Changes in Gene Expression in the Intact Human Heart

Downregulation of α-Myosin Heavy Chain in Hypertrophied, Failing Ventricular Myocardium

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Abstract

Using quantitative RT-PCR in RNA from right ventricular (RV) endomyocardial biopsies from intact nonfailing hearts, and subjects with moderate RV failure from primary pulmonary hypertension (PPH) or idiopathic dilated cardiomyopathy (IDC), we measured expression of genes involved in regulation of contractility or hypertrophy. Gene expression was also assessed in LV (left ventricular) and RV free wall and RV endomyocardium of hearts from end-stage IDC subjects undergoing heart transplantation or from nonfailing donors. In intact failing hearts, downregulation of β_1-receptor mRNA and protein, upregulation of atrial natriuretic peptide mRNA expression, and increased myocyte diameter indicated similar degrees of failure and hypertrophy in the IDC and PPH phenotypes. The only molecular phenotypic difference between PPH and IDC RVs was upregulation of β_2-receptor gene expression in PPH but not IDC. The major new findings were that (*a*) both nonfailing intact and explanted human ventricular myocardium expressed substantial amounts of α-myosin heavy chain mRNA (α-MHC, 23–34% of total), and (*b*) in heart failure α-MHC was downregulated (by 67–84%) and β-MHC gene expression was upregulated. We conclude that at the mRNA level nonfailing human heart expresses substantial α-MHC. In myocardial failure this alteration in gene expression of MHC isoforms, if translated into protein expression, would decrease myosin ATPase enzyme velocity and slow speed of contraction. (*J. Clin. Invest.* 1997. 100:2315–2324.) Key words: α-myosin heavy chain • β-myosin heavy chain • β-adrenergic receptors • atrial natriuretic peptide • sarcoplasmic reticulum Ca^{2+} ATPase

Introduction

Previous studies in aliquots of explanted human ventricular myocardium, typically removed at the time of cardiac transplantation, have indicated that the failing heart exhibits changes in gene expression at the mRNA (1–8) or protein gene product level (5–10). However, one of the problems with using explanted hearts to investigate changes in gene expression is that failing tissue is only available from hearts with end-stage myocardial disease, in which numerous factors (e.g., multiple drug therapy) may obscure true pathogenic changes in gene expression. Additionally, many important myocardial processes (such as concentric hypertrophy) may not be available for study in explanted human hearts. Another problem with using explanted hearts is that nonfailing control material is typically taken from brain-dead organ donors, which are exposed to a number of factors that could change gene expression, including markedly increased sympathetic activity and drugs used to maintain the circulation (11).

Previously, we have reported that multiple mRNAs may be quantified in endomyocardial biopsy-sized specimens (1), and more recently a few reports have documented that mRNA may be quantitated in actual endomyocardial biopsy samples (12, 13). We report here that multiple mRNA gene products of interest can be measured from endomyocardial biopsy specimens removed from nonfailing, hypertrophied, and failing human hearts. In some cases the results parallel those reported previously in explanted human myocardial tissue, and in other cases the results provide new insights into potentially important changes in gene expression that occur in hypertrophied or failing human hearts.

Methods

Patient material. Gene expression was measured in the intact human heart by removing endomyocardial tissue from the distal right ventricular (RV)[1] septum via endomyocardial biopsy (see below) in subjects with hypertrophy and myocardial failure and in control subjects. Two types of hypertrophy and failure were examined: RVs failing as a consequence of severe pressure overload from primary pulmonary hypertension (PPH, $n = 7$, 5 NYHA class III, 1 each class II and IV), and RVs failing in the context of biventricular failure from idiopathic dilated cardiomyopathy (IDC, $n = 29$, 21 class III, 5 class II, 3 class IV, P = NS for NYHA vs. PPH). Control RVs with normal or near-normal systolic function (RV ejection fraction [RVEF] ≥ 0.40) were available from several sources including subjects with cancer who were about to begin chemotherapy with IL-4 ($n = 4$) or liposomal adriamycin ($n = 1$). Only one of these chemotherapy-treated subjects had previously received anthracycline therapy. An additional two control subjects with normal systolic function and atypical chest pain or unexplained dyspnea on exertion were biopsied to rule out myo-

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1. *Abbreviations used in this paper:* ANP, atrial natriuretic peptide; EF, ejection fraction; IDC, idiopathic dilated cardiomyopathy; LV, left ventricle; MHC, myosin heavy chain; PPH, primary pulmonary hypertension; RT-QPCR, reverse transcription-quantitative PCR; RV, right ventricle; SRCA, sarcoplasmic reticulum calcium ATPase.

carditis or cardiomyopathy, and a final control subject with normal RV function (RVEF = 0.58) and minimal left ventricular (LV) dysfunction (LVEF = 0.47) was biopsied to rule out an infiltrative myocardial process 72 h after being resuscitated from a cardiac arrest that ultimately was attributed to coronary artery spasm. Six of the eight nonfailing controls were assessed as NYHA class I at the time of biopsy, one subject who had diastolic dysfunction and mild fibrosis on biopsy was class II, and the control subject who had arrested returned to class I several weeks after being evaluated. The IDC subjects were all on angiotensin-converting enzyme inhibitors, diuretics, and digoxin. PPH subjects were on diltiazem (n = 5), digoxin (n = 7), and diuretics (n = 7). No control subject was on cardioactive medications, including angiotensin-converting enzyme inhibitors. All subjects gave written consent for these studies, for research protocols approved by the Institutional Review Boards of the University of Utah and the University of Colorado Health Sciences Center.

RV endomyocardial biopsy and right heart catheterization. Right heart catheterization was performed from the right internal jugular vein as described previously (14). After cannulation of the right internal jugular vein, endomyocardial biopsy of the distal RV septum was performed with a Mansfield 2.2 mm jaw size (Boston Scientific Corp., Watertown, MA) bioptome under echocardiographic guidance to ensure proper positioning of the biopsy forceps. Six to eight samples of endomyocardium weighing 25–30 mg were taken, and allocated to β-adrenergic receptor measurements (10 mg), gene expression measurements (10 mg), routine light microscopy, and morphometrics. After endomyocardial biopsy, hemodynamics were measured using a balloon-tipped catheter.

Regional sampling from explanted human RVs. Six end-stage failing hearts with IDC removed from transplant recipients (age 38±7 yr) and six nonfailing hearts (age 45±5 yr, P = NS vs. IDC) harvested from would-be kidney transplant donors whose hearts could not be placed for transplant were used to assess regional gene expression in LVs and RVs. Explantation and transport of these hearts was as described previously (1, 7, 9). 10-g aliquots were removed from the mid-RV and -LV free walls and immediately placed in liquid N_2 for subsequent RNA analysis. Under direct visualization a 100–150-mg aliquot was removed from the distal RV septum using a Mansfield bioptome (simulated RV biopsy) and placed immediately in liquid N_2. The IDC hearts were from subjects treated before transplant with digoxin, diuretics, and angiotensin-converting enzyme inhibitors who were ambulatory outpatients (UNOS Status II) before transplant, and none had received intravenous inotropic therapy before transplant. These IDC hearts had severe biventricular failure, with LVEFs of 0.14± 0.02. The nonfailing control hearts were given intravenous dopamine at doses of 2–10 μg/kg/min for 4–12 h, and all hearts had estimated LVEFs of ≥ 0.45.

RNA extraction. Total RNA was extracted from two to four endomyocardial biopsies (a total of 4–8 mg) by the guanidinium thiocyanate phenol-chloroform method using RNA STAT-60, as described previously (1, 7). A double extraction was routinely used to eliminate small amounts of DNA contamination (see below).

Measurement of mRNA abundance by reverse transcription-quantitative PCR (RT-QPCR). mRNA abundance was measured by RT-QPCR according to previously described methods (1, 7). The RT-QPCR method is based on simultaneous reverse transcription and PCR amplification of a known amount of internal standard cRNA of slightly smaller size than the PCR product generated from the transcript of interest (1, 7, 14). The internal standard and the cDNA produced from the transcript of interest are then collinearly amplified in the same reaction tube, and the PCR products are quantified by using ^{32}P–end-labeled primers. From regions of the two curves that collinearly amplify, it is then possible to determine the original amount of unknown mRNA, provided that a known amount of internal standard is added. The PCR products are identified by direct PCR sequencing, and several (4–20) internal standard cRNAs can be built into a single internal standard construct driven by the bacteriophage T_7 RNA polymerase promoter (1). When possible the amplified regions of transcripts of interest are selected to cross-splice junctions so amplification of genomic material does not contaminate the assay. For intronless genes such as the adrenergic receptors, the extreme sensitivity (into the zeptomole range) of the assay can lead to genomic amplification (7), and an RT(−) control is run to detect this possibility.

Recently, we have modified the reverse transcription and PCR conditions for the β_1-receptor transcript to increase the efficiency of reverse transcription and amplification, to eliminate significant genomic contamination, and to obtain robust cDNA amplification in 30 cycles. These modifications include double extraction of RNA with RNA STAT 60™ (Tel-Test, Friendswood, TX), and multiple changes in the reverse transcription assay conditions, including adding 2% DMSO, using oligo d(T_{15}) primers to gain specificity for mRNA, increasing the concentration of Moloney murine leukemia virus reverse transcriptase by 50%, and carrying out the reverse transcriptase reaction in a thermocycler. The thermocycler protocol includes an initial 15 min at 37°C, then ramping to 45°C over 15 min, which is held there for an additional 15 min, and finally inactivating the transcriptase at 95°C for 10 min. With these modifications, a 202-bp region of the β_1-receptor cDNA reverse transcribed from mRNA in total RNA as the starting material can be amplified in ≤ 30 cycles of PCR, without genomic amplification. This now allows for measurement of β_1-receptor mRNA abundance in total RNA extracted from endomyocardial biopsy material. As determined using in vitro transcribed mRNA relative to the respective internal standard, the efficiency of reverse transcription was 7.5 times greater for β_2 mRNA compared with β_1, and the mRNA abundance of β_1-receptor mRNA determined by ribonuclease protection was 6–10 times that determined by quantitative RT-PCR. Therefore, in the RT-PCR determinations in the study the calculated amount of β_1-receptor mRNA was multiplied by 7.5.

Using this form of quantitative RT-PCR, three reverse transcriptase reactions were necessary to ensure collinear amplification of the internal standard and cDNAs of interest. Stated another way, it is necessary for the reverse transcribed internal standard and the unknown mRNA to be within 10-fold of one another before amplification in order to obtain collinearity. In general, one reverse transcriptase reaction each was used for low (β-adrenergic receptors), medium (atrial natriuretic peptide [ANP], α-myosin heavy chain [MHC]), and high (sarcoplasmic reticulum calcium ATPase [SRCA], β-MHC) abundance messages. The 5′ and 3′ primer sequences used to amplify the six cDNAs (15–19) of interest are given in Table I. The precision of this assay as assessed by repeat measurements on the same sample yields a coefficient of variation of 12.8% for β_1 mRNA, and 10–20% for all gene products we have tested (n = 8). All PCR products were confirmed to be the expected cDNA by subcloning the PCR product

Table I. Forward (F) and Reverse (R) Primer Sequences Used to Amplify the Six cDNAs of Interest

mRNA		Sequence	cDNA sequence coordinates	Product length
β_1-AR	F	CGAGCCGCTGTCTCAGCAGTGGACA	150–351	202
	R	GGTGGCCCCGAACGGCACCACCAGCA		
β_2-AR	F	ACTGCTATGCCAATGAGACC	548–905	358
	R	AGGTTATCCTGGATCACATG		
α-MHC	F	ATCAAGGAGCTCACCTACCAG	1327–1592	265
	R	CACTCCTCATCGTGCATTTTC		
ANP	F	CAACGCAGACCTGATGGATT	99–334	235
	R	TTAGGAGGGCAGATCGATCAGA		
β-MHC	F	ATCAAGGAGCTCACCTACCAG	1327–1662	335
	R	AGCTGTTACACAGGCTCCAG		
SRCA	F	TGTAACGCCCTCAACAGCTTG	2725–2954	229
	R	AACTTGAGCGTCTCATCCATG		

Table II. Myocardial Function and Hemodynamic and Demographic Characteristics among the Three Study Groups, ±SEM

Group (*n*)	LVEF*	RVEF*	RA	PWP*	PAP*	CI*	Age*	Gender M/F
			mmHg	*mmHg*	*mmHg*	*liters/min/m²*		
NF (8)	0.59±0.03	0.52±0.02	2.5±1.9	5.0±1.9	14.8±3.5	4.46±0.46	49.1±4.6	3/5
PPH (7)	0.53±0.03	0.29±0.02‡	9.1±2.3	6.1±1.2	49.6±4.8‡	2.06±0.18‡	36.6±2.8	0/7
IDC (29)	0.23±0.01‡§	0.33±0.03	6.7±1.1	15.0±1.6§	27.9±1.9§	2.49±0.14‡	55.0±2.0§	11/18

RA, right atrial mean pressure; *PWP*, pulmonary wedge mean pressure; *CI*, cardiac index. ‡$P < 0.05$ vs. NF (nonfailing); §$P < 0.05$ vs. PPH; *ANOVA $P < 0.05$.

using the PGEM-T vector system (Promega, Madison, WI), and then sequencing the cloned fragment.

Measurement of β_1- and β_2-adrenergic receptor density by radioligand binding. Total β-receptor density and β_1- and β_2-receptor subtypes were measured in biopsy material by using a single-point displacement of bound 300 pM [^{125}I]ICYP by 2×10^{-6} M CGP 20712A (β_1 subpopulation) or 10^{-6} M propranolol ($\beta_1 + \beta_2$ displacement) (20). This approach uses 9 assay tubes in comparison to the 33 tubes that are required for a full saturation curve plus CGP displacement. We have compared this "one-point" technique of determining β_1 and β_2 compared with computer modeling of full CGP 20712A competition curves in 25 hearts, and there was an excellent correlation ($r = 0.95$) between the two techniques (data not shown).

Light microscopic morphometry. Routine light microscopy was performed on the RV biopsy material removed from all control subjects, and a subset of 5 PPH and 10 IDC subjects. The tissue was fixed in 10% buffered formalin and embedded in paraffin. Hematoxylin and eosin and Massons' trichrome stained sections were prepared and examined by light microscopy. Additionally, biopsies were subjected to morphometric analysis in nine IDC subjects, in five subjects with PPH, and in seven control subjects. Morphometric analysis was performed using a Leitz microscope and a Hitachi KPC-501 video camera with the video images output to an Image1/AT image processing and analysis system operated with a Compaq Deskpro 386/25 PC. Microscopic fields of the sections were sampled systematically, with sufficient numbers sampled to ensure a coefficient of error (standard error/mean) of 5% or less (21). Myocyte width was measured in cells in which a nucleus was visible, with the measurement taken of the narrowest diameter of the cell across the nucleus.

Statistical analysis. Bivariate analyses were performed with Statview 512™ (Brainpower, Inc., Calabasas, CA) and multivariate and nonparametric analyses were performed with SAS (SAS Institute, Cary, NC). Unless otherwise specified, data are expressed as mean± standard error. The two-sided significance level was $P < 0.05$. Three-group comparisons were performed with ANOVA for continuous variables and by contingency table analysis for binary variables. With a significant three-group comparison, specific two-group comparisons were performed with the Scheffe or Bonferroni multiple comparisons procedures. When data were not normally distributed as assessed by the Shapiro-Wilk test, the ANOVA and *t* test results were confirmed by the Kruskal-Wallis and Wilcoxon tests, respectively. Univariate and multivariate relationships between continuous variables were assessed with stepwise linear regression, using $P = 0.15$ to enter and to remove.

Results

Gene expression in intact human hearts

SUBJECT DEMOGRAPHICS AND CARDIAC FUNCTION

Shown in Table II are the demographic and cardiac functional data for the three groups. As can be seen, PPH subjects were somewhat younger than the IDC subjects, who did not differ from nonfailing controls with respect to age. There were somewhat more women in the PPH group and more men in the IDC group, but these variations were not significant by multiple comparison contingency table analysis.

As can be seen in Table II, PPH subjects exhibited markedly elevated pulmonary artery pressures, consistent with the underlying diagnosis. For PPH subjects, LV filling pressure was normal, cardiac index was reduced, and right atrial pressure was mildly increased. Compared with PPH, subjects with IDC had lower pulmonary artery pressure and higher pulmonary wedge pressures. IDC subjects had biventricular dysfunction as deduced from markedly decreased LVEFs (0.23±0.01) and moderately decreased RVEFs (0.33±0.03). In contrast, PPH subjects had isolated RV failure by EF criteria, with normal LV function (LVEFs 0.53±0.03) and moderate to severely depressed RV function (RVEFs 0.29±0.02). Both the PPH and IDC groups had cardiac failure as assessed by cardiac index data, with the PPH group having slightly lower values than the IDC group.

MRNA ABUNDANCE MEASUREMENTS

β-Adrenergic receptors. Shown in Fig. 1 are mRNA abundance data for β_1- and β_2-adrenergic receptors in the three groups. As can be seen, compared with the nonfailing groups β_1-receptor mRNA abundance is reduced in both the PPH and IDC groups, by ~ 50% in the IDC group and 60% in the PPH group. Surprisingly, the PPH group exhibited an increase (by 85%) in β_2-adrenergic receptor mRNA abundance compared with the nonfailing group and by 152% compared with the IDC group.

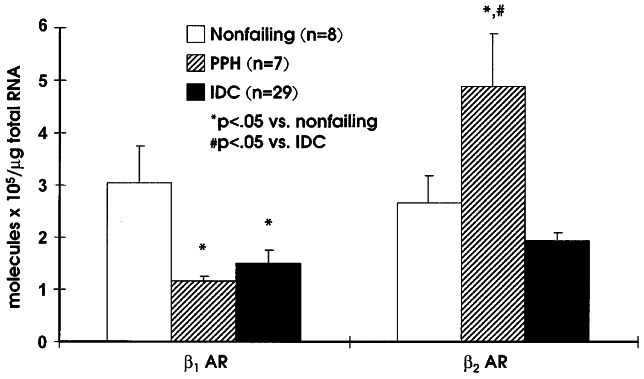

Figure 1. β_1- and β_2-adrenergic receptor mRNA abundance in nonfailing, PPH, and IDC in RV endomyocardium obtained by biopsy of the intact heart.

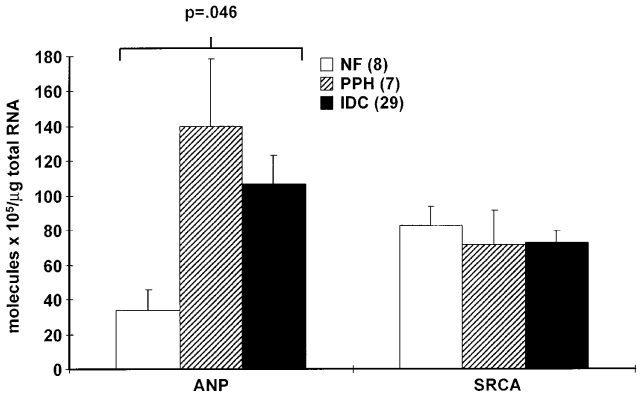

Figure 2. ANP and SRCA mRNA abundance in nonfailing, PPH, and IDC endomyocardium in RV endomyocardium obtained by biopsy of the intact heart.

ANP and SRCA. Shown in Fig. 2 are the mRNA abundance levels for ANP and SRCA. Although the ANOVA was statistically significant comparing the three groups, the Scheffe multiple comparison test comparing each individual group to the other two was not. Only one subject in the nonfailing group, the subject with mild diastolic dysfunction, had an ANP value (106×10^5 molecules/μg total RNA) which overlapped into the PPH and IDC groups. Therefore, the data are consistent with an increase in ANP gene expression in both the PPH and IDC groups, and in the one control heart that exhibited diastolic dysfunction and higher diastolic filling pressures.

There were no differences in expression of SRCA mRNA among the three groups.

α- and β-MHC. Fig. 3 gives PCR amplification curves for internal standards and α- and β-MHC cDNAs amplified from total RNA extracted from endomyocardial biopsies taken from a nonfailing and a failing (IDC) heart. As shown in Fig. 3 *A*, the amplification curve for α-MHC in RNA extracted from the nonfailing heart is to the left of the curve from failing myocardium, indicating a greater amount of reverse-transcribed α-MHC mRNA in the nonfailing sample. In Fig. 3 *B*, it can be seen that the opposite is true of β-MHC; that is, the curve for RNA extracted from nonfailing heart is to the right of the sample from failing heart. As can be seen in the grouped data shown in Fig. 4, the RV endomyocardium of the nonfailing hearts contained a substantial amount of the α isoform of MHC ($\sim 37.0 \times 10^5$ molecules/μg total RNA, range 10.7–72.2). The lowest α-MHC value of 10.7×10^5 molecules/μg total RNA was from the subject who had suffered a cardiac arrest 72 h before being biopsied, and who had mild LV dysfunction but normal RV function. However, as shown in Fig. 4, β-MHC is the dominant isoform in all three groups. Both PPH and IDC exhibited a marked decrease in α-MHC mRNA abundance, by 75% in PPH and 60% in IDC (Fig. 4). As can be seen in Fig. 4, compared with the nonfailing group there is a tendency for the abundance of β-MHC to be slightly higher in both PPH and IDC groups ($P = 0.06$ by ANOVA). As shown in Fig. 5, the percentage of total MHC represented by the α-MHC isoform decreased from 23.1 to 5.6% in PPH and to 7.6% in IDC (both $P < 0.001$). Also shown in Fig. 5 is the change in the percentage of β-MHC, which varies from 76.9% in nonfailing ventricles to 94.4% in PPH and 92.2% in IDC.

β-RECEPTOR MEASUREMENTS

Shown in Table III are β_1- and β_2-receptor measurements in a high-yield crude membrane preparation. As can be seen in Table III, β_1- and β_2-receptor density parallels the mRNA abundance measurements, with both PPH and IDC groups exhibiting a downregulation in β_1-adrenergic receptors, and the PPH subgroup exhibiting a strong tendency ($P = 0.053$) for an increased β_2-receptor density.

MORPHOLOGIC AND MORPHOMETRIC MEASUREMENTS

The biopsies from all subjects analyzed in the IDC and PPH groups revealed myocyte hypertrophy or hypertrophy and mild fibrosis. There were no lymphocytic infiltrates in any PPH or IDC biopsy, or in the nonfailing controls. Five of the nonfailing control biopsies were read as normal on routine light microscopy, without evidence of hypertrophy or fibrosis. Two subjects examined at baseline before starting IL-4 had mild hypertrophy noted on biopsy, and the subject with mild diastolic dysfunction and class II symptoms of dyspnea on exertion had hypertrophy and mild fibrosis detected on light microscopy.

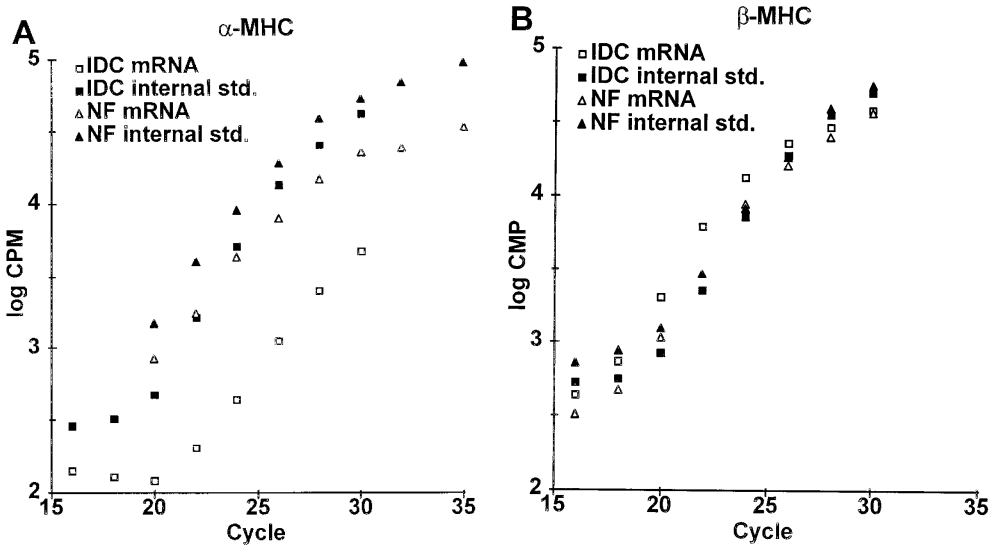

Figure 3. PCR amplification curves for internal standards and α- and β-MHC cDNAs amplified from total RNA extracted from endomyocardial biopsies taken from a nonfailing and a failing (IDC) heart. (*A*) Amplification curves for α-MHC and internal standard. (*B*) Amplification curves for β-MHC and internal standard.

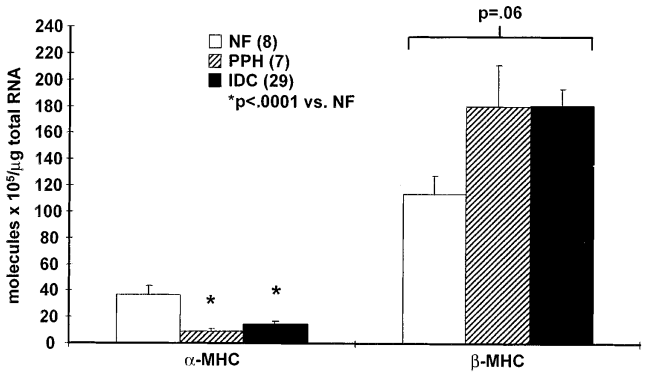

Figure 4. α- vs. β-MHC mRNA abundance in nonfailing, PPH, and IDC RV endomyocardium obtained by biopsy of the intact heart. As the data were not normally distributed, a Kruskal-Wallis test was also used, which yielded a $P < 0.05$ for both PPH and IDC groups compared with nonfailing groups.

The results of the light microscopic morphometric measurements of mean myocyte width are summarized in Table IV. As can be observed, myocyte width in the PPH group is increased compared with the nonfailing controls. The IDC group had intermediate myocyte width measurements that were not statistically significant versus either the control or the PPH group. There were no differences in myocyte volume fraction among the three groups.

Relation of α-MCH gene expression to myocardial function and regulation of other genes

Table V gives results of the univariate analysis of α-MHC mRNA abundance compared with the behavior of other genes and to other parameters including myocardial function, with the analysis conducted in the nonfailing plus IDC groups and in all three groups. As can be observed in Table V, α-MHC gene expression was only weakly related to age in the nonfailing plus IDC group, and unrelated to age when the younger PPH group was added. In nonfailing and IDC hearts the RV endomyocardial expression of α-MHC is directly and highly related to the LVEF but is only weakly and statistically insignificantly related to the RVEF. When the PPH group is added to the analysis, the relationship becomes weaker due to the fact that this group has normal LVEFs (Table III) but reduced α-MHC gene expression. Hemodynamic parameters are not closely related to α-MHC expression in either analysis; only mean PA pressure (inverse relationship) and cardiac index have r values > 0.25. In terms of the expression of other genes, in the three-group analysis the only mRNA that exhibits a good correlation is the β_1-adrenergic receptor. On multivariate analysis across the three groups LVEF ($P = 0.005$) and β_1-adrenergic receptor mRNA ($P = 0.0001$) were the only parameters that remained statistically significant.

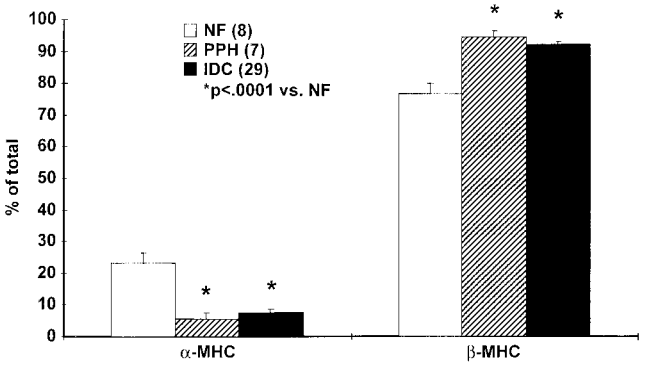

Figure 5. Percent expression of α- vs. β-MHC mRNA in nonfailing, PPH, and IDC RV endomyocardium obtained by biopsy of the intact heart. As the data were not normally distributed, a Kruskal-Wallis test was also used, which yielded a $P < 0.05$ for both PPH and IDC groups compared with nonfailing groups.

Table III. β-Adrenergic Receptor Measurements

	Receptor density, fmol/mg			Receptor %	
Group (n)	Total β*	β_1‡	β_2	β_1‡	β_2‡
NF (8)	74.5±12.3	57.7±10.4	16.8±3.6	77.0±4.1	23.0±4.1
PPH (7)	55.8±7.3	29.8±3.0§‡	25.9±5.4	56.9±6.9	43.1±6.9
IDC (27)	45.4±4.8§	29.7±3.9§	15.7±1.5	62.4±3.6	37.6±3.6

§ $P < 0.05$ vs. NF; *ANOVA $P < 0.05$; ‡Kruskal-Wallis test $P < 0.05$.

Regional gene expression in explanted human hearts

Table VI gives gene expression measurements in three different regions of failing and nonfailing explanted hearts. As can be observed, there is concordance of measurements in the three regions. Compared with nonfailing hearts there was a decrease in β_1-adrenergic receptor mRNA abundance in the LV and RV free wall as well as in the RV endomyocardium in the IDC ventricles. In contrast, there was no change in β_2-adrenergic receptor gene expression in any region of the IDC ventricles. ANP gene expression was increased in all three regions of IDC ventricles. SRCA expression was not statistically different between failing and nonfailing hearts, but values were 10–20% less than nonfailing controls in all three regions examined.

As shown in Table VI, explanted nonfailing hearts contained a substantial amount of α-MHC mRNA in all three regions examined. In the nonfailing LVs α-MHC abundance ranged from 36 to 64 molecules × 10^5/μg total RNA, with a percentage of total MHC ranging from 20 to 38 (average 29±3%). In the RV free wall α-MHC mRNA abundance and percentage of total MHC ranged from 39 to 74 molecules × 10^5/μg total RNA and 26–44%, respectively. In nonfailing ventricles α-MHC abundance and percentage of total MHC did not differ in the LV free wall, RV free wall, and RV endomyocardial sampling site. The mRNA abundance data are shown

Table IV. Histomorphometric Analysis of Endomyocardial Biopsies (±SEM)

Group	Myocyte width	Myocyte volume fraction
	μm	%
IDC (n = 9)	22.4±0.6	82.1±2.8
PPH (n = 5)	24.5±1.6*	78.0±7.4
NF (n = 7)	19.7±1.0	84.3±4.5

* $P < 0.05$ vs. NF.

Table V. Univariate Analysis of α-MHC mRNA Abundance versus Other Parameters

Parameter	Nonfailing, IDC		Nonfailing, IDC, PPH	
	r value	*P* value	*r* value	*P* value
1. Age	−0.35	0.03	−0.13	0.40
2. LVEF	0.67	0.0001	0.52	0.0007
3. RVEF	0.22	0.21	0.30	0.09
4. RA mean pressure	−0.02	0.92	−0.05	0.78
5. PA mean pressure	−0.11	0.55	−0.28	0.08
6. PW mean pressure	−0.14	0.45	−0.02	0.88
7. Cardiac index	0.29	0.11	0.34	0.04
8. Total β-AR density	0.14	0.43	0.10	0.52
9. β_1-AR density	0.17	0.33	0.19	0.23
10. β_2-AR density	−0.03	0.86	−0.14	0.38
11. β_1-AR mRNA	0.71	0.0001	0.71	0.0001
12. β_2-AR mRNA	0.57	0.0007	−0.08	0.62
13. ANP mRNA	−0.22	0.20	−0.26	0.11
14. SRCA mRNA	−0.09	0.65	−0.10	0.58
15. β-MHC mRNA	−0.17	0.36	−0.19	0.26

RA, Right atrial; *PA*, pulmonary artery; *PW*, pulmonary wedge; *AR*, adrenergic receptor.

in Table VI, and the α-MHC percentage of total MHC mRNA was 28±3, 34±3, and 29±3% in the LV free wall, RV free wall, and RV endomyocardium, respectively. As shown in Table VI, α-MHC gene expression was downregulated in all three regions of IDC ventricles, and β-MHC gene expression was increased in the RV and LV free walls. In terms of percentage of total MHC expression, α-MHC was decreased to 6.1±1.5, 5.6±0.8, and 6.3±0.9% (all $P = 0.0001$) in the LV free wall, RV free wall, and RV endomyocardium, respectively.

Discussion

Data presented in this study indicate that the expression of multiple genes can be measured in endomyocardial biopsy samples by RT-QPCR, for several ventricular myocardial phenotypes. The variant of RT-QPCR that we used is simultaneous amplification with an internal standard (SAIS), which has the advantage of using less RNA starting material than does competitive PCR (the other general approach for determining absolute levels of mRNA). This is because, in RNA extracted from human ventricular myocardium, with SAIS only three reverse transcription reactions are necessary, one each for low, medium, and high abundance messages. Since approximately half of our endomyocardial biopsy material was used to quantify β-adrenergic receptors, the number of mRNAs measured could have at least been doubled beyond the six reported here if receptors had not been measured.

Use of endomyocardial biopsy tissue allowed us to examine, for the first time, alterations in gene expression in hypertrophied human ventricular myocardium failing as the result of chronic severe pressure overload. Additionally, we were able to examine general alterations in gene expression in an ambulatory patient population of IDC myocardial failure that was not end-stage. The pressure-overloaded RV in PPH has been shown by others (22) as well as by our group (23) to evolve from concentric hypertrophy to chamber dilatation and failure, whereas the failing IDC RV is an example of volume-pressure overload and does not evolve through a phase of concentric hypertrophy (24). Despite the very different initiating insults in these two heart failure phenotypes, with one exception the failure-associated changes in gene expression were identical in the two groups. PPH RVs exhibited an increase in β_2-adrenergic receptor expression at both the mRNA and protein levels, but all other changes or trends in changes in gene expression—including upregulation in ANP, downregulation in β_1-adrenergic receptor, downregulation in α-MHC and upregulation in β-MHC—were quite similar in the two failure phenotypes. In addition, the degree of myocyte hypertrophy was judged to be histologically and morphometrically similar in the two myocardial failure groups. The downregulation in β_1-adrenergic receptor protein and mRNA, considered to be a phenotypic marker of systolic dysfunction (25), and upregulation in ANP gene expression, a phenotypic marker of hypertrophy (26), were predicted from previous results in explanted human hearts (1, 4, 6, 7, 9, 10, 27–29).

The most surprising and potentially most important new findings of this investigation are the expression of MHC isogenes in nonfailing and failing human RVs and LVs. MHC, or the thick filament of the sarcomere, contains in its head region the ATPase activity responsible for muscle contraction (30). Two MHC isoforms are present in mammalian hearts, α- and β-MHC. The α-MHC isoform is cardiac-specific and is three- to fourfold more enzymatically active (31, 32), while the less active β-MHC is present in the heart and in slow twitch skeletal muscle (30, 33). In humans, α- and β-MHC are closely related isogenes positioned sequentially on chromosome 14 (34). Although a substantial amount of α-MHC is present in human atrial myocardium (35–37), in human ventricular myocardium most previous studies have not detected significant (> 10%) α-MHC expression at the protein or mRNA levels (35, 36, 38, 39). Our data indicate that there is substantial expression of α-MHC mRNA in nonfailing ventricular myocardium, and

Table VI. Regional Gene Expression in Nonfailing (n = 6) and End-Stage Failing (IDC, n = 6) Human Ventricular Myocardium, mRNA Molecules × 10^5/μg Total RNA±SEM

Region	β_1-AR		β_2-AR		ANP		SRCA		α-MHC		β-MHC	
	NF	IDC	NF	IDC	NF	IDC	NF	IDC	NF	IDC	NF	IDC
RV endo	4.9±0.4	3.1±0.3*	2.5±0.3	2.0±0.3	34±2	121±26*	86±5	75±9	46±7	9.4±1.1*	111±7	142±5
RV FW	4.7±0.4	3.3±0.4*	2.1±0.5	1.8±0.3	32±2	92±18*	101±12	79±10	54±6	9.7±1.6*	104±11	163±6*
LV FW	4.8±0.6	2.9±0.6*	2.1±0.4	1.6±0.2	38±5	110±24*	68±9‡	56±6‡	46±9	8.9±1.9*	118±10	146±18*

* $P < 0.05$ vs. NF; ‡ $P < 0.10$ vs. RV endo or RV FW (ANOVA).

then a profound decrease in expression in myocardial failure. The downregulation in α-MHC was coupled with a reciprocal upregulation in β-MHC, as is observed in rodent hearts exhibiting changes in gene expression of MHC isoforms (40, 41). As was again noted in this study, the dominantly expressed MHC isoform in the human heart is β myosin, which in rodents is the fetal isoform (40–42). Rodents undergo a developmental change from β- to α-MHC as the dominant isoform, and then during hypertrophy or myocardial failure express as the dominant isoform β-MHC (41). Since most previous studies (35, 36, 38, 39) had suggested that human ventricular myocardium does not express a biologically significant amount of α-MHC and therefore does not undergo an isoform change in hypertrophy or failure, the MHC isogene expression data in this report and in our companion paper (43) were unanticipated. In fact, as originally designed the only reason MHC isogene expression was measured in the current study was to provide a method of normalization of other mRNAs.

Our data using SAIS quantitative RT-PCR to determine MHC mRNA abundance show that septal endomyocardium from nonfailing RVs contains 10–29% α-MHC, with an average value of 23% in eight RVs. In explanted nonfailing human hearts the mean percentage of α-MHC ranged from 28% in LV free wall to 34% in RV free wall, with RV septal endomyocardium containing 29% α-MHC. Therefore, the values for α-MHC/β-MHC mRNA expression did not differ across the three regions examined in nonfailing explanted hearts, and both the absolute abundance and percentage isogene expression were similar to values in endomyocardial biopsy samples taken from nonfailing intact hearts. The slightly higher values of α-MHC in the explanted hearts compared with the intact hearts were probably due to the fact that several of the intact heart controls were not completely normal, since they were derived from subjects with suspected myocardial disease. Collectively, these data indicate that nonfailing human ventricular myocardium exhibits a substantial amount of α-MHC gene expression at the mRNA level in all ventricular regions examined, and RV endomyocardial sampling may be used as a surrogate for the RV and LV free walls.

In both types of RV failure examined in the intact setting and in the explanted failing hearts, α-MHC was markedly downregulated and the β-isoform exhibited reciprocal upregulation. In intact hearts α-MHC was downregulated by 67% in IDC and 76% in PPH, which if translated into similar changes in protein expression would have significantly reduced the speed of contraction. Based on a threefold difference in speed of contraction in favor of the α-isoform (31) the changes in MHC gene expression would have accounted for respective decreases in shortening velocity of 21 and 24% in IDC and PPH RVs. These theoretical decreases in velocity of shortening corresponded to EFs of 0.32 in IDC and 0.29 in PPH, which represent moderate reductions in systolic function corresponding to moderate decreases in speed of contraction. In explanted end-stage failing LVs the downregulation in α-MHC of 80% would have theoretically reduced shortening velocity by 29%, in keeping with the severe LV systolic function (LVEFs of 0.14) in these transplant recipients. In RV endomyocardium and RV free wall of end-stage failing explanted hearts the predicted reduction in shortening velocity would have been by 29 and 34%, respectively. In other words, the predicted decrease in velocity of shortening based on changes in α- and β-MHC isoforms generally corresponds to the degree of systolic dysfunction in IDC and PPH RVs and IDC LVs. That downregulation in α-MHC and upregulation in β-MHC gene expression can contribute to the development of myocardial dysfunction and cardiomyopathy is supported by recent work from Robbins' laboratory, where mice with partial ablation (knockout heterozygotes) of the α-MHC gene have a 44% decrease in α-MHC mRNA abundance, a decrease in α-MHC protein of 25%, a 33% decrease in systolic function, and a cardiomyopathy consisting of sarcomeric alterations including hypertrophy and increased interstitial fibrosis (44).

Surprisingly, this report and the companion paper (43) are the first reported measurements of both α- and β-MHC mRNA gene expression in nonfailing and failing human hearts. Previous studies using Northern blotting and a rat α-MHC cDNA probe (2) which cross-hybridizes with rat β-MHC (45) reported no apparent difference in "MHC" expression between nonfailing and failing human hearts. Studer et al. (46), using Northern analysis and a β-MHC cDNA probe as a method of normalizing the expression of other genes, apparently found no difference between nonfailing and failing explanted human hearts. In collaborative quantitative PCR studies with Feldman's laboratory (1, 47) using quantitative RT-PCR we have reported statistically insignificant respective decreases and increases in α-MHC (47) and β-MHC (1) of 28%. Additionally, in a single hypothyroid patient with LV dysfunction Feldman's laboratory (12) reported an 11-fold increase in α-MHC mRNA gene expression with thyroid replacement and an increase in LVEF from 0.16 to 0.37. In view of the 96% homology of the α- and β-MHC cDNAs (19), it is not surprising that Northern blot analysis with either probe would not be able to detect differences in the expression of the two isoforms. As shown in this and the companion paper (43), quantitation of α- and β-MHC gene expression requires quantitative RT-PCR or ribonuclease protection assays.

For both α- and β-MHC, mRNA abundance and protein mass usually exhibit coordinate directional changes consistent with transcriptional regulation of gene expression (41). This concordance is observed in response to increased wall stress (19, 41, 48–50), during development (41, 51), with hormone administration (41, 50–52) and in response to gene ablation (44) or anthracycline treatment (53). However, exceptions to transcriptional regulation exist, such as in aortic banded rats (54) and rat cardiac myocytes subjected to contractile arrest (55). The rat α-MHC gene is known to have two potential mechanisms for posttranscriptional regulation, RNA processing and multiple polyadenylation sites (56). Thus it is not possible to directly infer changes in MHC protein levels from our mRNA data. Nevertheless, the observed decrease in α-MHC and increase in β-MHC gene expression correspond to multiple reports of decreased myosin (57, 58) or myofibrillar (59–61) ATPase activity in the failing human heart. Because it was assumed that there was no significant expression of α-MHC in nonfailing heart and no MHC isoform changes, this decrease in myosin or myofibrillar ATPase activity has been attributed to other factors that may regulate myosin ATPase activity, such as expression of variants of troponin T (62) or a reduction in regulatory light chains (57). Based on the data presented in this and the companion paper (43), there is reason to suspect that the failure-associated decrease in ATPase activity has its origin in the downregulation of α- and upregulation of β-MHC.

It should be pointed out that a few previous studies have reported a significant (> 10%) expression of α-MHC protein

in nonfailing human ventricular myocardium. Using selective antibodies Gorza et al. (37) reported that at least one control heart exhibited 14–24% of total MHC staining by the selective α-MHC antibody employed. However, these investigators found no decrease in α-MHC labeling with hypertrophy (37). Using gel electrophoresis, Alousi et al. (61) reported two distinct MHC bands, with a fast-migrating band presumably corresponding to α-MHC; however, these authors could not detect a difference between normal and failing hearts (61). A similar finding was reported by Takeda et al. (63), who reported in LV biopsy specimens a fast-migrating MHC band on gel electrophoresis that was 25–35% of the total, which paradoxically correlated with a decrease in myofibrillar ATPase activity in the same specimens. Kawana et al. (64) reported α-MHC fluorescence labeling in only a "few" myofibrils from normal human ventricular myocardium procured from autopsy specimens, but a striking increase in α-MHC positivity in ventricles from heart failure patients treated with the β-agonist dobutamine (64). Much of the problem with attempting to identify α- and β-MHC isoforms by gel electrophoresis is that in some species such as guinea pig—and perhaps human—the highly homologous ventricular α- and β-MHC isoform cannot be easily separated on pyrophosphate gels (65). This may be why ventricular α-MHC went undetected in the majority of previous human studies. Further studies will be required to determine if the MHC isoform mRNA measurements reported in both explanted hearts and biopsies removed from intact ventricles correlate with protein measurements. These measurements will have to be made by competitive RIA (65) using selective antibodies made from isoform-specific peptides.

One question which arises from the α-MHC gene expression data is the mechanism by which the mRNA is downregulated. As would be expected from the between-group mean data, α-MHC expression was directly related to myocardial systolic function as assessed by EF. In the nonfailing and IDC groups the relationship to LV function was much better than the RV function relationship. This was presumably because of the greater degree of precision of LVEF versus RVEF measurements using radionuclide techniques, inasmuch as when the PPH group, which had normal LVEFs, was added to the analysis the LVEF/α-MHC relationship worsened. These data suggest that better methods of measuring RV systolic function, such as by MRI or other techniques, will be required to more precisely examine the relationship between downregulation in α-MHC and depression of systolic performance. From the standpoint of potential signals for gene regulation, increased wall stress may play a role in the molecular regulation of α-MHC, as has been shown in rodent models (19, 41, 48–50). This study did not include a measurement of wall stress, which is influenced by pressure, chamber radius, and wall thickness. Such measurements are possible in the RV using MRI (23), and are being done in the next generation of these investigations. Finally, the close relationship between α-MHC and β_1-adrenergic receptor gene expression suggests that these two contractility-controlling genes are coregulated. However, there is currently no known regulatory paradigm which might account for this possibility, inasmuch as in the human heart the β_1-adrenergic appears to be regulated by changes in mRNA stability (66, 67) and despite the exceptions cited above MHC gene expression is generally thought to be predominately transcriptional (41).

In summary, in intact human ventricles the expression of multiple genes of interest can be reliably measured in endomyocardial biopsy specimens taken from multiple human cardiac phenotypes. Moreover, changes in gene expression in the RV endomyocardium reflect changes occurring in the RV free wall, and (when both ventricles are affected with the same disease process) in the LV free wall. Because the amount of starting material required for mRNA abundance measurements using SAIS RT-QPCR is low, and the expression of any gene with a known sequence may be examined, this method should prove to be an extremely useful tool in the characterization of myocardial disease processes and in assessing the long-term effects of pharmacologic therapy. The major new findings in this study are the substantial amount of α-MHC gene expression in nonfailing human ventricles (23–34%, depending on the region of sampling), and its marked downregulation coupled with an upregulation of β-MHC in two types of myocardial failure. Thus, in the human heart failing as the result of systolic dysfunction, changes in MHC isoforms are candidates for the molecular basis of myocardial failure, as had been predicted by previous studies in rodent models.

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