Selective Changes in Cardiac Gene Expression During Compensated Hypertrophy and the Transition to Cardiac Decompensation in Rats With Chronic Aortic Banding


Left ventricular hypertrophy (LVH) is associated with reinduction of the fetal program of gene expression. It is unclear whether this pattern of cardiac gene expression changes with the development of left ventricular decompensation and failure. To answer these questions, we quantified steady-state levels of mRNA by the polymerase chain reaction in the left ventricular myocardium of rats 8 and 20 weeks after ascending aortic banding. Clinical and hemodynamic assessment identified two distinct groups of animals 20 weeks after aortic banding. The first group (20-week nonfailed LVH) demonstrated substantial LVH but no depression in systolic developed pressure per gram left ventricular weight compared with the age-matched control group. In contrast, a second group of rats exhibited clinical signs of congestive failure as well as a marked diminution in left ventricular developed pressure per gram. Assessment of the levels of mRNA encoding a panel of cardiac proteins demonstrated a greater than twofold increase in β-myosin heavy chain mRNA and an approximately sixfold increase in atrial natriuretic factor mRNA in left ventricular myocardium of all three groups (8-week LVH, 20-week nonfailed LVH, 20-week failed LVH) when compared with appropriate age-matched control groups. In contrast, Ca$^{2+}$-ATPase mRNA levels were decreased by 50% only in the left ventricular myocardium of animals with both clinical signs and hemodynamic indexes consistent with cardiac decompensation (20-week failed LVH). These results suggest that in rats with ascending aortic banding the hypertrophic phenotype is associated with a selective reinduction of the fetal gene program, which persists even after the development of left ventricular failure. Furthermore, the hypertrophic gene program that accompanies hypertrophy and failure is dissociated from changes in Ca$^{2+}$-ATPase expression. The decrease in Ca$^{2+}$-ATPase mRNA levels may be a marker of the transition from compensatory hypertrophy to failure in these animals.

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KEY WORDS • Ca$^{2+}$-ATPase • heart failure • mRNA

In response to chronic pressure overload, the heart undergoes an increase in mass independent of a change in the number of functional myocytes. This adaptive change is characterized by the preservation of systolic pressure development and the extent of muscle shortening, a decrease in the maximum velocities of shortening and lengthening of the muscle, and a slowing of relaxation. Although the heart is initially able to compensate for the increase in load, recent studies in animal models suggest that a gradual transition occurs from compensated hypertrophy to decompensated heart failure. A similar transition is not uncommon in patients with systemic hypertension or aortic stenosis, in whom gradual chronic pressure overload of the left ventricle results in myocardial hypertrophy with subsequent cardiac dilatation and failure.

It is well known that the adaptive changes in myocardial function that occur after the initiation of hemodynamic stress are associated with alterations in the steady-state levels of mRNA encoding cardiac proteins. In rodents, myocardial hypertrophy secondary to pressure overload is characterized by a recapitulation of the fetal pattern of gene expression as evidenced by a decrease in the levels of α-myosin heavy chain mRNA, a reciprocal increase in β-myosin heavy chain (β-MHC) mRNA, and a shift from a V1 to a V3 predominant myosin heavy chain isoform distribution. In addition, the development of compensated hypertrophy has been associated with an increase in the ventricular levels of the mRNAs encoding atrial natriuretic factor (ANF), and angiotensin converting enzyme and a diminution in the levels of phospholamban and Ca$^{2+}$-ATPase mRNA. The changes in both ANF, angiotensin converting enzyme, and Ca$^{2+}$-ATPase mRNAs are associated with comparable alterations in the amounts of the ANF peptide, angiotensin converting
enzyme activity, and Ca\(^{2+}\)-ATPase protein, respectively. Although it has not been definitively proven that the changes accompanying load-induced hypertrophy are transcriptional, a recent study suggests that the accumulation of β-MHC gene product is regulated primarily through transcriptional mechanisms.\(^{19}\)

Although numerous studies have assessed gene expression in rodents with compensated left ventricular hypertrophy (LVH) secondary to aortic banding or systemic hypertension, critical questions regarding the mechanisms that regulate alterations in cardiac gene expression during the development of hypertrophy remain unanswered.\(^{20}\) Specifically, little information is available regarding the potential changes in gene expression that accompany the transition from compensated hypertrophy to decompensated heart failure. Therefore, the present studies were designed to answer two important questions regarding the adaptive responses of the rat myocardium during the development of hypertrophy and failure after banding of the ascending aorta: (1) Is hypertrophy accompanied by a selective or nonselective regulation of cardiac-specific genes? (2) Is the hypertrophic gene program modified during the transition from compensated hypertrophy to decompensated heart failure?

**Materials and Methods**

**Preparation of Animals**

Male Wistar rats were obtained from Charles River Breeding Laboratories, Wilmington, Mass. After induction of anesthesia with intraperitoneal methohexitol sodium (Brevital), aortic stenosis was created in weanling rats (body weight, 60 to 70 g; age, 3 to 4 weeks) by placing a stainless-steel clip of 0.6-mm internal diameter on the ascending aorta via a thoracic incision (LVH groups). Age-matched control animals underwent a left thoracotomy without placement of the clip. All rats were subsequently fed normal rat chow (Purina, St Louis, Mo) and water ad libitum. Aortic-banded animals and age-matched sham-operated control animals were used 8 or 20 weeks after surgery. Isolated hearts from animals in all groups were subjected to hemodynamic evaluation followed by preservation of the heart tissue for mRNA analysis.

**Perfusion Technique and Hemodynamic Evaluation**

Rats were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg), body weight was recorded, and the thorax was rapidly opened. Each animal was inspected for clinical markers of heart failure, including the presence of ascites, pleural or pericardial effusion, and left atrial enlargement. Within 20 seconds, the heart was removed and perfused by a constant flow pump (MasterFlex, Cole-Parmer Instrument Co, Chicago, Ill) through a short cannula inserted into the aortic root just below the level of the aortic clip, and hearts were perfused using a protocol that has been described previously in detail.\(^{15,21}\) A collapsed latex balloon, slightly larger than the left ventricular chamber such that no measurable pressure was generated by the balloon itself over the range of volumes used, was placed in the left ventricle, and left ventricular pressure was measured with a Statham p23Db pressure transducer (Gould Instruments, Eastlake, Ohio). Coronary perfusion pressure was measured from a side arm of the aortic perfusion cannula, and the coronary flow rate was measured by timed collection of samples of coronary venous effluent.

Hemodynamic evaluation was performed as previously described.\(^{15,21,22}\) The hearts were perfused for a stabilization period of 20 minutes at a paced heart rate of 240 beats per minute, which was continued throughout the experiment. In hearts from rats with aortic stenosis, coronary flow rate was adjusted to achieve a mean coronary perfusion pressure of 100 mm Hg and was then held constant throughout the experiment. In sham-operated control animals, coronary flow rate was adjusted to achieve a mean coronary perfusion pressure of 80 mm Hg and was also held constant throughout the experiment. These differing levels of coronary flow and perfusion pressures were chosen in recognition of the difference between the in vivo coronary perfusion pressure to which the control and aortic stenosis groups were chronically exposed and because prior experiments with these hearts showed that this approach would achieve comparable myocardial perfusion flow rates per gram of ventricular weight.\(^{15,22,23}\)

Left ventricular balloon volume was adjusted to achieve a left ventricular end-diastolic pressure of 10 mm Hg in all groups. This level of end-diastolic pressure was chosen because it allowed the study of the control hearts and the hearts with aortic stenosis at comparable left ventricular balloon volumes at a single common operational point on the left ventricular pressure-volume curves.

At the end of the stabilization period, measurements of left ventricular systolic and diastolic pressure, coronary perfusion pressure, and coronary flow rate were recorded. After hemodynamic evaluation, hearts were rapidly removed from the perfusion apparatus, the right and left ventricles were dissected and weighed, and the samples were snap-frozen in liquid nitrogen and stored in sterile tubes at -80°C.

**Analysis of Steady-State Levels of mRNA**

Total mRNA was extracted from frozen left ventricular myocardium by use of either cesium chloride gradients\(^{24}\) or acid guanidinium thiocyanate/phenol/chloroform (RNAzol B, Tel-Test, Inc, Friendswood, Tex).\(^{25,26}\) The concentration of RNA in each sample was assessed spectrophotometrically, and all samples were stored at -70°C until use. To prevent variations in processing from affecting the experimental results, the samples from each group (eg, 8-week LVH group) were processed at the same time. Furthermore, analysis was only made within an experimental group (eg, 8-week LVH group versus 8-week sham-operated control group).

Assessment of mRNA levels in left ventricular myocardium was performed using the quantitative polymerase chain reaction (PCR) method described previously.\(^{25}\) In brief, first-strand cDNA was synthesized by reverse transcription of 1 μg total RNA using oligo-dT primers according to manufacturer’s instructions (Boehringer Mannheim Corp, Indianapolis, Ind). The cDNA was then amplified in a TempCycler (Coy Laboratory Products Inc, Ann Arbor, Mich) with 2.5 U *Thermus aquaticus* DNA polymerase (Boehringer Mannheim) and 100 μL of 10 mM Tris-HCl containing...
50 mM KCl, 1.5 mM MgCl₂, 0.001% (wt/vol) gelatin, 200 μM of each dNTP, and 13 pmol of specific 5' and 3' primers that had been used previously for PCR amplification of human cardiac genes. Each reaction also contained varying quantities (10 to 30 pg) of an internal cDNA standard prepared by reverse transcription of the in vitro transcription product of a synthetic DNA template. The 3' primer of each primer pair was end-labeled with [γ-³²P]ATP using T₇ poly nucleotide kinase (Pharmacia LKB Biotechnology Inc, Piscataway, NJ). Amplification temperatures were 40°C for phospholamban and 50°C for Ca²⁺-ATPase. The identities of the PCR products were confirmed by sequence analysis.

Ten microliters of each PCR reaction mixture was removed during successive cycles of amplification (12 to 30) and electrophoresed in a 3% (wt/vol) NuSieve GTG/0.5% (wt/vol) SeaKem LE (FMC Corp, Rockland, Me) gel containing Tris acetate/EDTA and ethidium bromide. A Hae III digest of φ×174 DNA (BRL Life Technologies, Inc, Gaithersburg, Md) was used as a molecular size standard. The gel was visualized with indirect UV irradiation and photographed, and appropriate bands representing amplification products of the cDNA of interest and amplification products of the internal cDNA standard were cut out from the gel. Radioactivity in the bands was determined by Cerenkov counting. The amount of radioactivity in each excised gel band was plotted against the number of PCR cycles. A complete cycle was plotted for each reaction, and only those points which represented the linear part of the plot were used for quantification.

To confirm the results obtained using the quantitative PCR technique, Northern blot analysis was also performed. Total cellular RNA (40 μg) from individual hearts was size-fractionated by electrophoresis on a 1.5% agarose-formaldehyde gel and transferred to nylon membranes (Gene Screen, Dupont-NEN Research Products, Boston, Mass) by pressure transfer (Posiblot pressure blower, Stratagene Inc, La Jolla, Calif). Membranes were prehybridized at 42°C overnight in 50% formamide, 5× Denhardt's solution, 0.2% sodium dodecyl sulfate (SDS), and 100 μg/mL salmon sperm DNA and hybridized in the same solution containing specific DNA probes at 42°C for 18 to 20 hours. After hybridization, the blots were washed in varying concentrations of standard saline citrate (SSC) and SDS. The blots then exposed Kodak X-OMAT AR film with an intensifying screen for 0.25 to 7 days at -80°C. The relative amounts of each mRNA were determined by laser densitometry, and densitometric scores of specific mRNAs were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as previously described. DNA probes used in this study included (1) a 0.7-kb Pst I fragment of the cDNA encoding the rabbit cardiac slow-twitch skeletal muscle sarcoplasmic reticulum Ca²⁺-ATPase, (2) an 84-bp synthetic oligonucleotide complementary to the coding region of rat ANF, and (3) a 1.3-kb Pst I fragment of the cDNA encoding rat GAPDH. The cDNA probes were radiolabeled with [α-³²P]dCTP (New England Nuclear, Boston, Mass) using a random-priming method and the synthetic oligonucleotide probes with T₇ poly nucleotide kinase and [γ-³²P]ATP (New England Nuclear).

Statistical Analysis
Comparisons were made between groups of animals with aortic banding and their age-matched and sham-operated control groups using Student's t test. Comparisons among all six experimental groups for developed pressure per gram were obtained using analysis of variance, and within-group differences were assessed by Dunnett's test. All data are presented as mean±SEM.

Results
Characterization of Animals
Animals were killed 8 or 20 weeks after ascending aortic banding. At 8 weeks, body weight was similar in sham-operated age-matched control animals and banded animals. Two separate groups of rats underwent aortic banding or sham operations. The surgery in these two groups was performed by the same investigator but was carried out at different times, and the animals varied in both age (3 to 4 weeks) and size (60 to 70 g). In one group, the banded animals showed no clinical changes associated with heart failure, and these animals were denoted as 20-week nonfailed LVH. In the second group, the banded animals uniformly were found to have small pericardial effusions, pleural effusions, ascites, and marked left atrial enlargement. These clinical signs were consistent with a transition from compensated LVH to cardiovascular decompensation and congestive failure. These animals were denoted as 20-week failed LVH. All three experimental groups had an age-matched sham-operated control group.

As seen in Table 1, left ventricular weight was greater in rats with LVH secondary to aortic banding, compared with age-matched sham-operated control rats in all three groups of animals (8-week LVH, 20-week nonfailed LVH, and 20-week failed LVH groups). In addition, the left ventricular weight to body weight ratio, an index of hypertrophy that accounts for differences in body weight, was increased in all three groups of banded rats when compared with sham-operated control rats. The left ventricular weight to body weight ratio was lower in the 20-week nonfailed LVH group compared with the 20-week failed LVH group.

 Hemodynamics
Peak systolic pressure. As seen in Table 1, left ventricular peak systolic pressure was higher in hearts from 8-week banded animals and 20-week nonfailed animals in comparison with the respective age-matched and sham-operated control hearts. In contrast, peak systolic pressure was similar in the 20-week failed LVH group and the age-matched control group.

Developed pressure per gram left ventricle. In this isovolumic heart model, left ventricular systolic developed pressure normalized per unit of left ventricular mass is proportional to left ventricular systolic wall stress. Therefore, developed pressure per gram left ventricle was calculated for all hearts and is shown in Table 1. Developed pressure per gram left ventricle was similar in both 8-week banded hearts and in 20-week nonfailed LVH hearts when compared with the respective age-matched control hearts. In contrast, the developed pressure per gram left ventricle was significantly lower in the 20-week failed LVH hearts when compared with appropriate control hearts. Similarly, comparison
TABLE 1. Characterization of Animals and Hemodynamics of Isolated Hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>LVW (g)</th>
<th>LVW/BW (g/kg)</th>
<th>PSP (mm Hg)</th>
<th>DevP (mm Hg/g)</th>
<th>Flow (mL/min^-1·g^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Wk LVH</td>
<td>10</td>
<td>481±18</td>
<td>1.891±0.09</td>
<td>3.94±0.13</td>
<td>197±8</td>
<td>100±6</td>
<td>12.2±0.5</td>
</tr>
<tr>
<td>8-Wk sham</td>
<td>11</td>
<td>467±14</td>
<td>1.251±0.07</td>
<td>2.67±0.12</td>
<td>137±4</td>
<td>104±5</td>
<td>12.5±0.5</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>NS</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>20-Wk non-F LVH (compensated hypertrophy)</td>
<td>6</td>
<td>638±12</td>
<td>2.220±0.13</td>
<td>3.49±0.23</td>
<td>215±9</td>
<td>94±7</td>
<td>11.8±1.0</td>
</tr>
<tr>
<td>20-Wk sham</td>
<td>6</td>
<td>585±10</td>
<td>1.563±0.05</td>
<td>2.67±0.05</td>
<td>158±5</td>
<td>95±5</td>
<td>11.2±0.4</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;.007</td>
<td>&lt;.009</td>
<td>&lt;.006</td>
<td>&lt;.003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>20-Wk F LVH (decompensated hypertrophy/failure)</td>
<td>7</td>
<td>491±10</td>
<td>2.070±0.07</td>
<td>4.22±0.15</td>
<td>137±3</td>
<td>65±5*</td>
<td>11.5±0.4</td>
</tr>
<tr>
<td>20-Wk sham</td>
<td>7</td>
<td>571±20</td>
<td>1.328±0.05</td>
<td>2.32±0.05</td>
<td>127±11</td>
<td>92±7</td>
<td>12.7±0.3</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;.0035</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
<td>&lt;.02</td>
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</tr>
</tbody>
</table>

n, Number of rats in each group; BW, body weight; LVW, left ventricular weight; PSP, left ventricular peak systolic pressure; DevP, developed pressure ([systolic−diastolic pressure]/LVW); flow, coronary flow rate/LVW; LVH, hearts with left ventricular hypertrophy studied 8 weeks after ascending aortic bandings; non-F LVH, nonfailing hearts with left ventricular hypertrophy studied 20 weeks after aortic banding; F LVH, failing hearts studied 20 weeks after aortic banding; P, probability value resulting from unpaired Student's t test between hypertrophied groups and their age-matched control groups; NS, P>.05. Values are mean±SEM. *DevP analyzed by analysis of variance including all six groups indicates statistically significant lower DevP in 20-wk F LVH group compared with all other groups.

of developed pressure per gram in all six experimental groups by analysis of variance confirmed the statistical significance of the decrease in developed pressure per gram in the 20-week failed LVH hearts.

Coronary flow. By study design, coronary flow was adjusted to achieve a mean coronary perfusion pressure of 80 mm Hg in the sham-operated control groups and a mean coronary perfusion pressure of 100 mm Hg in the banded groups. This resulted in myocardial perfusion rates per gram left ventricular weight that were similar among all groups, as shown in Table I. By maintaining perfusion rates at similar levels, appropriate comparisons could be made between the various experimental groups. Therefore, the lower developed pressure per gram left ventricle observed in hearts in the 20-week failed LVH group could not be accounted for by lower myocardial perfusion rates in this group.

Steady-state Levels of mRNA in the Left Ventricular Myocardium

Although the quantitative PCR technique has been used in samples of human left ventricular myocardium,26–28 evaluations were performed to ensure that the technique was applicable to rat mRNA. Under the conditions outlined, each PCR reaction yielded only two bands, one representing the product of interest and the second representing the amplification product of the internal cDNA standard. Because primer pairs were designed to amplify regions of the cDNA that spanned splice junctions, sample contamination by genomic DNA could not effect the experimental results. However, the gene encoding phospholamban is intronless. Therefore, to exclude the presence of contaminating genomic DNA when assessing the levels of this mRNA, samples were amplified after deleting reverse transcriptase from the reaction constituents. No bands were detected in these samples, suggesting the absence of significant genomic contamination. The relation be-

between the amount of control RNA and the concentration of total rat myocardial RNA was established independently for each pair of primers so that the slopes of the curves for the rat heart cDNA and the internal standard template cDNA would be similar (Fig 1). Therefore, the amplification efficiency of the two templates could be assumed to be the same within the exponential range. Furthermore, a curve was constructed for each primer pair of interest by amplifying varying amounts of total RNA and internal RNA standard for a predetermined number of cycles. The number of amplification cycles used to construct each of these standard curves was determined from the amplification curve and was in the exponential (linear) range. These curves were used to ensure that amplification was linear with increasing concentrations of both RNA standard and the RNA of interest over a range of several orders of magnitude. With the exception of the α subunit of the stimulatory guanine nucleotide-binding protein (αG), the reaction concentrations used previously for samples of human heart were applicable to rat ventricular myocardium. In the case of αG, mRNA levels were substantially lower in human heart than in rat heart, necessitating an increase in the concentration of the standard construct for αG, from 15 to 30 pg.

The sensitivity of the quantitative PCR assay differed slightly for each mRNA; however, the sensitivity of each assay was at least 5×10^4 molecules mRNA/μg total RNA. The assay was reproducible, with repetitive measurements from a single sample having a coefficient of variation of 12%. Small, though significant, differences were noted between the mRNA levels in the three sham-operated control groups (Table 2). This difference could not be attributable to assay variability because the assay was highly repeatable. More likely, this disparity was due to differences in tissue handling and processing. Previous experiments have demonstrated that, because of the high sensitivity of this assay system,
Fig 1. Plots showing quantitative assessment of Ca$^{2+}$-ATPase, β-myosin heavy chain (βMHC), the α subunit of the stimulatory guanine nucleotide-binding protein (αG), and atrial natriuretic factor (ANF) mRNAs. One microgram of total RNA and an appropriate amount of RNA standard were reverse-transcribed, and the resulting cDNA was amplified for varying cycles in the presence of selected radiolabeled primers. Plots demonstrate mRNA measurements in representative samples of RNA from the left ventricular myocardium of rats with left ventricular hypertrophy and decompensation following 20 weeks of aortic banding (hatched lines) or age-matched sham-operated control myocardium (solid lines). Closed symbols represent the radioactivity in the amplification product from the RNA standard; open symbols represent the radioactivity in the amplification product of total RNA.

Small variations in tissue processing and handling can result in differences in mRNA levels. For this reason, all tissues from each experimental group and its age-matched control group were processed at the same time. Furthermore, statistical comparisons were performed within and not between experimental groups. mRNA was extracted from the 8-week LVH group (and the appropriate sham control group) using the acid guanidinium thiocyanate/phenol/chloroform technique. Although contamination of total RNA by small quantities of tRNA could have a small effect on calculation of the absolute level of an mRNA, it would not be expected to affect the experimental results, because all comparisons were made within the group.

As seen in Table 2, compensated hypertrophy was associated with a twofold increase in the steady-state

<table>
<thead>
<tr>
<th>TABLE 2. mRNA Levels by Quantitative Polymerase Chain Reaction</th>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>8-Wk LVH</td>
</tr>
<tr>
<td>8-Wk sham</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>20-Wk non-F LVH</td>
</tr>
<tr>
<td>(compensated hypertrophy)</td>
</tr>
<tr>
<td>20-Wk sham</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>20-Wk F LVH</td>
</tr>
<tr>
<td>(decompensated hypertrophy/failure)</td>
</tr>
<tr>
<td>20-Wk sham</td>
</tr>
<tr>
<td>P</td>
</tr>
</tbody>
</table>

PHLB, phospholamban; MHC, myosin heavy chain; αG, α subunit of stimulatory guanine nucleotide-binding protein; ANF, atrial natriuretic factor; LVH, hearts with left ventricular hypertrophy studied 8 weeks after ascending aortic banding; non-F LVH, nonfailing hearts with left ventricular hypertrophy studied 20 weeks after aortic banding; F LVH, failing hearts studied 20 weeks after aortic banding; NS, not significant. Numbers in parentheses indicate the number of rats in each group. Values are mean±SEM.
levels of β-MHC mRNA and a sixfold increase in the level of ANF mRNA when compared with sham-operated control values. There was a notable absence of a change in the levels of Ca²⁺-ATPase, phospholamban, and αG, mRNAs in the left ventricular myocardium from these animals relative to the sham-operated control animals. Similar results were seen 20 weeks after aortic banding in animals without clinical signs of congestive heart failure. However, in the group of animals that demonstrated obvious clinical signs of heart failure (20-week failed LVH), the levels of Ca²⁺-ATPase mRNA were approximately 50% less than in age-matched and sham-operated control animals. Additionally, the substantial increases in the levels of β-MHC mRNA and ANF mRNA persisted in these animals with clinical and hemodynamic evidence of heart failure. As seen in Fig 2, there was a linear relation (r = 0.57, P < 0.01) between Ca²⁺-ATPase mRNA levels in the 20-week failed LVH and 20-week nonfailed LVH myocardium and systolic function (developed pressure per gram). Because of differences in the mRNA levels in the sham-operated control groups for the two (20-week failed and nonfailed LVH) groups, values were expressed as a percentage of the mean of the sham-operated control value for each group.

To confirm our findings by PCR, Northern blot analysis was performed using the cDNA encoding the rabbit slow-twitch Ca²⁺-ATPase, the oligonucleotide ANF, and the cDNA encoding GAPDH, a constitutively expressed glycoet enzyme used as an internal control. The ratios of signal intensities for Ca²⁺-ATPase and GAPDH mRNA and the ratios of signal intensities for ANF and GAPDH mRNA were calculated by use of laser densitometry and are shown in Table 3. A fourfold to fivefold increase in ANF was observed both in hearts with compensatory hypertrophy (8-week LVH) and in hearts with failure (20-week failed LVH) when compared with the age-matched control hearts. In contrast, Ca²⁺-ATPase mRNA levels were similar in 8-week hearts with compensatory hypertrophy and age-matched control hearts but were decreased by approximately 30% in 20-week failed LVH hearts when compared with the appropriate control hearts. These changes in Ca²⁺-ATPase mRNA levels in the 20-week failed LVH group as measured by Northern blot analysis were similar, although quantitatively less than those assessed using the quantitative technique (Table 2). Also corroborating the PCR analysis, substantial increases (fourfold to fivefold) in ANF gene expression were noted in both the 8-week LVH and 20-week failed LVH groups when compared with age-matched control groups. A representative Northern blot for Ca²⁺-ATPase is shown in Fig 3.

**Discussion**

The results of the present study demonstrate that in the rat with chronic pressure overload due to banding of the ascending aorta (1) compensatory LVH is associated with a substantial increase in the steady-state levels of the mRNAs encoding β-MHC and ANF, (2) the gene program that characterizes compensated hypertrophy persists during the transition to heart failure, and (3) in contrast, steady-state levels of Ca²⁺-ATPase mRNA decrease only in the presence of hemodynamic changes and clinical findings consistent with cardiac decompensation and congestive failure. These results are consistent with previous studies that demonstrated changes in the levels of the mRNAs encoding both β-MHC and ANF in rats with constriction of the abdominal aorta. However, our results challenge the notion that Ca²⁺-ATPase mRNA levels are decreased only after the development of decompensation and clinical signs of congestive failure in rats with ascending aortic banding is disparate from earlier studies where there was no change in Ca²⁺-ATPase mRNA levels in young rats 1 month after coarctation of the abdominal aorta at a level between the renal and superior mesenteric arteries. However, this decrease in Ca²⁺-ATPase mRNA level was only observed in a subgroup of animals with severe LVH as defined by a left ventricular weight to body weight ratio of >2.6. However, our results are consistent with studies in which mild hypertrophy was
associated with diminished Ca\textsuperscript{2+}-ATPase mRNA levels only in senescent rats.\textsuperscript{29}

It is unlikely that the differences between our results and those published previously can be attributed to differences in the techniques used to assess mRNA levels. The quantitative PCR technique has been well validated in our laboratory\textsuperscript{26-28} and by others\textsuperscript{35,36} and provides quantitative assessment of mRNA levels. Preliminary studies unambiguously confirmed the identity of the amplification products by sequence analysis, verified that the levels of detected mRNA species were within the linear range of the assay, and, in the case of phospholamban mRNA measurements, excluded the presence of contaminating genomic DNA in the samples. Furthermore, full (12- to 30-cycle) amplification curves were performed with each experimental sample, and data were obtained within the linear portion of the curve that spanned at least six amplification cycles. That the RNA standard and the RNA of interest were amplified with identical efficiencies was confirmed by the colinearity of the amplification curves for the control RNA and sample RNA. In addition, the use of Northern blot analysis to assess relative steady-state levels of the mRNA encoding Ca\textsuperscript{2+}-ATPase and ANF confirmed the results obtained by quantitative PCR. Therefore, it is more likely that the results of the present study differ from previous results because of intrinsic differences in the study populations.

Several factors might have contributed to potential differences in our study population and those reported earlier. In previous studies, hypertrophy was defined by the relation between heart weight and body weight at varying times after aortic coarctation. However, in the present study, we have separated compensatory hypertrophy from decompensated hypertrophy with failure by assessing the functional capacity of the ventricular myocardium as well as the clinical status of the animals. This has allowed us to discriminate two groups of animals, the 20-week nonfailed LVH and the 20-week failed LVH groups. Whereas the degree of hypertrophy did not predict levels of Ca\textsuperscript{2+}-ATPase mRNA, only the animals with clinical signs of congestive failure and diminished left ventricular systolic function demonstrated decreases in levels of Ca\textsuperscript{2+}-ATPase mRNA. These results point out the importance of performing hemodynamic evaluations to complement molecular and biochemical measurements.

A second possible explanation for our inability to demonstrate a decrease in Ca\textsuperscript{2+}-ATPase mRNA levels before the onset of decompensation and congestive failure might relate to the fact that our model of ascending aortic constriction is substantially different from the model of abdominal aortic constriction. Although both experimental approaches produce the pressure-overload phenotype, abdominal aortic banding but not ascending aortic banding (B.H. Lorell, unpublished observations) is associated with systemic hypertension. Therefore, the release of neurohumoral agents, growth factors, or proto-oncogenes\textsuperscript{37} from noncardiac tissues might effect changes in Ca\textsuperscript{2+}-ATPase gene expression during compensated hypertrophy in the animals with abdominal banding but influence the expression of Ca\textsuperscript{2+}-ATPase only after the onset of clinical failure and the accompanying decrease in renal blood flow in the group with ascending aortic coarctation. Additionally, substantial hypertrophy developed after only 4 weeks of abdominal aortic banding, whereas we assessed mRNA levels in the presence of somewhat lower levels of hypertrophy 8 and 20 weeks after ascending aortic banding. Therefore, we cannot exclude the possibility that an overlap between normal heart growth and hypertrophy during 5 months of banding in our animals precluded seeing a decrease in Ca\textsuperscript{2+}-ATPase gene expression until the onset of diminished left ventricular function. The extent of hypertrophy in our animals was somewhat less than that reported by others. Although we cannot exclude the possibility that this lesser degree of hypertrophy precluded seeing a decrease in Ca\textsuperscript{2+}-ATPase gene expression in animals without failure, it should be noted that the extent of hypertrophy was comparable in all three experimental groups (ie, 8-week LVH, 20-week nonfailed LVH, and 20-week failed LVH groups) despite their different ages.

Previous studies have demonstrated a close correlation between the levels of the mRNAs encoding Ca\textsuperscript{2+}-ATPase,\textsuperscript{18} ANF,\textsuperscript{14} and β-MHC\textsuperscript{10} and the levels of their gene products in the ventricular myocardium exposed to chronic pressure overload. Although it is likely that the mRNA levels in the present study also reflect the concentrations of the appropriate proteins, we cannot exclude the possibility that either translational or posttranslational modifications could regulate the expression of these gene products. Furthermore, we have measured the steady-state levels of the mRNAs encoding a panel of proteins of interest. Therefore, we cannot
determine whether the changes in steady-state levels are the result of modifications in gene transcription or alterations in the stability of the mRNA.

A characteristic pattern accompanying hypertrophy in small animals is a recapitulation of the fetal phenotype, because both β-MHC and ANF gene expression are expressed at high levels during fetal development but at low levels with maturity. However, a critical question that has remained unanswered is whether hypertrophy results in the upregulation of all cardiac-specific genes. In the present studies, we have demonstrated that, in rats with ascending aortic constriction, the switch to the fetal gene program is not ubiquitous. Both Ca\(^{2+}\)-ATPase and phosphoramidon are expressed at lower levels in fetal shear than in adults, and Ca\(^{2+}\)-ATPase is expressed at lower levels in the fetal rat. However, only ANF and β-MHC mRNA levels were different among compensated hypertrophy in rats with proximal aortic banding. Therefore, in rats with ascending aortic constriction, the hypertrophic phenotype is associated with a selective rather than a promiscuous induction of the fetal gene program. These results would suggest that distinct cis- and trans-acting elements regulate cardiac-specific expression of Ca\(^{2+}\)-ATPase. Specific promoter elements have been identified that contribute to cardiac-specific gene regulation during the development of the hypertrophic phenotype. However, although the 5' upstream regions of the mammalian Ca\(^{2+}\)-ATPase and phosphoramidon have been isolated, the regulatory regions within these genes have not been identified.

Of further importance is the finding that the gene program that characterizes myocardial hypertrophy persisted during the transition of left ventricular dysfunction and clinical failure. These results might suggest that other factors contribute to the transition from hypertrophy to failure. However, it is also possible that the progressive increase in myocyte hypertrophy may in itself lead to failure by progressive impairment of contract and relaxation. Finally, in the rat with ascending aortic banding, steady-state levels of Ca\(^{2+}\)-ATPase mRNA appear to provide a marker for the development of left ventricular decompensation and failure. This observation is consistent with the recent findings that failing human myocardium is characterized by altered Ca\(^{2+}\)-ATPase gene expression and changes in intracellular Ca\(^{2+}\) handling that may contribute to abnormal excitation-contraction coupling. The isolation and characterization of the signals that regulate Ca\(^{2+}\)-ATPase gene expression during the transition from compensatory hypertrophy to failure in rats with ascending abdominal aortic banding may help further to explain the molecular pathophysiology of this process.

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