Hemodynamic Stresses Induce Endothelial Dysfunction and Remodeling of Pulmonary Artery in Experimental Compensated Heart Failure

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Background — We hypothesized that, in compensated heart failure (HF), hemodynamic perturbations and their consequences exist in pulmonary artery (PA) despite the absence of any perturbation in thoracic aorta (TA).

Methods and Results — The left coronary artery was ligated in 20 male Wistar rats with compensated HF. Four months after ligation, these rats were compared with 20 sham-operated control rats. Blood pressure, velocity, viscosity, luminal diameter, and wall tensile and shear stresses were determined in PA and TA. Arterial rings were mounted in a myograph for ex vivo study. Endothelial nitric oxide synthase (eNOS) mRNA expression was determined in lung and aorta. Sections of PA and TA were used for histomorphometric study. In PA from rats with compensated HF, (1) blood pressure and wall tensile stress increased, whereas blood velocity and wall shear stress decreased; (2) contractions to KCl were not altered, but maximal contraction to phenylephrine and EC50 decreased; (3) endothelium-dependent relaxation to acetylcholine and basal NO activity were blunted, whereas endothelium-independent relaxation was preserved; (4) eNOS mRNA levels and eNOS transcription in lung nuclei decreased; and (5) medial cross-sectional area, thickness, smooth muscle cell number, elastin, and collagen contents increased. Conversely, no such changes were found in TA from rats with compensated HF.

Conclusions — In compensated HF induced by small myocardial infarction, hemodynamics, vascular wall function, and structure are altered in PA but preserved in TA. These results indicate that the pulmonary vascular bed is an early target of regional circulatory alterations in HF. (Circulation. 2000;101:2764-2770.)

Key Words: stress ■ endothelium ■ remodeling ■ vasculature ■ heart failure ■ arteries

Left ventricular failure due to infarction has hemodynamic consequences both to pulmonary circulation upstream and aortic circulation downstream. Nevertheless, for the same cardiac output, hemodynamic changes differ between pulmonary artery and thoracic aorta; blood pressure increases upstream to failing left ventricle and tends to decrease downstream. The pulmonary artery and thoracic aorta differ both structurally and functionally. The pulmonary arterial bed is a capacitive vascular system in which small changes in pressure induce large changes in dimensions.1 Ontkean et al2 showed that endothelial dysfunction was present in both pulmonary artery and aorta at a decompensated stage of chronic heart failure. Huang et al3 showed decreased expression of angiotensin-converting enzyme, a protein mainly expressed by endothelial cells, in lung. Sakai4 et al showed an increase in endothelin expression in lung without change in kidney.

Nevertheless, to the best of our knowledge, no information is available about the functional and structural status of pulmonary artery versus thoracic aorta in the early compensated stage of heart failure (HF). At this stage, pulmonary artery and thoracic aorta are submitted to different local hemodynamics. Therefore, we examined differential effects of mechanical factors on structure and function of large arteries by studying in the same animal pulmonary circulation and thoracic aorta.

We used the model of left ventricular myocardial infarction of small size, in which cardiac output was not detectably perturbed at rest, and demonstrated that pulmonary hypertension precedes any change in aortic hemodynamics and may be responsible for pulmonary endothelial dysfunction and wall remodeling.

Methods

Experimental Design
Male Wistar rats (IflaCreo, Lyon, France) that weighed 225±25 g were used. Left ventricular infarction was produced by ligation of the left descending anterior coronary artery.3 In sham operations, the heart was manipulated in the same way. Four months after the surgical procedure, rats were anesthetized for hemodynamic study. Only rats with compensated HF...
(ie, with normal cardiac index at rest ≥20 mL/min per 100 g) were included in the study. These rats (20 of 40 operated) underwent the pharmaco logical and histomorphometric study described below (sham-operated rats, n=20; compensated HF rats, n=20).

The present investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996), Bethesda, Md.

**Hemodynamic Study**

Four months after undergoing surgical coronary artery ligation, rats were anesthetized with pentobarbital (50 mg/kg IP), intubated, and ventilated. Thoracic aorta and pulmonary artery blood velocity and diameter were measured in vivo in closed chest by use of an echocardiographic apparatus with a 10-MHz frequency probe (E-acte AU 4 Ideo). Thoracic aortic lumen diameter was measured in a bidimensional longitudinal parasternal view 5 mm beyond insertion of the aortic valve. Pulmonary artery lumen diameter was measured in a bidimensional axial parasternal view 5 mm beyond the insertion of the pulmonary valve. Mean blood velocity was measured with a pulsed Doppler coupled with an echocardiographic in the thoracic aorta and pulmonary artery at the same levels of measurement as for diameters.

As described elsewhere, a microtip catheter was introduced into the right carotid artery and advanced into the ascending aorta. A left thoracotomy was performed, and the pulmonary artery was dissected free. Another microtip catheter was placed in the pulmonary artery to record simultaneously aortic and pulmonary arterial pressures. The system was allowed to stabilize for 5 minutes. Left thoracotomy did not modify aortic pressure. All parameters were calculated on a beat-to-beat basis for 30 seconds and averaged. Aortic blood then was collected to measure blood viscosity.

Pulse pressures were calculated as the difference between systolic and diastolic blood pressures. Blood flow was calculated as the product of blood velocity and arterial lumen cross-sectional area. Cardiac output was calculated as the product of aortic integral time and diastolic blood pressures. Blood flow was calculated as the product of blood velocity and arterial lumen cross-sectional area.

**Infarct-Size Determination**

Animals received a lethal dose of pentobarbital, and heart, lungs, and aorta were removed. Heart and ventricles were weighed. The left ventricle was opened with an incision along the septum from base to apex. Myocardial infarction size was measured.

**Viscosity Measurement**

Whole-blood viscosity was measured at 37°C with a Brookfield model LVT cone-plate viscosimeter at different shear rates; plasma viscosity was measured at 37°C with Ostwald capillary viscosimeters.

**Pharmacological Study**

Rings of aorta and pulmonary artery 3 mm in length were mounted in an organ bath. After determination of maximum response to K+ (60 mmol/L)-cumulative concentration–response curves to phenylephrine (10−9 to 10−4 mol/L) and acetylcholine (10−7 to 10−3 mol/L) were established. Basal nitric oxide (NO) production was assayed by adding Nω-nitro-L-arginine methyl ester (L-NAME; 10 μmol/L) to rings precontracted with phenylephrine (10% maximum). Maximum arterial dilation was obtained with sodium nitroprusside (SNP; 10−4 mol/L). Rings were blotted dry and weighed (in milligrams) at the end of the experiments, and results were expressed as millinewtons force per milligram tissue for phenylephrine and percentage relaxation for acetylcholine.

**Comparative Reverse Transcription–Polymerase Chain Reaction of Endothelial NO Synthase mRNA Expression**

RNAs were extracted from frozen lung and aorta. Reverse transcription (RT) was performed for 1 hour at 37°C on 200-ng rat-lung RNA in the presence of 1 mg of oligo d(T)12-18. Primers for endothelial NO synthase (eNOS) polymerase chain reaction (PCR) included 5′-TTC CGG CGT GCA CCT GAT CCT TAA-3′ (sense) and 5′-AAC ATG TGT CCT TGC TCG AGG CA-3′ (antisense) and were designed to allow amplification of a 340-bp fragment. Primers for GAPDH, which amplify a 299-bp mRNA region, included 5′-GTG AAG GTC GGA GTC AAC-3′ (sense) and 5′-GTT GAA GAC GCC AGT GGA GTC-3′ (antisense). Quantification of PCR products was performed by use of [γ-32P] primers. Twenty-eight cycles of PCR for lung and 29 for aorta were performed. For GAPDH, number of cycles was 24 for lung and 27 for aorta. Products were electrophoresed. Bands corresponding to the amplified fragments were excised, dissolved in periodic acid, and counted. eNOS mRNA expressions were normalized to that of GAPDH mRNA.

**Run-On Assay of eNOS**

Nuclei from lungs (n=5 per group) were isolated as described by Boggaram and Mendelson. Each transcription-elongation reaction was performed in the presence of 20×106 isolated nuclei. The nuclear suspension was incubated with 10 mmol/L each of CTP, ATP, GTP, and 32P-labeled UTP (800 Ci/mmol; IsotopChim). Samples were extracted, precipitated, and resuspended in Tris-EDTA buffer. Equivalent amounts of radioactive RNA (7×106 cpm) from sham and compensated HF lungs were added to slot blots. Hybridization to denatured probes (10 μg) dot-blotted on nylon membranes was performed at 52°C for 2 days. Probes used were 10 μg of the plasmid-containing insert of eNOS cDNA or 10 μg of GAPDH cDNA. Bluescript KS plasmid and pGEM plasmid were used as controls. After hybridization was complete, blots were washed and exposed to Kodak Biomax MS film with an intensifying screen at −80°C for 15 to 21 days. Relative intensity of eNOS band was determined as ratio of eNOS to GAPDH intensity.

**Morphological Study**

Thoracic aorta and pulmonary artery were fixed in situ under mean arterial blood pressure by a perfusion of 4% formaldehyde for 30 minutes. Segments of these 2 vessels, 0.5 to 1 cm in length, were then removed, dehydrated, and embedded in paraffin. Longitudinal and transverse sections (5 μm thick) were stained with Sirius red for collagen fibers, orcein for elastin, and neutral red for nuclei. Slides were analyzed in an automatic-image analyzer. Repeated measurements were pooled and averaged in corresponding stained sections of aortic and pulmonary arterial wall media of each animal.

**Statistical Analysis**

Results are expressed as mean±SE. The experimental design allowed us to use 1-way and 2-way ANOVA to show differences due to compensated HF and interaction between compensated HF and arterial localization of the measurement (pulmonary versus aortic parameters). Differences between groups were evaluated with Scheffé’s F test.

**TABLE 1.** Body and Heart Weights

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Compensated HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>533±5</td>
<td>532±11</td>
</tr>
<tr>
<td>Heart/BW, mg/g</td>
<td>2.19±0.04</td>
<td>2.63±0.11†</td>
</tr>
<tr>
<td>Left ventricle/BW, mg/g</td>
<td>1.64±0.02</td>
<td>1.86±0.04‡</td>
</tr>
<tr>
<td>Right ventricle/BW, mg/g</td>
<td>0.55±0.03</td>
<td>0.77±0.08*</td>
</tr>
</tbody>
</table>

Values are mean±SE. BW indicates body weight.

*P<0.02, †P<0.01, ‡P<0.001 vs sham-operated control group.
Results

Cardiac and Hemodynamic Characteristics
Infarct size averaged 27±2% of the left ventricle in rats with compensated HF. Cardiac index was similar in compensated HF and in sham-operated controls (23±1 versus 22±1 mL/min per 100 g, respectively; P=NS). Left ventricular infarction was associated with right ventricular hypertrophy (40±14%; P<0.02) and pulmonary hypertension (38±7%; P<0.01) without change in aortic blood pressure (Tables 1 and 2). Blood viscosity was similar in rats with compensated HF and controls (0.026±0.001 versus 0.027±0.001 poise, respectively).

Pulmonary arterial diameter, wall tension, and tensile stress increased, whereas blood velocity and wall shear stress decreased in rats with compensated HF (Table 2). No hemodynamic changes were found in thoracic aorta. A powerful statistical interaction occurred between compensated HF and the pulmonary arterial site for measurement of arterial diameter (F=16; P<0.001), blood velocity (F=9; P<0.01), and wall shear stress (F=14; P<0.001).

Pharmacological Studies

Pulmonary Artery
Maximal contraction to KCl of pulmonary artery rings from rats with compensated HF was similar to controls (1.8±0.5 versus 1.9±0.3 mN/mg, respectively). Concentration-response curves to phenylephrine obtained in pulmonary artery rings from rats with compensated HF were signifi-
cantly desensitized compared with control curves; maximal contraction response and EC50 were decreased (1.6±0.1 versus 2.6±0.2 mN/mg, P<0.01, and 3.2±0.6 versus 5.7±0.8×10⁻⁸ mol/L, P<0.05), respectively, in rats with compensated HF (Figure 1A).

Maximal relaxation to acetylcholine was reduced in rats with compensated HF (49±8 versus 74±7% in controls; P<0.05). EC50 for relaxation was ~4-fold greater in rats with compensated HF (116±42 versus 27±7×10⁻⁸ mol/L in controls; P<0.05; Figure 2A). Antagonism of basal NO activity to induce pulmonary-ring contraction by L-NAME was significantly reduced in rats with compensated HF (0.56±0.10 versus 1.28±0.31 mN/mg in controls; P<0.05). Endothelium-independent relaxation to SNP was not significantly different between the 2 groups of rats (91±2% in HF versus 94±6% in controls).

**Thoracic Aorta**

Maximal contraction to KCl (3.1±0.3 in HF versus 2.6±0.4 mN/mg in controls) as well as the concentration-response curves to phenylephrine (Figure 1B), and the response to L-NAME (1.75±0.4 versus 1.8±0.3 in HF versus controls, respectively) of thoracic aorta rings obtained from rats with compensated HF and from controls were similar.

Concentration-response curves to acetylcholine and the endothelium-independent relaxation to SNP (92±8 in HF versus 87±5% in controls, NS) were similar in the 2 groups of rats (Figure 2B).

**eNOS mRNA Expression**

Because eNOS expression has been shown to be dependent on shear stress, we used eNOS mRNA expression as a marker of endothelial dysfunction. Two-way ANOVA showed that eNOS mRNA levels evaluated by comparative RT-PCR were significantly higher in lung than aorta (F=80; P<0.001). A significant interaction occurred between the site of analysis (lung or aorta) and compensated HF (F=13; P<0.001); eNOS mRNA levels did not differ in the aorta (F=0.2; P=NS) but significantly differed in the lung (F=13; P<0.01; Figure 3A). These results were confirmed by run-on assay, which showed significantly decreased in vitro transcription of eNOS by lung nuclei from rats with compensated HF (n=5; t=4.9, P<0.05) (Figure 3B).

**Histomorphometry**

Pulmonary hypertension secondary to myocardial infarction induced an increase in medial cross-sectional area, thickness,
arterial pulse pressure was similar in compensated HF and increased in rats with compensated HF, whereas pulmonary values. Nevertheless, mean pulmonary arterial pressure was and wall shear stress decreased in comparison with control diameter, and wall tension increased, whereas blood velocity artery. Therefore, pulmonary arterial blood pressure, lumen affected in the aorta but were modified in the pulmonary ar-terial wall function and structure may exist in the pulmonary rest, hemodynamic perturbations with consequences on arte-

Discussion
The objective of the present study was to show that, in compensated HF, when cardiac output is not yet modified at rest, hemodynamic perturbations with consequences on arterial wall function and structure may exist in the pulmonary artery when aortic hemodynamics and wall structure remain unmodified.

In the compensated HF group, hemodynamics were not affected in the aorta but were modified in the pulmonary artery. Therefore, pulmonary arterial blood pressure, lumen diameter, and wall tension increased, whereas blood velocity and wall shear stress decreased in comparison with control values. Nevertheless, mean pulmonary arterial pressure was increased in rats with compensated HF, whereas pulmonary arterial pulse pressure was similar in compensated HF and control groups. Indeed, because in each rat blood flow was identical in aorta and pulmonary artery, the observed decrease in pulmonary-wall shear stress and a part of the increase in pulmonary-wall tensile stress were most likely due to changes in pulmonary artery luminal dimensions. Because of low stiffness (or high compliance) of the pulmonary arterial vasculature, small changes in blood pressure induce large increases in pulmonary arterial dimensions. This influences shear stress but also amplifies the increase in tensile stress by means of application of the law of Laplace. Therefore, pressure-dependent and dimension-dependent early changes in mechanical stresses observed in pulmonary circulation could modify local expression and activity of autacoid factors such NO or the intracellular signaling response to these factors. Differences in alterations of vasomotor tone and vascular structure between pulmonary artery and thoracic aorta in rats with compensated HF could be a consequence of local hemodynamically induced changes in wall shear and tensile stresses.

Changes in resistance vessels have been reported previously. Because our present study was aimed at investigating the effect of changes in mechanical stresses on the structure of large arteries, we have no data concerning behavior of systemic and pulmonary resistance vessels and skeletal muscles.

Given that the maximal response to KCl was unchanged and the response to phenylephrine decreased, we suggest that the intrinsic contractility of pulmonary artery is not affected, whereas the sensitivity and maximal response to exogenous α1-adrenergic stimuli are reduced in compensated HF. Specific local changes in hemodynamics and wall structure could modify signals within the target cells in response to catecholamines. The fact that the dose-response curve to phenylephrine was modified in pulmonary artery but not in aortic rings provides evidence for this hypothesis and confirms previous data.

Pulmonary artery endothelial function was blunted in rats with compensated HF. The same results were reported in rats with decompensated HF. Unchanged relaxation to SNP in rats with compensated HF suggests that the ability of smooth muscle to respond to NO was preserved. Thus, the defect in NO activity reported in the pulmonary artery of rats with compensated HF could be due in part to diminished NO production or release by endothelial cells. Basal NO activity was assessed by measuring the increase in force that occurred when arterial rings were exposed to L-NAME. Decreased response to L-NAME in the pulmonary artery rings from rats with compensated HF suggests that basal NO activity was reduced under these experimental conditions. Similar results were reported at a decompensated stage.

One physiologically important stimulus for the release of NO is the value of shear stress. Therefore, we measured eNOS expression as a possible marker of hemodynamically induced endothelial dysfunction. We showed that eNOS mRNA levels were similar between controls and compensated HF in aorta and significantly different in lung. This last result was confirmed by run-on assay, which showed a decrease in transcription in the lung in compensated HF. Previous studies performed to explore endothelial dysfunc-
tion used large myocardial infarctions,17,18 which induced significant decreases in cardiac output. It is possible that a chronic decrease in shear stress, which develops as a consequence of low cardiac output, alters endothelial cell function so that production of NO is reduced.19 In agreement with our results, Le Cras et al reported that a reduction in pulmonary artery blood flow with unchanged blood pressure induced reduction in eNOS expression in normoxic adult rat lung,20 whereas eNOS expression is not altered in response to increased pulmonary flow.21 In contrast to these results but in agreement with the present results, Bauersachs et al17 did not detect any significant decrease in eNOS mRNA expression in aorta of myocardial infarcted rats, and Varin et al18 recently reported a decrease in eNOS mRNA expression in the skeletal muscle in decompensated HF. Nevertheless, because we used lung tissue to evaluate eNOS expression, cellular sources other than endothelium could contribute to this difference in eNOS expression. Therefore, in the present study, a decrease in wall shear stress might alter endothelium-dependent vasodilation. Pulmonary hypertension induced by left ventricular infarction was associated with a significant increase in pulmonary artery luminal diameter that contributed to reduced pulmonary artery wall shear stress and increased tensile stress. These 2 factors may lead to the endothelial dysfunction observed in the present study. The inactivation of NO by superoxide anions could also participate in endothelial dysfunction.17

Previous studies reported an impairment in endothelial function in thoracic aorta of rats with myocardial infarction-induced HF.2,15,17 However, in these studies, congestive HF was due to large myocardial infarction and resulted in a marked alteration in aortic hemodynamics. Differences in results between these studies and the present one thus are probably due to a smaller infarct size in the present investigation, which permitted the coexistence of preserved aortic hemodynamics with an altered pulmonary artery circulation.

In parallel with the alteration in vasoreactivity, arterial wall remodeling was assessed in thoracic aorta and pulmonary artery. The main findings are absence of thoracic aorta remodeling and expansive pulmonary artery remodeling in

<table>
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<th>TABLE 3. Histomorphometric Parameters</th>
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<tr>
<td><strong>Site</strong></td>
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<td><strong>Model Effect</strong></td>
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<tr>
<td>Media CSA, $10^3 \mu m^2$</td>
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<tr>
<td>Sham</td>
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<tr>
<td>Compensated HF</td>
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<tr>
<td>Media wall thickness, $\mu m$</td>
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<tr>
<td>Sham</td>
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<tr>
<td>Compensated HF</td>
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<tr>
<td>Elastin content, %</td>
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<td>Sham</td>
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<tr>
<td>Compensated HF</td>
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<td>Elastin content per CSA, $10^3 \mu m^2$</td>
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<td>Compensated HF</td>
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<td>Collagen content per CSA, $10^3 \mu m^2$</td>
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<td>Elastin/collagen</td>
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<td>No. of SMC nuclei per CSA</td>
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<td>SMC nuclei size, $\mu m^2$</td>
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<tr>
<td>Compensated HF</td>
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Values are mean±SE. CSA indicates cross-sectional area; SMC, smooth muscle cell.

*P<0.05, †P<0.01 vs sham-operated control group.
rats with compensated HF. Therefore, differential hemodynamic stresses and strains are probably one of the main determinants of differential remodeling between pulmonary artery and aorta in compensated HF.

In conclusion, in compensated HF induced by left ventricular infarction, (1) hemodynamics, vasomoticity, and vascular wall structure are altered in the pulmonary artery but remain unmodified in the thoracic aorta and (2) pulmonary alterations may be related to chronic decrease in shear stress and increase in tensile stress. These results indicate that the pulmonary circulation may be one of the first regional targets of hemodynamic changes in compensated HF.

Acknowledgments

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