Progression from hypertrophic to dilated cardiomyopathy in mice that express a mutant myosin transgene

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Am J Physiol Heart Circ Physiol 280: H151–H159, 2001.—A mouse model of hypertrophic cardiomyopathy (HCM) was created by expression of a cardiac α-myosin transgene including the R103Q mutation and a deletion of a segment of the actin-binding domain. HCM mice show early histopathology and hypertrophy, with progressive hypertrophy in females and ventricular dilation in older males. To test the hypothesis that dilated cardiomyopathy (DCM) is part of the pathological spectrum of HCM, we studied chamber morphology, exercise tolerance, hemodynamics, isolated heart function, adrenergic sensitivity, and embryonic gene expression in 8- to 11-mo-old male transgenic animals. Significantly impaired exercise tolerance and both systolic and diastolic dysfunction were seen in vivo. Contraction and relaxation parameters of isolated hearts were also decreased, and lusitropic responsiveness to the β-adrenergic agonist isoproterenol was modestly reduced. Myocardial levels of the G protein-coupled β-adrenergic receptor kinase 1 (β-ARK1) were increased by more than twofold over controls, and total β-ARK1 activity was also significantly elevated. Induction of fetal gene expression was also observed in transgenic hearts. We conclude that transgenic male animals have undergone cardiac decompensation resulting in a DCM phenotype. This supports the idea that HCM and DCM may be part of a pathological continuum rather than independent diseases.

characterized by ventricular chamber dilation with normal or decreased wall thickness and impaired systolic function, which often manifests as heart failure (33). Hypertrophic cardiomyopathy (HCM) is characterized by abnormal cardiac hypertrophy, fibrosis, and myofibrilar disarray (33). Systolic function is typically normal or enhanced, but cardiac relaxation is impaired due to the thickened, fibrotic ventricular walls. Patients can remain asymptotic for many years, or they can display symptoms and consequences of outflow tract obstruction, diastolic dysfunction, or atrial fibrillation, including sudden cardiac death (39).

It has been estimated that 20% of idiopathic DCM and 70% of HCM is familial (26, 33), and recent studies have elucidated the genetic basis of some of these cases. Eight different disease genes have been linked to familial HCM (2, 28). Significantly, all of the eight genes encode distinct molecular components of the cardiac sarcomere, the fundamental force-generating unit of heart muscle fibers. Linkage analysis in families with idiopathic DCM has been less informative, in part because of the later onset of disease, but recently a mutation in cardiac actin, a component of the sarcomeric thin filament, was linked to DCM (32). Mutations in cardiac actin have also been associated with familial HCM (28). In hamsters, both HCM and DCM are caused by mutations in the same gene, δ-sarcoglycan, which encodes a protein of the dystrophin-associated glycoprotein complex (34). In addition, cases in which HCM has progressed to DCM have been reported (18, 42); this decompensation occurs in ~10–15% of patients (37, 38). An important question raised by these findings is whether HCM and DCM are inherently independent diseases or whether these diseases are part of the same pathological spectrum (2).
Valuable insight into the pathogenesis of familial HCM has been gained from studies of the β-myosin heavy chain (β-MyHC) gene. Over 50 mutations at this disease locus have been linked to HCM, and it has been estimated that β-MyHC mutations account for one-third of the familial HCM cases (39). Nearly all of the β-MyHC mutations associated with HCM lie in the “head” region of the heavy chain (2), which includes both the ATPase and actin-binding regions critical for generating muscle force. These mutations are predominantly missense mutations or short deletions that do not disrupt the genetic reading frame, but they instead appear to produce full-length mutated myosin molecules that become incorporated into the sarcomere (40). The advent of mouse models for HCM demonstrated that mutant MyHCs could disrupt the cardiac muscle apparatus, causing a stress on the heart, which results in HCM (10, 45). We engineered a mutant α-MyHC transgene (the normal murine myocardium is almost exclusively α-MyHC) that includes a well-characterized R1445Q missense mutation associated with markedly reduced survival in humans (45). An additional deletion of amino acids 468–527 in the actin-binding domain bridged by nine nonmyosin amino acids allowed the mutant protein to be distinguished electrophoretically from endogenous mouse MyHC. Targeted expression of this transgene in the mouse heart was achieved by using a rat α-MyHC promoter, and 10–12% of total MyHC in purified myofibrils from transgene-positive animals was the mutant protein (45). At 3 mo of age, the hearts of transgene-positive animals display hypertrophy of both right and left ventricles, with a pattern of myocyte hypertrophy, myocellular disarray, interstitial fibrosis, and small vessel coronary disease that closely replicates the human histopathology (45). Areas of severe myocyte damage, examined at the electron microscope level, contained degenerating myofibrils and collagen deposits (45). A gender difference was also observed in these animals. Cardiac hypertrophy increases with age in female animals, but older male animals have dilated left ventricular (LV) chambers, suggesting a more severe phenotype resembling DCM.

The purpose of this study was to determine whether DCM is part of the phenotypic presentation of this transgenic mouse model of HCM. We hypothesized that older male animals would demonstrate not only morphological indications of DCM but also functional and biochemical defects, including systolic dysfunction and adrenergic desensitization. Exercise testing, echocardiography, and isolated heart studies determined cardiac function. Because adrenergic signal abnormalities are well established in DCM, we assessed the sensitivity of isolated hearts to the adrenergic agonist isoproterenol. In addition, we measured adrenergic receptor density, adenyl cyclase activity, and the levels and activity of G protein-coupled receptor kinases (GRK) in heart extracts. We also assessed the expression of genetic markers of cardiac hypertrophy in the HCM animals.

METHODS

Experimental animals. Mice heterozygous for the mutant myosin transgene (45) were backcrossed with C57/B16 mice to generate experimental animals (HCM) and nontransgenic (NTG) littermate controls. The transgene coding region consists of a rat α-MyHC cDNA containing a G1445A point mutation, resulting in Arg483Gln, and a deletion of amino acids 468–527 bridged by the addition of nine nonmyosin amino acids: SerSerLeuProHisLeuLysLeu. Male offspring were genotyped by PCR and allowed to reach 8–11 mo of age under identical conditions, when some of the mice were selected for noninvasive echocardiography and exercise testing. Separate groups of age-matched male mice were euthanized for isolated heart experiments, histology, pharmacology, or RNA extraction. All of the animals were handled according to approved protocols of the University of Colorado.

Treadmill exercise. The mice were exercised on a custom-built eight-lane treadmill with an infrared detection system similar to that previously described (9). The mice were acclimated to the treadmill at a 7° incline with one 15-min low-speed (5–7 m/min) session without the shock grid and two 15-min sessions with the shock grid (5–7 m/min and 20 m/min). The mice were exercised once daily at 20 m/min for 60 min over a 2-wk period. If a mouse became exhausted during exercise, it was removed from the apparatus. Exercise tolerance was measured by counting the average number of infrared beam breaks per minute for each animal over all exercise sessions. To determine exercise endurance, the animals were exercised once at a high speed (27 m/min) for 60 min, and the time at which each animal became exhausted was recorded.

Echocardiography. We performed transthoracic echocardiography with the use of a System Five echocardiography machine (Vingmed, Horton, Norway) with a 10-MHz phased-array transducer. Each mouse was injected intraperitoneally immediately before imaging with successive 0.05- to 0.3-ml doses of 20 mg/ml tribromoethanol (Avertin) until mild sedation was achieved. The chest was shaved, and the mouse was positioned on its abdomen on a 1.25-cm-thick acoustic standoff pad. Heart rates were monitored by electrocardiography during image acquisition. M-mode recordings were acquired in an M-mode format. To maximize temporal resolution, images were displayed off-line from the original R0 sampling information for measurements by using Echo-Pack software (Vingmed). Measurements from three cardiac cycles per animal were averaged.

Histology. Hearts were rapidly excised after cervical dislocation and placed in phosphate-buffered saline while still beating to allow blood to be pumped out of the cardiac chambers and coronary vessels. The hearts were then placed in a 10:1 volume of 10% Formalin to tissue for fixation. The fixed hearts were embedded in paraffin, sectioned, and stained with Mason's trichrome according to standard protocols. The first subatrial section from each heart was digitized, and the internal and external LV areas from each heart were traced manually and measured with the use of Scion Image software (Scion, National Institutes of Health).

Isolated heart preparations. For determination of isolated heart function, ejecting heart preparations were performed as previously described (13, 14, 31). Hearts with rates under 300 beats/min were paced at that rate (3/5 NTG and 3/7 HCM hearts). Baseline measurements were taken after the establishment of steady-state conditions, and preload was altered over a range of cardiac work from 200 to 350 mmHg·ml·min^-2 to generate Starling curves for each heart. Sensitivity to the adrenergic agonist isoproterenol was deter-
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mained with additional hearts by using an isovolumic preparation similar to that previously described for the rat heart (24). Briefly, each heart was excised and retrograde perfusion was established, and then a highly compliant custom-made latex balloon was inserted into the left ventricle via the mitral valve. Balloons were sized and shaped to match the dimensions of dilated HCM hearts. The balloon was attached to an airtight catheter filled with distilled water and connected to pressure tubing which housed a 3-Fr transducer (Millar Instruments, Houston, TX), and the balloon was inflated to yield an end-diastolic pressure of 5 mmHg. The hearts were paced at 360 beats/min, and LV pressures were monitored during steady-state conditions and at 1, 3, and 5 min after exposure to 1 μmol/l isoproterenol.

β-Adrenergic receptor density, adenylyl cyclase activity, and GRK immunoblotting and activity. The mice were euthanized by cervical dislocation, and the hearts were excised and placed in phosphate-buffered saline while beating to pump blood out of the myocardium. Left ventricles were dissected and frozen at −80°C within 10 min of death. The functional state of β-adrenergic receptors in the HCM and NTG hearts was determined as described previously (20, 23, 27). Briefly, we measured total β-adrenergic receptor binding on myocardial membranes with the use of the nonselective β-adrenergic ligand [125I]iodocyanopindolol (20). Membrane adenylyl cyclase activity was determined under basal conditions and in the presence of isoproterenol or NaF (23, 27). The myocardial levels of β-adrenergic receptor kinase 1 (β-ARK1) and GRK5 were determined by immunoprecipitation, followed by immunoblotting of detergent-solubilized extracts (20, 23). Total GRK activity in the myocardial membranes was determined by using rhodopsin-enriched rod outer segment membranes as an in vitro substrate, and [γ-32P]ATP incorporation into rhodopsin was determined (20, 23).

RNA analysis. Total RNA was extracted from the LV myocardium previously frozen as described above by using TriZOL reagent (GIBCO-BRL). The expression of α-MyHC, β-MyHC, α-skeletal actin (s-ACT), and atrial natriuretic factor (ANF) mRNA were determined by using a slot blot with previously described oligonucleotide probes (22, 41). Small variations in loading were corrected by normalization to mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistics. Data are presented as means ± SE. The number (n) of mice used is indicated. Statistical analysis was performed by Student’s t-test for paired comparisons between HCM and NTG mice. Starling curves and isoproterenol dose-response values were tested by ANOVA.

RESULTS

To quantify the extent of ventricular dilation in older male HCM animals, hearts from age-matched HCM and NTG mice were dissected and fixed in formaldehyde. The first subatrial section from each heart was digitized, and internal LV chamber area was directly measured. The “external LV chamber area” was defined as the area within a closed region circumscribing the entire left ventricle myocardium, including the interventricular septum. Both the internal LV chamber area (1.41 ± 0.14 vs. 3.21 ± 0.51 mm², NTG vs. HCM, respectively, P < 0.005) and the ratio of the internal to the external LV chamber area (9.27 ± 0.81 vs. 18.77 ± 1.99, P < 0.01) were significantly increased in the HCM hearts (Fig. 1). Foci of myofibrillar disarray and fibrosis were observed in HCM hearts, as described in detail previously (45).

Exercise intolerance is one of the hallmarks of DCM. HCM is linked to sudden cardiac death in young athletes. Furthermore, exercise intolerance has been found in cases of symptomatic HCM with diastolic dysfunction alone as well as in cases that have progressed to dilation and systolic dysfunction (39). We assessed exercise tolerance in the HCM mice by using a custom-built treadmill equipped with infrared beams above the shock stimuli at the rear of the treadmill belt. We first measured the ability of mice to keep pace with a treadmill belt moving at 20 m/min on a 7° incline. Compared with NTG, the HCM mice were exercise intolerant, as indicated by a significantly higher number of beam breaks per minute during the exercise test (Fig. 2). When the mice ran at a higher speed (27 m/min) for 60 min, HCM mice showed significantly depressed exercise endurance compared with NTG controls (Fig. 2). Whereas all of the six control mice completed 60 min of exercise at this speed, only one of eight HCM mice was able to complete the...
entire exercise session. One HCM mouse died after 34 min of exercise, six failed from exhaustion between 35 and 52 min, and only one was able to complete the full 60-min session (Fig. 2).

To assess in vivo hemodynamics, we studied HCM and NTG mice by transthoracic echocardiography at comparable near-physiological heart rates (560 ± 10 beats/min). Systolic function, measured as percent fractional shortening, was significantly decreased in the HCM mice (Fig. 3). Because of the high murine heart rate, standard Doppler parameters for assessment of diastolic function were not measurable. Therefore, we chose to assess diastolic function by evaluating the rate of relaxation of the posterior wall of the left ventricle, measured directly from the digitized M-mode images as the slope of a line tangent to the posterior wall during diastole. The posterior wall relaxation slope of the HCM mice was significantly depressed, suggesting impaired in vivo relaxation (Fig. 3). The internal diameter of the left ventricle in diastole was modestly increased in the HCM animals as measured from M-mode images, but the difference from NTG hearts was not significant.

To assess myocardial function directly in the absence of neurohumoral or peripheral cardiovascular effects, we conducted isolated ejecting heart experiments. A small number of transgenic and control hearts were excised and cannulated via the pulmonary veins and aorta to establish an isolated system in which the hearts performed measurable preload-dependent pressure work against a tightly controlled afterload. Under identical load conditions, both contractility and relaxation, measured by the first derivative of pressure development over time (+dP/dt) and first derivative of pressure relaxation over time (−dP/dt), respectively, were decreased, and time to peak pressure (TPP) and half-time to relaxation (RT½) were increased in the HCM mice (Table 1). The maximal systolic pressure developed by the HCM hearts was also diminished (Table 1). These changes were modest, ranging from 9 to 16%, but were statistically significant as indicated. Despite an overall reduction in both +dP/dt and −dP/dt over a range of cardiac work obtained by varying the preload, HCM hearts were able to increase both +dP/dt and −dP/dt to the same relative extent as controls, suggesting that the Starling response is preserved in these hearts (Fig. 4).

Abnormalities in β-adrenergic signaling have been well documented in heart failure, including DCM, and recently β-adrenergic receptor downregulation, abnormal adrenergic control of the force-frequency relation, and reduced catecholamine reuptake have been reported in primary HCM (6, 21, 35). To measure the β-adrenergic responsiveness of HCM hearts, we used an isolated isovolumic preparation, in which a balloon is inserted into the left ventricle and inflated to a constant diastolic pressure. Isovolumic pressure changes are recorded as the heart is stimulated to contract. In this preparation, the effects of adrenergic activation on coronary perfusion are minimized by retrograde perfusion. Additionally, endogenous pacemaker activity is abolished by crushing the atria, permitting direct assessment of myocardial inotropic and lusitropic responsiveness to the adrenergic agonist without chronotropic effects. We measured the isovolumic response of transgenic and NTG hearts to a single
maximal dose of isoproterenol (1 μM) at 1-, 3-, and 5-min time points. Systolic responsiveness (+dP/dt) of the HCM hearts to isoproterenol was not impaired (Fig. 5). However, the lusitropic response, −dP/dt, of the HCM hearts, was significantly diminished (Fig. 5). The initial relaxation in response to the β-agonist was normal in HCM hearts, but this was followed by an additional augmentation of relaxation after 1 min in the NTG but not the HCM hearts.

To further explore β-adrenergic function through biochemical assays, homogenates from HCM and control hearts were assayed for β-adrenergic receptor density, adenylyl cyclase activity, and GRK levels and activity. β-Adrenergic receptor density and membrane adenylyl cyclase activity in HCM hearts, under basal, sodium fluoride-stimulated, and isoproterenol-stimulated conditions, were not significantly altered compared with NTG hearts (data not shown). Myocardial β-ARK1 levels, determined by immunoprecipitation of cytosolic extracts, were significantly higher in HCM hearts compared with controls (Fig. 6). Protein levels of a second GRK found in the heart, GRK5, were not different in HCM hearts (Fig. 6). β-ARK1 exerts its regulatory activity at the myocardial membrane, so we also measured total membrane GRK activity in membrane extracts and found a significant increase in HCM hearts (Fig. 6).

The initial relaxation in response to the β-agonist was normal in HCM hearts, but this was followed by an additional augmentation of relaxation after 1 min in NTG but not the HCM hearts.

Table 1. Baseline parameters in isolated working heart preparation

<table>
<thead>
<tr>
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<th>NTG</th>
<th>HCM</th>
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<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Age, mo</td>
<td>8.4 ± 0.2</td>
<td>8.7 ± 0.1</td>
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<tr>
<td>Body wt, g</td>
<td>30.5 ± 1.0</td>
<td>27.3 ± 0.8</td>
</tr>
<tr>
<td>Heart wt (both ventricles), mg</td>
<td>196 ± 8.2</td>
<td>202 ± 3.9</td>
</tr>
<tr>
<td>Heart wt/body wt, mg/g</td>
<td>6.5 ± 0.32</td>
<td>7.4 ± 0.23*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>337 ± 11.5</td>
<td>346 ± 11.6</td>
</tr>
<tr>
<td>Mean aortic pressure (afterload), mmHg</td>
<td>50.3 ± 0.22</td>
<td>50.0 ± 0.24</td>
</tr>
<tr>
<td>Left IVP, mmHg</td>
<td>99.0 ± 2.3</td>
<td>89.1 ± 1.2†</td>
</tr>
<tr>
<td>Systolic</td>
<td>−5.0 ± 1.1</td>
<td>−4.2 ± 1.4</td>
</tr>
<tr>
<td>End diastolic</td>
<td>9.5 ± 0.8</td>
<td>10.1 ± 1.8</td>
</tr>
<tr>
<td>Cardiac output (venous return; preload), ml/min</td>
<td>5.04 ± 0.02</td>
<td>5.03 ± 0.01</td>
</tr>
<tr>
<td>Aortic output</td>
<td>3.04 ± 0.26</td>
<td>2.65 ± 0.28</td>
</tr>
<tr>
<td>Coronary flow</td>
<td>1.97 ± 0.23</td>
<td>2.34 ± 0.28</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>15.0 ± 0.5</td>
<td>14.6 ± 0.5</td>
</tr>
<tr>
<td>Cardiac work (left ventricle), mmHg·ml·min⁻¹</td>
<td>251.8 ± 0.8</td>
<td>250.2 ± 0.8</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>3,737 ± 72</td>
<td>3,399 ± 56†</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>−3,599 ± 61</td>
<td>−3,017 ± 171*</td>
</tr>
<tr>
<td>TPP/unit pressure, ms/mmHg</td>
<td>0.455 ± 0.007</td>
<td>0.493 ± 0.005†</td>
</tr>
<tr>
<td>RT1/2/unit pressure, ms/mmHg</td>
<td>0.445 ± 0.010</td>
<td>0.504 ± 0.017*</td>
</tr>
<tr>
<td>Pα2, mmHg</td>
<td>653 ± 52</td>
<td>656 ± 33.3</td>
</tr>
<tr>
<td>Pβ2, mmHg</td>
<td>179.6 ± 8.8</td>
<td>251 ± 35.5</td>
</tr>
<tr>
<td>MVO2, μl O2·min⁻¹·g⁻¹</td>
<td>153.8 ± 16.5</td>
<td>149 ± 19.9</td>
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Values are means ± SE. n, number of mice for each group; HCM, mice with hypertrophic cardiomyopathy; IVP, internal venous pressure; NTG, nontransgenic control mice; TPP, time to peak pressure; +dP/dt and −dP/dt, first derivative of pressure development and relaxation, respectively, over time; RT1/2, half-time to relaxation; Pα2 and Pβ2, partial arterial and venous, respectively, pressures; MVO2, myocardial oxygen consumption. *P < 0.05; †P < 0.01.
It has been previously shown that younger, hypertrophic HCM mice express two genetic markers of compensatory hypertrophy, ANF and s-ACT (44, 45). These genes are normally expressed during embryonic heart development but not in adult myocardium, and their induction has been well established in cardiac hypertrophy (4, 36). α-MyHC is the predominant isoform in murine myocardium until birth, when β-MyHC is preferentially expressed; reversion to the embryonic α-MyHC isoform is also observed in cardiac hypertrophy (see Ref. 30 for a review). We were interested in assessing the expression of embryonic genes in the hearts of older dilated male HCM animals. Total RNA extracted from LV tissue was used for analysis. Slot blots were hybridized with α-MyHC, β-MyHC, ANF, s-ACT, or GAPDH probes, and the signals were normalized to GAPDH levels. Expression of β-MyHC, ANF, and s-ACT was significantly increased in the HCM ventricles; α-MyHC expression was significantly decreased (Fig. 7). RNAse protection of LV RNA with a probe that protects fragments of different length from α-MyHC, β-MyHC, and the mutant MyHC transgene mRNA confirmed an increased ratio of β-MyHC to total endogenous MyHC in the HCM hearts (data not shown). These RNA results strongly suggest that an embryonic gene expression program is still active in the decompensated hearts of the older transgenic male animals.

**DISCUSSION**

HCM and DCM are clinically recognized as distinct diseases, although a progression from HCM to ventricular dilation with systolic and diastolic dysfunction has been observed in a subset of the HCM population (18, 37, 38, 42). These clinical findings suggest that the two cardiomyopathies may actually be part of the same pathological spectrum. The data presented here demonstrate that phenotypic progression from HCM to DCM occurs in mice expressing a mutant myosin transgene.

The hearts of 8-mo-old male HCM animals are visibly dilated (Fig. 1). Myocardial mass is significantly increased (Table 1), indicating that eccentric hypertrophy of the heart has resulted in increased LV chamber dimensions without increased wall thickness (Fig. 1). The increased chamber volume may provide some benefit in terms of a larger stroke volume, but the elevated systolic and diastolic stress would be detrimental to cardiac function over the long term.

One of the primary symptoms of DCM is exercise intolerance. By 8 mo, the HCM mice show a significantly impaired ability to keep pace with a moving treadmill, and exercise endurance was also significantly decreased (Fig. 2). These results suggest that cardiac function in the HCM mice is impaired enough to cause significant physiological consequences. DCM is also characterized by systolic dysfunction, which may be accompanied by diastolic abnormalities. In contrast, HCM patients generally display diastolic dysfunction, with normal or even elevated systolic function (39). Both systolic and diastolic dysfunction were observed in HCM hearts in vivo by echocardiography. Isolated ejecting heart studies confirmed that both systolic and diastolic anomalies are intrinsic to the HCM myocardium, independent of neurohumoral or peripheral effects. Although +dP/dt and −dP/dt were uniformly depressed in HCM hearts over a wide range of cardiac work, both indexes increased to the same extent as controls in response to increased load (Fig. 4).
The preserved Starling response in these HCM hearts is not surprising because even end-stage failing human hearts have the ability to respond to enhanced preload with an increase in force development (46). The ability of the HCM hearts to increase contractility normally, despite depressed overall contractility, argues against an intrinsic inability of the HCM hearts to respond to increased demand. Instead, it is likely that defects in the regulation of excitation-contraction coupling contribute to the in vivo functional impairment observed in the HCM mice.

The cardiac β-adrenergic signaling system is important for the regulation of excitation-contraction coupling in the heart, and abnormalities in this signaling system commonly occur with ventricular remodeling and cardiac decompensation (8). Like hypertrophy, adrenergic activation is part of the compensatory response to cardiac damage, and persistent β-adrenergic stimulation can lead to desensitization of this G protein-coupled receptor system (3, 16). In the isolated isovolumic heart experiments, the HCM mice exhibited significantly diminished relaxation in response to the infusion of the β-adrenergic agonist isoproterenol (Fig. 5). This moderate desensitization in lusitropic function might be expected to have a relatively larger impact on overall cardiac function because small deficits in myocardial relaxation would in turn affect ventricular filling and result in diminished cardiac output. Desensitization of adrenergic responsiveness is consistent with the finding of significantly increased levels and activity of β-ARK1 in HCM hearts. β-ARK1 acts to uncouple β-adrenergic receptors from downstream effectors, including adenyl cyclase and cardiac contractility (23, 25). In the HCM mice, myocardial protein levels of β-ARK1 but not GRK5 were increased. In failing human hearts, β-ARK1 mRNA and GRK activity are elevated approximately two- to threefold (43), but myocardial levels of GRK5 are unaltered (Iaccarino and Koch, unpublished observations). Similar observations have been made in other animal models of heart failure, such as cardiomyopathic hamsters (see Ref. 19 for a review). The increased β-ARK1 in the HCM hearts could lead to physiological adrenergic uncoupling, consistent with the isolated heart results.

Although we observed a diminished lusitropic response to isoproterenol in the HCM animals, no difference in myocardial membrane adenyl cyclase responsiveness was observed. It is possible that the sensitivity of the adenyl cyclase assay was not sufficient to detect the modest change in adrenergic responsiveness observed at the whole organ level. Alternatively, coupling between the β-adrenergic receptors and adenyl cyclase may be intact in the HCM hearts despite elevated β-ARK1 levels. Considering our observation that the relaxation deficit observed in HCM hearts after isoproterenol exposure was time dependent (Fig. 5), it is interesting to speculate that the primary adrenergic abnormality in the HCM hearts may be a defect in one of the distal components of relaxation, such as phospholamban or troponin phosphorylation. In a study (15) that used myocardial tissue from human HCM patients, isometric contraction and relaxation were markedly prolonged, and the calcium transients of the HCM myocardium exhibited two distinct components, in contrast with controls. Studies of Ca2+ transients in isolated cardiac myocytes from HCM hearts are in progress.

Analysis of total RNA extracted from LV tissue of HCM animals revealed a pattern of embryonic gene expression consistent with cardiac hypertrophy (Fig. 7). β-MyHC, ANF, and s-ACT were significantly induced, and α-MyHC expression was significantly decreased. These findings suggest that the fetal gene expression program that initially supports compensatory hypertrophy is maintained as the hearts progress to decompensated, eccentric hypertrophy.

One of the major problems in understanding HCM is the difficulty in acquiring myocardial specimens from human patients, and this problem has been an impetus for the generation of several different mouse models. Despite different genetic backgrounds, the HCM mice described here share several phenotypic traits with the α-MyHC<sup>403/+</sup> model of HCM (10). The αMyHC<sup>403/+</sup> mice carry an Arg<sup>403</sup>Gln (R<sup>403</sup>Q) missense mutation on one allele. In contrast, the HCM mice studied here have two normal α-MyHC alleles but express an additional mutant α-MyHC transgene that includes the same R<sup>403</sup>Q missense mutation along with a deletion of amino acids 468–527 bridged by the addition of nine nonmyosin amino acids (45). Myocardial sections from both models show the classic histopathology of HCM. The nature of cardiac hypertrophy is somewhat different between the two models in that the HCM mice display both LV and right ventricular hypertrophy (45), whereas in the α-MyHC<sup>403/+</sup> mice only left atrial weights are increased. Both the α-MyHC<sup>403/+</sup> mice (17) and the HCM mice are exercise intolerant. Cardiac dysfunction was evident in both HCM and α-MyHC<sup>403/+</sup> mice but with different phenotypic presentations. The α-MyHC<sup>403/+</sup> mice display predominantly diastolic abnormalities, with accelerated systolic kinetics (11). The kinetics of both cardiac contraction and relaxation are impaired in older HCM mice. Interestingly, a gender difference is apparent in both models. Male α-MyHC<sup>403/+</sup> mice more consistently display left atrial enlargement and histopathology than female mice (10). Female HCM mice typically show a progressive ventricular hypertrophy at 8 mo of age without the chamber dilation seen in male mice (45). Gender differences have also been described in the prevalence and presentation of human cardiac disease, including aortic stenosis (5), idiopathic DCM (7), and HCM (12). The advent of mouse models that also display gender differences may help to elucidate the gender-specific factors that impact the differential response of male and female hearts to similar stresses.

The HCM mice described here are the first mouse models of a sarcomeric protein mutation to show a progression from HCM to DCM. The data presented above demonstrate functional and biochemical defects in 8- to 11-mo-old male HCM mice consistent with DCM, with ventricular dilation, systolic and diastolic...
dysfunction, exercise intolerance, and adrenergic desensitization. These animals have undergone cardiac decompensation similar to that of the subgroup of human HCM patients who progress from primary hypertrophy to symptomatic DCM and systolic dysfunction. The HCM mice do not appear to develop overt heart failure at this time point, and the extent of the cardiac dysfunction seen in the HCM mice might be described as mild or moderate compared with the severe acute abnormalities in other murine models of heart failure, such as muscle Lin-II, Isl-1, and Mec-3 protein null or calcineurin overexpression (1, 29). However, the progression from HCM to DCM in the HCM mice occurs over a lifespan period of ~30–50%, which compares favorably with the kinetics of cardiac decompensation in humans. These data support the idea that HCM and DCM may be part of the same pathological process. A mouse model that recapitulates the transition that caused an undiagnosed primary HCM. Identification of a mouse model that recapitulates the transition from HCM to DCM may ultimately provide valuable insight not only into HCM but also the more general phenomenon of cardiac decompensation.

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