Comparison of Hearts With 2 Types of Pressure-Overload Left Ventricular Hypertrophy

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Abstract—Comparisons of myocardium remodeled by the 2 most common causes of left ventricular hypertrophy (LVH), hypertension and aortic constriction, are limited. We hypothesized that important differences may exist in the myocardium of hearts with these 2 origins of “pressure overload” LVH. Accordingly, we studied isolated hearts from 3 groups of Dahl salt-sensitive rats, controls, and hearts with matched amounts of LVH secondary to either hypertension or aortic constriction. Isovolumic LV function and myocardial energetics (31P nuclear magnetic resonance spectroscopy) were measured as coronary flow was lowered to 16% of baseline for 48 minutes. During this low-flow ischemia, isovolumic end-diastolic pressure, a measure of LV stiffness, increased to 52±4 mm Hg in controls and 51±6 mm Hg in aortic banded hearts but to only 35±5 mm Hg in hearts with hypertensive LVH. In all hearts, the P resonance in the 31P nuclear magnetic resonance spectrum, whose position indicates myocardial pH, split into 2 peaks during low-flow ischemia, which indicates distinct regions of pH 6.9 (moderate acidosis) and pH 6.2 (severe acidosis). Concentrations of ATP, PCr, Pi, and H+ of the moderately acidic region were not different among groups. However, the size of the severely acidic region was smallest in the hypertensive LVH hearts, and in all 3 groups, the size of this region correlated (r²=0.65 to 0.80) with the degree of LV stiffening. We conclude that in Dahl rats, LVH secondary to hypertension protects against ischemia-induced diastolic dysfunction by minimizing the size of the region of severe acidosis. (Hypertension. 2000;35:1167-1172.)

Key Words: hypertrophy, left ventricular ■ hypertension, essential ■ stenosis aortic ■ ischemia ■ metabolism

Left ventricular hypertrophy (LVH) can have many origins, which may include hypertension, aortic stenosis, and congenital or genetic defects. LVH secondary to hypertension and aortic stenosis are often considered together to be “pressure overload” hypertrophy, although studies that directly compare hearts hypertrophied secondary to these 2 causes are limited.1-2 Because hypertension and aortic stenosis place different mechanical loads on the heart, present the heart with different neuroendocrine environments, and result in chronically different coronary artery perfusion pressures, it seems likely that differences exist in the biochemistry of myocardium remodeled by hypertension and aortic stenosis. How these differences in remodeling affect the myocardial response to ischemia is of particular clinical interest. Although it is generally thought that hypertrophied myocardium, especially the subendocardium, has a poor tolerance to ischemia, the causes of this phenomenon, and which forms of LVH it applies to, are not known.3-6

Accordingly, the goal of the present study was to determine whether hearts with LVH secondary to hypertension respond differently to low-flow ischemia than do hearts with the same degree of LVH secondary to aortic constriction. To examine the mechanism underlying any differences between the groups, the concentrations of ATP, phosphocreatine (PCr), inorganic phosphate (P), and H+ were monitored with 31P NMR spectroscopy throughout the study. Isolated rat hearts were perfused with a solution that contained a normal hemocrit and the metabolic substrates of glucose, lactate, and free fatty acids. To facilitate comparisons between groups, we studied Dahl salt-sensitive rats. With this model, an equal degree of LVH could be induced by means of hypertension or aortic constriction in a strain of rat that could also serve as a nonhypertrophied control. Hearts with LVH were studied at only one time point during the natural history of LVH.

Methods

Experimental Groups and Protocol
Three groups of Dahl-salt sensitive rats were studied at 12 weeks of age: a control group and 2 groups that developed LVH by means of different origins. The control group (n=6) consisted of sham-operated rats that were maintained on a low (0.1%) salt diet from 8 to 12 weeks of life and did not develop hypertension or LVH. The second group (n=11) was placed on a high salt diet (8%) from 8 to 12 weeks of life to induce hypertension and LVH. In the third group of rats (n=10), LVH was induced by proximal aortic banding at age ~4 weeks.3 These aortic-banded rats were maintained on a low-salt...
diet for the subsequent 8 weeks and did not develop hypertension. Pilot data had indicated that at 12 weeks of age, approximately equal amounts of LVH could be induced using these 2 different interventions. Tail-cuff systolic pressures were recorded in each animal before euthanatization.

The protocol for studying the isolated hearts consisted of 32 minutes of baseline perfusion followed by 48 minutes of low-flow ischemia in which coronary flow was reduced to 16% baseline. LV pressure was continuously monitored, and values for LV systolic and diastolic pressures were recorded every 4 minutes. Two nuclear magnetic resonance (NMR) spectroscopic measurements were made during the 8 minutes before coronary flow was decreased. Once coronary flow was decreased, 4 sequential NMR measurements were made. Three more consecutive measurements were made during the final 12 minutes of low-flow ischemia.

**Experimental Preparation**

An isolated, isovolumic rat heart (Langendorff) preparation perfused with a red blood cell–containing perfusate was used. Hearts were rapidly excised from rats and cannulated by the aorta on a constant-flow perfusion apparatus. Flow was set at ~2.5 mL min⁻¹ g⁻¹ heart weight in each heart on the basis of pilot experiments in which this level of perfusion was found to yield a coronary perfusion pressure of 80 to 100 mm Hg. A fluid-filled latex balloon attached to a Statham P23db pressure transducer (Gould) was inserted through the mitral valve into the LV. The balloon was filled until an LV end-diastolic pressure (EDP) of 10 mm Hg was achieved, and the mitral valve into the LV. The balloon was filled until an LV end-diastolic pressure (EDP) of 10 mm Hg was achieved, and the balloon volume was then held constant so that changes in EDP reflected changes in LV diastolic compliance. Salt-bridge pacing wires consisting of PE-160 tubing filled with 2% agarose and 4 mol/L KCl and tipped with 4 cm of nonmagnetic wire were positioned to make contact with the heart. Hearts were paced at a rate of 5.8 Hz (350 bpm). Hearts were inserted into a 20-mm-diameter glass NMR tube. Coronary perfusion pressure and LV pressure were monitored with a MacLab data acquisition system. All data were sampled at 200 Hz and stored on a hard disk.

**Perfusion Solution**

The perfusion solution consisted of packed bovine red blood cells resuspended in a phosphate-free modified Krebs-Henseleit solution at a hematocrit of 40%. The modified phosphate-free Krebs-Henseleit buffer contained (in mmol/L) NaCl 118, KCl 4.7, CaCl₂ 2.0, MgSO₄ 1.2, NaHCO₃ 25.5, glucose 5.5, lactate 1.0, NaEDTA 0.5, insulin 15 μU/mL, and palmatic acid 0.4 in combination with 4% BSA (No. A7030, Sigma Chemical Co). The perfusate was equilibrated with 20% oxygen/3% carbon dioxide/77% nitrogen to achieve a PO₂ of ~140 mm Hg and pH 7.4. To make the red-cell perfusate feasible for 31P-NMR spectroscopy, KH₂PO₄ was not included in the Krebs-Henseleit buffer. To reduce the extracardiac signal originating from red blood cells in the NMR-sensitive volume, a solution of mannitol (0.2 mol/L) was superfused around the heart at twice the coronary flow rate to provide rapid removal of venous effluent.

**NMR Spectroscopy**

Myocardial energetics were studied with 31P NMR spectroscopy. Briefly, spectra were collected with the resonance frequency for 31P of 161.94 MHz in a GE-400, 9.4-T spectrometer at a pulse width of 27 μs, to give a 60° flip angle. An interpulse delay of 2.14 seconds was used, which enabled 104 scans to be collected in each 4-minute period. Individual free-induction decays were zero-filled and weighted with a 20-Hz line-broadening decaying exponential before Fourier transformation.

**Data Analysis and Statistics**

The area of the P, PCr, and [γ-P] of ATP resonances for each 31P spectrum was measured by use of commercially available software (NMR1). From fully relaxed spectra (interpulse delay, 10 seconds) we determined that the area under the P, PiCr peaks needed to be corrected for partial saturation by multiplying them by 1.15 and 1.2, respectively. Area units were converted to intracellular concentrations with the assumption that [ATP] was 10.8 mmol/L in each heart during the control period as previously reported. Setting the area under the [γ-P] of the ATP peak during the control period of each heart equal to 10.8 mmol/L provided a method of conversion of the PCr and Pi Cr area units into concentrations. Intracellular pH was measured by comparing the chemical shift between P, and PCr resonances.

To calculate metabolite concentrations when the P, resonance area had split into 2 distinct peaks required that the fraction of the heart in each of the 2 metabolically distinct regions be calculated. The fraction in the low-pH (severely ischemic) region was calculated as the number of molecules of P, in this region divided by the concentration of P, in this region. The number of molecules of P, in the severely ischemic region was calculated from the resonance area of the P, peak at pH 6.2. The concentration of P, in the severely ischemic region was assumed to be equal to the total phosphate concentration ([P,] + [PCr] + 3×[ATP]) during the baseline period (~60 mmol/L). This assumption was based on prior work that showed that all PCr and ATP are hydrolyzed under conditions of ischemia severe enough to decrease pH to 6.2. To calculate metabolite concentrations in the moderately ischemic region, we assumed that all ATP and PCr were in the region of the heart that was not severely ischemic.

Ten to fifteen seconds of physiological data were analyzed at 4-minute intervals throughout the protocol in each heart. All values for physiological data and energetics are expressed as mean±SEM. One-way ANOVA was performed at the end of the baseline period and at the end of low-flow ischemia with Statview II statistical program. For all statistical tests, P<0.05 was considered statistically significant.

**Results**

Systolic arterial blood pressure measured in vivo was significantly higher in the hypertensive group (204±25 mm Hg) than in the sham (136±1 mm Hg) or banded (153±10 mm Hg) groups. The degree of LVH, as assessed by the ratio of LV weight to body weight, was well matched in hypertensives (3.1±0.13 mg/g) and banded rats (3.2±0.08 mg/g) and significantly greater than in shams (2.7±0.17 mg/g).

**Contractile Function of Isolated Hearts**

At baseline, coronary flow was 2.37±0.13 mL min⁻¹ g⁻¹ in shams, 2.64±0.13 mL min⁻¹ g⁻¹ in banded rats, and 2.49±0.10 mL min⁻¹ g⁻¹ in hypertensives (P=NS), which indicated a close matching of perfusion levels among the groups. This condition resulted in a significantly higher coronary perfusion pressure in banded (96±1 mm Hg) and hypertensive (96±1 mm Hg) rats than in shams (81±1 mm Hg), which indicated a higher coronary vascular resistance in the hypertrophied hearts. In the isolated hearts, baseline LV systolic pressures were significantly greater in the banded (119±5 mm Hg) and hypertensive (111±5 mm Hg) groups than in shams (81±1 mm Hg). During low-flow ischemia, coronary flow per gram of heart was well matched among the 3 groups: 0.38±0.02 mL min⁻¹ g⁻¹ in shams, 0.41±0.02 mL min⁻¹ g⁻¹ in banded rats, and 0.42±0.02 mL min⁻¹ g⁻¹ in hypertensives (P=NS). During the first 5 minutes of low-flow ischemia, coronary perfusion pressure decreased to 19 to 25 mm Hg, after which it gradually increased such that at the end of the
low-flow ischemia, coronary perfusion pressure was 28 to 35 mm Hg in each group of hearts. Coronary perfusion pressure during the low-flow ischemia did not differ among the 3 groups. After 10 minutes of low-flow ischemia, EDP began to increase in all 3 groups, which indicated LV stiffening (Figure 1). EDP increased less after 48 minutes of low-flow ischemia in the hypertensive group (EDP of 35±5 mm Hg) than in the banded (51±6 mm Hg) or sham (52±4 mm Hg) groups. Not only was diastolic function better in hearts with hypertensive LVH at the end of low-flow ischemia, but LV developed pressure was significantly larger (36±2 mm Hg) than in shams (28±3 mm Hg) or banded rats (29±3 mm Hg).

Cardiac Energetics

In Figure 2 are representative 31P NMR spectra at baseline, after 46 minutes of low-flow ischemia, and after 24 minutes of reperfusion in a heart with LVH secondary to aortic banding. At baseline, no significant differences existed among the 3 groups of hearts in [ATP], [Pi], or pH, but [PCr] was significantly lower in the hypertensive hearts than in shams (Figures 3 and 4).

During low-flow ischemia, the Pi resonance of each heart split into 2 distinct peaks at 4.0 and 4.9 PPM (Figure 2). Because the position of the Pi resonance reports pH, the fact that 2 distinct Pi peaks developed in each heart indicates that 2 regions of different pH developed, in this case regions of pH 6.2 (4.0 PPM) and pH 6.9 (4.9 PPM) (Figure 4). As soon as the 4.0-PPM peak appeared in each heart, the heart was modeled as 2 metabolically distinct regions, 1 of moderate ischemia (pH 6.2) and 1 of severe ischemia (pH 6.9). The percentage of the heart in the 6.2 pH region at the end of the low-flow ischemia was less in the hypertensives (16±3%) than in the shams (32±2%) or banded rats (26±2%) (Figure 5). The position of this Pi resonance was relatively stable during the final 35 minutes of low-flow ischemia in all groups, which indicates that the pH of this region was stable. A strong positive correlation occurred between EDP and the fraction of the heart in the severely ischemic region in all groups (r²=0.65 in shams, 0.80 in banded rats, and 0.67 in hypertensives).

The metabolic characteristics of the pH 6.9 region of the heart are shown in Figures 3 and 4. The gradual decrease in
groups of hypertrophied hearts as well as in controls, severe metabolic inhomogeneity existed during low-flow ischemia. Third, this inhomogeneity was characterized by a region of severe ischemia, the size of which strongly correlated with the loss of LV compliance.

LVH and Ischemia-Induced Diastolic Dysfunction
Many previous studies including those from our laboratory have demonstrated that the hypertrophied heart responds relatively poorly to ischemia.3–6 Therefore, our finding that hypertension-induced LVH can result in improved ischemic tolerance was somewhat surprising. Despite the perception that LVH worsens ischemic tolerance, the experimental literature indicates that LV function during ischemia can be better, worse, or the same when hypertrophied and nonhypertrophied hearts are compared, depending on the experimental preparation.1,3–6,12–15 Key factors that affect the response are the stage of LVH (compensated versus decompensated), type of insult (hypoxia, zero-flow ischemia, low-flow ischemia), type of coronary perfusate (buffer or blood), and whether LV function is assessed during or after the insult. An example of the importance of the stage of LVH comes from Gaasch et al,14 who reported that a moderate degree of LVH secondary to aortic banding did not affect the response to ischemia, but that as LVH progressed to the early stage of heart failure, ischemic tolerance became worse than at baseline. This finding of poor ischemic tolerance during the late stages of LVH is consistent with results of many other studies, including a preliminary report in Dahl salt-sensitive rats, and likely contributes to the perception that all LVH worsens ischemic tolerance.12

Metabolic Inhomogeneity During Low-Flow Ischemia
Research has long recognized that hypoperfusion of the heart results in inhomogeneous changes in myocardial ATP and PCr concentrations with larger decreases in the subendocardium than in the subepicardium.16–18 However, little is known about the extent of this metabolic inhomogeneity, whether it is affected by LVH, or its effect on LV function. In the present study, we report that during low-flow ischemia, regions of identical pH (6.2 and 6.9) developed in both hypertrophied and control hearts. Thus, neither LVH nor the origin of LVH affected the magnitude of the intramyocardial pH gradient. The fact that 2 well-defined, narrow P resonance (as shown in Figure 2) were present and not more resonances or 1 broad plateau, indicated that myocardial pH was not normally distributed during low-flow ischemia but instead was distributed bimodally in all hearts. This suggests that the region of severe acidosis existed in close proximity to the region of moderate acidosis with little if any transition zone.

This magnitude of metabolic gradient within the myocardium has not previously been reported, probably because all previous reports of regional myocardial energetics during hypoperfusion have been done in models with little or any increase in EDP.16–18 An increase in EDP to near or above the coronary perfusion pressure may be needed to cause the
degree of metabolic inhomogeneity that we observed. Also, previous studies have used techniques for quantification of regional myocardial energetics that measure the average composition of a myocardial region and thus underestimate the degree of metabolic inhomogeneity within the heart.\textsuperscript{16–18} The metabolic inhomogeneity we observed appears to have important functional consequences, given that the size of the severely ischemic region correlated strongly with the loss of compliance (increase in EDP) in each of the 3 groups of hearts studied. Whether the smaller region of severe ischemia in hypertension-induced LVH was the cause of the lower EDP in this group or the result of it cannot be determined from our data. Regardless, our data suggest the occurrence of a self-reinforcing "vicious cycle" during low-flow ischemia: energetic deterioration causes a loss of LV compliance, which causes an increase in EDP, leading to restriction of subendocardial blood flow and thus further energetic deterioration. The rate at which this cycle proceeds was apparently lower in hearts with hypertension-induced LVH.

### Split P\textsubscript{i} Resonance

Ours is not the first study to report that the P\textsubscript{i} resonance in the \textsuperscript{31}P spectrum can split in the heart.\textsuperscript{11,19,20} Previous studies in buffer-perfused rat hearts have reported splitting of the P\textsubscript{i} resonance during zero-flow perfusion\textsuperscript{19,20} and reperfusion after zero-flow perfusion.\textsuperscript{11} In the present study, both the size and energetic characteristics of each metabolic region could be calculated if 2 well-supported assumptions were made. The assumptions were (1) that all high-energy phosphates (ATP and PCr) in the severely ischemic region were hydrolyzed and (2) that the P\textsubscript{i} thus produced remained in this region and was not "washed" out. This second assumption was supported by our observation that total myocardial phosphate did not change during low-flow ischemia. The assumption that all ATP and PCr in the severely ischemic region were hydrolyzed was supported by the observation that the pH in this region was in the range reported during conditions in which total depletion of ATP and PCr occurs.\textsuperscript{11,19} Additionally, during reperfusion, the pH of this region demonstrated no tendency to recover and P\textsubscript{i} was rapidly washed out, which suggests that the region was no longer metabolically active. Any low level of ATP that remained in the severely ischemic region during low-flow ischemia would cause a relatively small systematic error in the calculation of the size of this area. Our use of \textsuperscript{31}P NMR spectroscopy to measure cardiac energetics did not allow localization of the metabolically distinct regions of the myocardium. However, studies have consistently demonstrated that blood flow and high-energy phosphate levels decrease more in the subendocardium than in the subepicardium during brief coronary occlusion.\textsuperscript{17,18,21} It is therefore likely that the region of severe ischemia that we observed is the subendocardium, although we cannot claim this definitively.\textsuperscript{22}

### Experimental Model

Our experimental model was designed to mimic several of the important aspects of clinical myocardial ischemia that are often overlooked in experimental studies. First, our coronary perfusate contained a normal hematocrit and oxygen content. This allowed us to simulate the approximate rates of coronary flow and oxygen delivery reported in the ischemic myocardium of patients who have an acute myocardial infarction.\textsuperscript{23} Second, the coronary perfusate contained physiological concentrations of the metabolic substrates normally consumed by the heart, namely, glucose, lactate, and free fatty acids. This was deemed important because it is becoming increasingly clear that the myocardial response to ischemia is highly dependent on the concentrations of the metabolic substrates present during the ischemia.\textsuperscript{24} One limitation of the present study is that it was cross sectional and thus characterized hearts at only 1 time during the complex natural history of LVH.

### Acknowledgments

The present research was supported by National Research Service Award HL-09259 (KWS) and National Institutes of Health SCOR grant HL-55993.

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Hypertension. 2000;35:1167-1172
doi: 10.1161/01.HYP.35.5.1167
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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