepinephrine, is argued against not only by the increased norepinephrine ratio, but also by a 32\% decrease in the maximal contraction in rings without endothelium from rats with heart failure. These results are supported by the findings with the \( \alpha \)-specific agonist methoxamine, which also produced a 34\% decrease in the maximal contraction in denuded rings from heart failure rats over that in controls. Thus, increased vascular smooth muscle responsiveness does not appear to play a role in the increased vasoconstriction to norepinephrine observed in our study.

The second possible mechanism, a downregulation of the vasodilating \( \alpha \)-adrenergic receptors, was investigated with the specific \( \alpha \)-agonist clonidine. Norepinephrine can cause vasodilation through the \( \alpha \)-adrenergic receptor endothelium-mediated nitric oxide pathway,\textsuperscript{41} but clonidine induced no vasodilation in rings from either of the groups of rats. This finding is consistent with those of others,\textsuperscript{42} who have noted minimal or no \( \alpha \)-adrenergic receptor-mediated vasodilation in rat aorta. Previous
studies in other vessels from different species with heart failure demonstrate either no change in α₁-adrenergic receptor reactivity in the dog or human peripheral vasculature or increased endothelium-dependent relaxation in response to stimulation of α₁-adrenergic receptors in dog coronary arteries. In addition, the increased vascular responsiveness was still evident with the α₁-adrenergic receptor agonist methoxamine. Thus, a specific α₁-receptor defect does not seem to be the cause for the increased responsiveness to norepinephrine in rats with heart failure.

Decreased endothelium-dependent relaxation has already been reported in studies of heart failure, and thus, the third possible mechanism, a generalized defect in either the stimulated or basal EDRF pathway, seemed likely. In femoral arteries of dogs and pulmonary arteries of rats with heart failure, significantly decreased acetylcholine-induced relaxations were noted, although relaxation to nitrroglycerin was preserved. Studies in humans with severe heart failure have consistently demonstrated abnormalities in endothelium-mediated relaxation as assessed by changes in the coronary microvasculature, forearm blood flow, and superficial femoral artery. We selected the time of 1 week after myocardial infarction for our study because our previous study had demonstrated that there was no evidence of endothelial dysfunction as assessed by acetylcholine-induced relaxation at this time. This finding is confirmed in this study by the lack of any difference between heart failure and sham-operated rats in the relaxation response to acetylcholine. Thus, a generalized abnormality in stimulated EDRF is not supported.

However, the use of agents that stimulate EDRF release does not allow an evaluation of the basal release of this factor, which may involve a different mechanism. In this study, the effect of baseline nitric oxide release on the vascular response in intact rings was tested with the nitric oxide synthase inhibitor L-NAME. L-NAME completely eliminated the attenuating effect of the endothelium on the contractile response to norepinephrine and methoxamine, suggesting that decreased basal release of nitric oxide was responsible for the decreased modulating effect of the endothelium in heart failure. These findings are confirmed by the markedly decreased cGMP levels noted in rings from heart failure rats, as shown in Fig 4. Nitric oxide has been shown to promote vasodilatation via the stimulation of intracellular cGMP production and thus, intracellular cGMP levels have been widely used as indications of nitric oxide release. The incubation of the aortic ring segments for at least 60 minutes allowed the effects of other agents that can increase cGMP levels, such as atrial natriuretic factor, to abate, although it has been demonstrated that the contribution of endogenous atrial natriuretic factor to basal aortic cGMP is minor compared with that of nitric oxide. Measurements in segments both with and without endothelium allowed an assessment of the amount of cGMP production induced by the endothelium itself. Thus, it appears that decreased basal nitric oxide release is responsible for the increased responsiveness of the intact rings to norepinephrine. Other studies have reported that endothelium-dependent basal nitric oxide release also appears to be impaired in the coronary circulation of rats and in the systemic circulation of dogs with heart failure. However, ours is the first study, to the best of our knowledge, that demonstrates that a dissociation of the stimulated and basal release of nitric oxide can lead to pathophysiological abnormalities.

Conclusions
This study demonstrates that the increased vascular responsiveness to norepinephrine in intact vessels from rats with heart failure is the result of decreased basal nitric oxide release, despite preserved responses to acetylcholine and sodium nitroprusside. The dissociation of basal and stimulated nitric oxide release may play a pathophysiological role at an early stage of heart failure, and further studies will be needed to elucidate the significance of these findings for patients with CHF.

Acknowledgments
We wish to gratefully acknowledge the superb technical assistance of Danièle Rothé and Evelyne Dornbierer.

References


47. Rapoport RM, Murad F. Agonist induced endothelium-dependent relaxant in rat thoracic aorta may be mediated through cyclic GMP. Circ Res. 1983;52:352-357.


Increased vascular responsiveness to norepinephrine in rats with heart failure is endothelium dependent. Dissociation of basal and stimulated nitric oxide release.

J R Teerlink, G A Gray, M Clozel and J P Clozel

Circulation. 1994;89:393-401
doi: 10.1161/01.CIR.89.1.393

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/1/393

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/