Hemodynamic Regulation of Myosin Heavy Chain Gene Expression

Studies in the Transplanted Rat Heart

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Abstract

Cardiac work is a major determinant of heart size and growth. Heterotopic cardiac isografts are hemodynamically unloaded and undergo atrophy. To determine the molecular changes that occur as a result of hemodynamic unloading, we have studied the rate of synthesis of total cardiac proteins and myosin heavy chain (MHC) and the expression of the myosin heavy chain gene as reflected in the messenger RNA levels for α - and β -MHC isoforms. 72 h after transplantation there is a significant decrease in left ventricular size accompanied by a 27% decrease in the rate of total cardiac protein synthesis and a 53% decrease in the rate of myosin heavy chain synthesis. In contrast to isografts 14 d after transplantation which have a decrease in protein synthetic capacity, simultaneous measurements of 18S ribosomal RNA and myosin messenger RNA suggest that after 3 d the decrease in synthesis is due to a change in the efficiency of protein translation. While the working in situ heart expresses primarily α -MHC mRNA (97%) hemodynamic unloading leads to a 43% decrease in α -MHC mRNA concentration and the de novo expression of the β -MHC mRNA. Total MHC mRNA (α plus β) concentration analyzed by a quantitative S_1 nuclease protection assay was similar in the two groups of hearts. Thus, in association with hemodynamic unloading there are changes in cardiac myosin heavy chain content as a result of both gene transcription and protein translation mechanisms. (*J. Clin. Invest.* 1992; 89:68–73.) Key words: cardiac protein synthesis • cardiac work • gene regulation

Introduction

Heart size and growth are determined in large part by cardiac work (1–3). In response to an increase in hemodynamic load there is a prompt increase in both total and specific contractile protein synthesis reported for hearts in vivo and in vitro and for cardiac myocytes in culture (2–4). The molecular changes that accompany this increase in cardiac protein synthesis have been

well studied (2, 3, 5, 6). When cardiac work is reduced, as occurs with heterotopic cardiac transplantation, the heart undergoes negative growth (atrophy) with a reduction in the rate of protein synthesis (7, 8). In addition to the quantitative decrease in left ventricular myosin content, we have previously observed that 14 d after transplantation there is a shift in the myosin isoenzyme expression with the appearance of the enzymatically less-active myosin ATPase (V_3) (7). This expression is similar to that reported for hypothyroidism, aging, and for hearts exposed to increased afterload, and reflects the expression of the specific gene coding for the β -myosin heavy chain (MHC)¹ (5, 9–12).

In the present study we have examined the role of cardiac unloading and atrophy on myosin heavy chain gene expression. To do this we have examined both myosin heavy chain protein synthesis as well as the simultaneous expression of the specific isoforms for myosin heavy chain messenger RNA. These studies identify both a quantitative as well as a qualitative shift in myosin gene expression and allow us to further identify the steps in the regulation of protein synthesis that are important mediators of cardiac growth and plasticity (2, 10, 11, 13).

Methods

Cardiac transplantation. Inbred male Lewis rats weighing 140–160 g were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA. Infrarenal heterotopic cardiac transplants were performed as previously described using a modification of the technique of Ono and Lindsey (7, 14). Anastomosis of the end of the donor heart aorta to the side of the recipient abdominal aorta was followed by connection of the pulmonary artery to the inferior vena cava to return myocardial blood flow. The aortic valve of the transplanted heart was maintained competent and the workload of the transplant was decreased (7, 8, 15). Heart weights were measured at the time of death, total heart weight includes the left and right ventricles after removal of the atria and great vessels, left ventricular weight includes the intraventricular septum.

Protein synthesis. Left ventricular protein synthetic rates were determined in vivo by constant infusion of [³H]leucine as previously described (16). A 2.5-ml solution of 0.4 mCi/ml L-[4,5-³H]leucine (120 Ci/mmol) Amersham Corp., Arlington Heights, IL), 100 mM leucine, 10 U/ml heparin, and 150 mM NaCl was continuously infused for 6 h through the femoral vein of restrained awake animals. After the infusion period the rats were killed, blood was collected for serum leucine-specific radioactivity determinations and the hearts were removed, washed in cold PBS (4°C) and the left ventricles were dissected free and frozen in liquid nitrogen.

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1. Abbreviations used in this paper: H, host or in situ heart; LV, left ventricle; MHC, myosin heavy chain; RV, right ventricle; T, transplanted or isograft heart.

Serum samples were deproteinized by the addition of an equal volume of 10% TCA, amino acids were isolated by cation exchange chromatography, and serum-specific radioactivity (dpm/nmol) was measured (16, 17). Left ventricular tissue was homogenized in 19 vol of 100 mM KCl and 250 μ M thioglycolate, pH 6.8. An aliquot of the homogenate was used for protein content determinations (18). A separate aliquot was extracted with 10% TCA, washed four times in 1.5 ml of 10% TCA, once with ethyl ether, and then hydrolyzed in 6 N HCl at 110°C for 24 h under vacuum. The hydrolysate was concentrated by evaporation and leucine-specific radioactivity in total cardiac protein was determined by the 14 C-dansyl chloride dilution method (16).

Myosin heavy chain was isolated from the remaining homogenate by centrifugation of the homogenate at 10,000 *g* for 10 min. The sediment was washed by suspension and recentrifugation as previously described. Myofibrillar proteins were then separated by SDS-PAGE, and the bands corresponding to myosin heavy chain isoforms (α - and β -myosin heavy chain) were excised and hydrolyzed. Leucine-specific radioactivity (dpm/nmol) was assayed by isotope dilution (16).

Fractional synthetic rates (K_s ; day $^{-1}$) of left ventricular total protein and myosin heavy chain were determined as previously described by Everett et al. (16) using the formula, $K_s = P^*/F^*[t/(1 - e^{-K_f t}) - 1/K_f]$, where P^* is the incorporated leucine-specific radioactivity (dpm/nmol), F^* is the serum leucine-specific radioactivity (dpm/nmol), t is the time of infusion (days), and K_f is the first-order rate constant for the rise in serum leucine-specific radioactivity to plateau (74.3/d). Protein synthetic rates (R_s ; mg/d per LV) were generated by multiplying the fractional synthetic rate by the left ventricular total protein or myosin heavy chain content. Myosin was assayed as previously described (4, 19) and myosin heavy chain content was calculated assuming that it constitutes 80% of the weight of the total myosin molecule.

Total cellular DNA and RNA determinations. Isolated left ventricles that had been frozen in liquid nitrogen were pulverized with a mortar and pestle, and an aliquot was homogenized in 10–20 vol of solution containing 150 mM NaCl, 5 mM EDTA, 1.0% SDS, 50 mM Tris-HCl, pH 8.3, and 1.2 mg/ml proteinase K (20). Samples were incubated at 25°C for 10 min. The mixture was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (50:48:2) followed by one extraction with chloroform-isoamyl alcohol (96:4). The final aqueous phase was adjusted to 0.2 M sodium acetate and total nucleic acids were precipitated by the addition of 2.2 vol of 100% ethanol at –20°C. The precipitate was recovered by centrifugation and washed twice with 70% ethanol, dried, and resuspended in 1 mM EDTA pH 7.0 (8).

The DNA concentration was determined by fluorometric analysis using Hoechst dye 33258 (Polysciences, Inc., Warrington, PA) and compared to a standard curve of calf thymus DNA ranging from 0.05 to 1.0 μ g/ml in a solution of Hoechst dye 0.1 μ g/ml, 0.1 M NaCl, 1.0 mM EDTA, and 10 mM Tris pH 7.4. Relative fluorescence was measured using a fluorometer (Gilford Instrument Laboratories, Inc., Oberland, OH) at an excitation wavelength of 458 nm and an emission wavelength of 365 nm (21).

Total nucleic acid content was determined by spectrophotometric absorbance at a wavelength of 260 nm. Only when the absorbance ratio of A_{260}/A_{280} was between 1.8–2.0 were the samples considered of sufficient purity for analysis. The RNA content was derived by the difference between total nucleic acid and DNA content. The efficiency of RNA extraction using this methodology has been previously documented. Ribosomal 18S RNA and total MHC mRNA levels were measured by dot-blot analysis using serial dilutions of total cellular RNA (22). Total MHC mRNA levels were quantitated by hybridization of the dot-blots to a radiolabeled 486 bp *Ava* I cDNA fragment from a highly conserved region of both α - and β -MHC mRNA's (pMHC-5 provided by A. Barrieux). Specific radioactivity of the probe was 12×10^6 dpm/pmol and was added to a final concentration of 10^6 dpm/ml hybridization solution. After hybridization the dot-blots were washed at a final stringency of $0.2 \times$ SSPE + 1% SDS at 65°C. 18S RNA was quantitated by hybridization to a 2-kb 18S rDNA fragment (23) radiolabeled by random priming methodology to a specific radioac-

tivity of 5×10^8 dpm/pmol. Dot-blots were washed at a stringency of $0.05 \times$ SSPE + 1% SDS at 65°C. The dots were excised with a hole punch and the radioactivity measured by liquid scintillation counting. The number of 18S molecules per LV was derived as previously described (8).

Poly(A) $^+$ RNA was isolated by oligo (dT) cellulose chromatography (Gibco-Bethesda Research Laboratories, Bethesda, MD) using standard methodology (22).

Northern blot analysis of MHC mRNA. Total cellular RNA was extracted from frozen pulverized left ventricles by a modification of the acid guanidinium thiocyanate-phenol-chloroform method described by Chomczynski and Sacchi (24). After homogenization in guanidinium-thiocyanate and extraction with phenol-chloroform, the aqueous phase was reextracted with phenol/chloroform (1:1) followed by extraction with an equal volume of chloroform. The RNA was then precipitated from the aqueous phase with an equal volume of isopropanol at –20°C. The pellet was collected by centrifugation, washed with 70% ethanol, air dried, and dissolved in sterile water. The concentration of RNA was determined spectrophotometrically.

Total cellular RNA was size fractionated by electrophoresis on 1% agarose gels containing 2.2 M formaldehyde, 20 mM *N*-morpholino propane-sulfuric acid, pH 7.0, 5 mM sodium acetate, and 1 mM EDTA. The RNA was then transferred to nylon membranes (Duralon UV; Stratagene, Inc., La Jolla, CA) by capillary blotting (22) and immobilized on the membrane by exposure to UV light (Stratagene). These membranes were prehybridized for 8 h at 42°C in a solution containing 50% formamide (vol/vol), 5 $\times$ Denhardt's solution, 1% SDS, 5 $\times$ SSPE (0.75 M NaCl, 50 mM sodium phosphate, pH 7.4, 5 mM EDTA) and 100 μ g/ml denatured salmon sperm DNA. Hybridization of the RNA to either cDNA or oligonucleotide probes was carried out in the same prehybridization solution at 42°C for 18 h. In order to standardize for loading variability among lanes, the Northern blots were routinely hybridized to an 18SrDNA probe as described above.

Total MHC mRNA levels were measured by hybridization to pMHC-5 cDNA probe as described for the dot-blot analysis and quantitated by densitometric scanning (over a linear range) of the autoradiograms (8). Each measurement was normalized to 18S rRNA, which was also quantitated by densitometry. α - and β -MHC mRNAs were detected by hybridization to 40-base oligonucleotide probes complementary to unique 3' untranslated regions of the mRNAs (Oncogene Science, Inc., Manhasset, NY). Alpha-tubulin mRNA was identified by similar hybridization conditions to a 40-base probe from the same manufacturer. Specific radioactivities of the probes, end labeled with [γ - 32 P]ATP (New England Nuclear, Boston, MA) using T_4 polynucleotide kinase, were routinely 4×10^6 dpm/pmol. After hybridization the membranes were washed at a final stringency of $2 \times$ SSPE and 1% SDS at 65°C for 30 min, and then exposed to x-ray film.

MHC mRNA analysis by S1 nuclease protection assay. In an S1 nuclease mapping assay, a single oligonucleotide probe was used to quantitate both the α - and β -MHC mRNAs in the same sample of cellular RNA. The method used was a modification of that described by Waspel et al. (10). A 61-base oligonucleotide probe was synthesized on a Biosearch Model 8600 DNA Synthesizer (San Rafael, CA) and purified by PAGE. The probe was complementary to a 38-base common-coding sequence at the carboxyl terminal of both α - and β -MHC mRNAs (nt 5763–5800) (25), and to an additional 8-bases, complementary only to β -MHC mRNA. The oligomer probe, 3' labeled with [α - 32 P]Cordycepin 5' triphosphate (New England Nuclear) was hybridized in molar excess to 10 μ g total cellular LV RNA. Comparison among experimental groups was possible by using oligomer probe derived from a single labeling reaction. The hybridization reaction was carried out in a 60- μ l vol containing 50% formamide, 40 mM Pipes, pH 6.5, 400 mM NaCl, 1 mM EDTA, 10 μ g yeast RNA, overlaid with 40 μ l mineral oil and incubated at 40°C for 15 h. S1 nuclease digestion was conducted at 37°C for 2 h in 400 μ l final vol containing 280 mM NaCl, 30 mM sodium acetate, pH 4.6, 1 mM zinc acetate, 5% (vol/vol) glycerol and 800 U enzyme (Gibco-Bethesda Research Laboratories). The reaction was stopped by the addition of 5 μ g yeast tRNA, ammonium

Table I. Left Ventricular Total Protein and MHC Synthesis Rates

	Protein content	[³ H]Leucine protein specific radioactivity	Fractional synthetic rate K_s	Protein synthetic rate R_s
	mg/LV	dpm/nmol	day ⁻¹	mg/d per LV
Total cardiac protein				
In situ	52±2	69±10	0.13±0.03	6.6±1.5
Transplant	41±1**	71±12	0.13±0.04	4.8±1.1*
Myosin heavy chain				
In situ	7.8±0.37	69±6	0.12±0.02	0.97±0.09
Transplant	5.8±0.22*	45±5*	0.08±0.02*	0.46±0.12*

* Significantly less than paired in situ $P < 0.05$; ** $P < 0.01$; $n = 5$.

acetate, and EDTA, and then extracted once with phenol/chloroform (1:1) and the nucleic acids precipitated with 2 vol ethanol at -20°C . The precipitate was collected by centrifugation, washed with 70% ethanol, dried, and dissolved in 7 μl gel-loading buffer containing 80% formamide. The digestion products were resolved by electrophoresis on a 10% polyacrylamide/7 M urea gel and identified by autoradiography. MHC mRNA species were quantitated by excising the appropriate bands from the gels and counting the radioactivity, or by densitometric scanning of the autoradiograms.

The specificity of this assay was ascertained by using total RNA purified from hearts of rats made hypothyroid by thyroidectomy in which the MHC mRNA was predominantly beta or from 8-wk-old growing euthyroid animals in which alpha predominated. Fig. 2 shows the protected fragments corresponding to the MHC mRNAs. The major hybridizing band plus the band immediately above and below were excised for quantitation by scintillation counting. Northern blot analysis using total cellular RNA extracted from rat ventricles confirmed the specificity of the S1 oligomer probe for the MHC mRNA (data not shown).

Statistical analysis. Results are expressed as means±SEM unless stated otherwise. Statistical differences between paired mean values were evaluated by Student's t test, and a two-tailed t test was used for comparing the means of nonpaired data (26).

Results

The heterotopically transplanted hearts maintained structural and functional integrity as has been previously documented (7, 14, 27). The period of surgical warm ischemia that has been shown to be an important determinant of cell viability was always less than 20 min (28). These hemodynamically unloaded and denervated hearts maintained a spontaneous mean sinus heart rate of 326 bpm, which was significantly slower than the simultaneously measured in situ heart rate of 390 bpm. Because the aortic valve remained competent the left ventricle did not fill normally and the heart was mechanically unloaded (7, 8, 15).

Cardiac mass, protein, and nucleic acid content. To assess the changes in cardiac mass (total heart and LV) we have compared the isograft to the in situ heart size from weight matched animals at day 0 and to the in situ paired heart at the time of sacrifice. 3 d after transplantation the unloaded transplanted hearts (RV plus LV) decreased in size from 455 ± 3 (day 0) to 379 ± 10 mg, whereas the in situ hearts grew to 475 ± 10 mg such that the in situ heart weight to body weight ratio for these growing animals was maintained. There was a decrease in LV size of the transplant to 298 ± 8 mg compared to the in situ weight of 355 ± 5 and 366 ± 8 mg at days 0 and 3, respectively ($P < 0.001$).

The decrease in LV mass of the isograft was accompanied by a similar decrease in total protein content to 41 ± 1 mg when compared with 52 ± 2 mg/LV for the in situ heart (Table I). The concentration of protein (mg/g wet wt) was no different between the host and transplanted hearts (142 ± 3 vs 137 ± 2 , respectively).

Transplanted heart LV total RNA content ($\mu\text{g/LV}$) was reduced by 10% from 551 ± 18 μg measured at day 0 to 499 ± 20 μg after 3 d ($P < 0.05$). Ribosomal 18S RNA content was similarly reduced. The values reported in Table II are those for LV total RNA and LV 18S RNA simultaneously measured in the in situ and transplanted hearts 3 d after surgery. Although there were significant reductions in protein content, total cellular RNA, and 18S rRNA in the transplant, total DNA content remained unchanged.

Total cardiac protein and myosin heavy chain synthesis. To address the possible mechanism(s) by which hemodynamic unloading of the transplanted heart resulted in a marked decrease in cardiac mass, we measured the synthesis rates for total cardiac proteins and for the myosin heavy chains.

[³H]Leucine specific radioactivity in the serum was $2,489\pm294$ dpm/nmol, and was assumed to be equal to the cardiac leucyl-tRNA specific radioactivity in both the in situ and transplanted hearts during the entire course of the constant intravenous infusion. Fractional synthetic rates (day^{-1}) were determined by measuring leucine specific radioactivity in total cardiac protein, and in electrophoretically purified myosin heavy chains. Based on these measurements, the K_s value for myosin heavy chain in the transplant was 60% of the rate determined for the in situ heart ($P < 0.05$) (Table I). When protein synthetic rates were calculated based on the total LV protein pool and the LV myosin heavy chain content, both total LV protein and myosin heavy chain synthetic rates (mg/d/LV) were significantly reduced in the transplanted heart to 72 and

Table II. Left Ventricular Total RNA, 18S RNA, and DNA Content

	Hearts	
	In situ	Transplant
RNA content ($\mu\text{g/LV}$)	567±22	499±22*
RNA concentration (mg/gLV)	1.55±0.06	1.68±0.09
18S content (molecules/LV) $\times 10^{10}$	5.79±1.32	4.88±0.81*
DNA content ($\mu\text{g/LV}$)	569±24	547±25

* $P < 0.05$ vs in situ, $n = 10$.

47% of the in situ synthetic rates, respectively (Table I). Because these protein synthetic rates were decreased to a greater extent than was the decrease in total cellular RNA content (12%, Table II), this suggests that the capacity of protein synthesis was not rate limiting but that protein synthetic efficiency was altered.

Because the transplanted heart has decreased in size by 21% when compared to the in situ heart ($P < 0.05$), we also calculated the total protein and MHC synthetic rates per gram of tissue. When expressed in this fashion the total cardiac protein synthesis rate (R_p) was 13.9 ± 2.1 mg/d per g of tissue in the host and 12.7 ± 1.8 in the transplant. The MHC synthetic rates per gram LV tissue were 2.0 ± 0.44 and 1.23 ± 0.31 ($P < 0.05$) in the host and transplant, respectively.

Myosin heavy chain gene expression. The percentage decrease in the rate of myosin heavy chain synthesis was greater than that of total cardiac proteins in the 3-d transplanted heart, which suggested that myosin heavy chain protein synthesis was altered either at a pretranslational or transcriptional step. To further assess this possibility we measured total MHC mRNA levels as well as the relative distribution of the two MHC gene transcripts, α and β , by Northern blot analysis and by a S1 nuclease protection assay.

Fig. 1 shows representative host and transplant RNA samples on a Northern blot that was sequentially hybridized to α - and β -MHC specific oligonucleotide probes, to the MHC cDNA probe pMHC-5, and to an 18S rDNA probe to standardize for the amount of sample loaded in each lane. Total MHC mRNA was measured by the pMHC-5 cDNA probe, which hybridized equally to α - and β -mRNA species. Quantitation by densitometric scanning of the MHC mRNA and normalization to 18S rRNA on the same Northern blot showed no significant differences in the cellular concentration of total MHC mRNA between host and transplanted hearts (Table III). This result was further confirmed by quantitative dot-blot hybridization analysis and by the S1 nuclease protection assay (Table III), in which host and transplanted heart RNA samples were compared within a single labeling experiment.

Hybridization to α - and β -MHC specific oligonucleotide probes showed that the 3-d hemodynamically unloaded trans-

Figure 1. Northern blot analysis of MHC mRNA. 5 μ g of total ventricular RNA from paired samples of host (H) and transplanted (T) hearts were hybridized sequentially to pMHC-5 cDNA probe, α - and β -MHC specific probes and 18S rDNA probe. The migration of 18S and 28S RNA is indicated. Autoradiography was for 24 h with pMHC-5 and alpha MHC probes, 48 h with β -MHC, and 2 h with 18S rDNA probes.

Table III. Left Ventricular MHC mRNA Expression Measured by S1 Nuclease Analysis, Dot Blot, and Northern Blot Hybridization

	Hearts	
	In Situ	Transplant
S1 nuclease assay		
α -MHC/ α - + β -MHC [†]	0.93 ± 0.03	$0.48 \pm 0.06^*$
α - + β -MHC/10 μ g RNA [‡]	196 ± 21	188 ± 46
Dot blot hybridization [§]		
pMHC/18S	3.44 ± 0.25	3.66 ± 0.06
Northern blot hybridization [†]		
pMHC/18S	1.23 ± 0.02	1.14 ± 0.17

Data are mean $\pm$ SEM, * $P < 0.01$ vs in situ; $n = 5$ for S1 Nuclease, $n = 3$ for dot blot, $n = 4$ for Northern blot analysis; [†] measured by densitometric scanning of autoradiograms; [§] measured by scintillation counting of dots or excised bands (dpm).

planted heart expressed the β -MHC gene de novo and that the cellular content of α -MHC mRNA was lower than in the host in situ heart. To determine the relative levels of α - and β -MHC mRNA expression in the same RNA sample from either host or transplanted hearts, we used an S1 nuclease protection assay. Quantitation of the MHC mRNA species by densitometric scanning of the protected fragments in the autoradiograms as shown in Fig. 2 revealed that α -MHC mRNA constituted $93 \pm 3\%$ of the total MHC mRNA in the in situ heart and only $48 \pm 6\%$ in the 3-d transplant ($P < 0.01$). β -MHC expression that was negligible in the host working heart was markedly induced in the unloaded transplant to constitute 50% of the total MHC mRNA.

To determine if the decrease in total cardiac protein synthesis (R_p) could possibly be the result of a general decrease in all mRNA species we measured the expression of a representative cytoskeletal gene, α -tubulin. Northern blot analysis of 5 μ g of either total RNA or poly(A)⁺ RNA showed no differences in

Figure 2. S1 nuclease analysis of MHC mRNA. A single 3' end-labeled 62 nt synthetic probe was hybridized to 10 μ g total LV RNA from hypothyroid (HYPO) and euthyroid (EU) rats, and 3-d host (H) and transplanted (T) hearts. The probe formed hybrids with a 46-nt fragment of beta MHC mRNA and a 38-nt fragment of alpha-MHC mRNA.

alpha-tubulin mRNA concentration between paired host and transplanted heart samples. When expressed as optical density (absorbance area, AU $\times$ mm) per microgram poly(A)⁺ RNA the results were 0.28 ± 0.08 and 0.33 ± 0.10 for the host and transplanted hearts, respectively.

Discussion

The cardiac response to work is a dynamic process (1–3, 12). The heart responds to an increase in work with a prompt rise in both total, as well as specific, protein synthesis (2, 3). The mechanisms responsible for these changes have been described (2, 5, 6, 12, 28). In contrast, the response to hemodynamic unloading with cardiac atrophy is less well studied (7, 8, 27).

The heterotopically transplanted cardiac isograft is a functionally competent (29) and histologically normal heart that undergoes rapid and marked cardiac atrophy (7, 15, 30). As early as 3 d after hemodynamic unloading the isograft has decreased in size by 15% when compared to the size at the time of transplantation and by 20% when compared to the recipient animal's in situ working heart. We have shown in this and in previous studies that this negative growth is accompanied by a rapid decline in the rate of total cardiac protein synthesis (7, 8, 30). To further assess the mechanisms by which changes in hemodynamic load alter cardiac protein synthesis (2–4), we undertook in the present study to measure the synthesis rate of a major contractile protein, myosin heavy chain, and to quantify the cellular content of the messenger RNAs that code for this protein.

Based upon our current understanding of the cellular regulation of protein synthesis, it is possible to alter synthetic rate at the level of gene transcription or protein translation (28). Transcriptional level regulation implies changes in mRNA synthesis (31) while translational regulation can result from changes in the capacity for peptide synthesis (ribosomal RNA content) (32, 33) or in the efficiency of translating messenger RNA into protein (34). In the present study we observed a 53% reduction in the rate of MHC synthesis at a time when MHC mRNA content per microgram of total RNA was unaltered and total ribosomal LV RNA content was reduced by only 15%. This combination of changes leads us to conclude that translational, rather than transcriptional level regulation is responsible for the initial decrease in myosin heavy chain synthesis observed after hemodynamic unloading in the transplanted heart.

The data demonstrating a maintenance of MHC mRNA as a proportion of total cellular RNA were derived from both Northern blots using a cDNA probe complementary to both α and β MHC as well as with an S1 nuclease assay that allowed comparison of MHC mRNA content between paired samples. Because total LV RNA content ($\mu\text{g/LV}$) was decreased in the transplanted heart, MHC mRNA content decreased proportionately. However, this decrease was of a significantly smaller magnitude than the decrease in the MHC protein synthetic rate.

Prior studies have observed that in response to changes in thyroid hormone (9, 31, 35) or insulin levels (36), aging (9), and cardiac overload leading to cardiac hypertrophy (5, 11, 31) there is a shift in the MHC mRNA isoforms. This has been linked to a transcriptional change in MHC gene expression (10). In the present study of hearts undergoing hemodynamic unloading in euthyroid, young growing rats we observed de

novo induction of β -MHC transcription concomitant with a reduction in the expression of the alpha isoform. We have previously shown that this leads to increased V_3 myosin content (7). Thus, while the total amount of MHC synthesis appears to be primarily regulated at a translational level, the specific isoform expressed appears to result from transcriptional changes.

Cardiac protein synthetic capacity varies as a function of total cellular RNA (2, 3, 28, 32, 33). Efficiency is measured as the rate of synthesis per unit rRNA (8, 33). In contrast to studies of transplanted hearts 7 and 14 d after surgery when the decline in total cardiac protein synthesis is due to changes in synthetic capacity (8), after 3 d there appears to be a decrease in protein synthetic efficiency. Thus, immediately after the onset of changes in hemodynamics there is a selective reduction of MHC translation, while at later time points the capacity for total protein synthesis is reduced as the heart reaches a new steady state size.

Similar to prior reports from this laboratory (8) the fractional synthetic rate (K_s) of total cardiac proteins was not different between host and transplanted hearts. This implies that hemodynamic unloading may also affect protein degradation, as a mechanism leading to cardiac atrophy. 3 d after transplantation we observed that the K_s for the MHC, a protein with a relatively long half life was significantly reduced. Conversely, relatively small increases in the synthetic rate of short-lived proteins could have profound effects on the measured rates for total protein synthesis (37). It is generally assumed that the kinetics of protein synthesis are independent of the amount of protein in the protein pool and that, during muscle growth or atrophy, changes in the protein pool size can occur independently of changes in the fractional protein synthetic rates (38).

In the present studies a reciprocal relationship appears to exist between the α - and β -MHC gene such that the total level of expression is maintained. While similar to that observed under experimental conditions of varying thyroid hormone status (39), the current changes occur in the absence of changes in serum levels of T_4 or T_3 (7), reinforcing the potential importance of hemodynamic factors in mediating gene transcription. Because the isograft is denervated and beats at a slower rate (30) we cannot exclude the role of neurohumoral or heart rate-related parameters in altering myosin gene expression.

The heterotopic cardiac isograft appears to offer an opportunity to study the potential mechanisms by which myocytes alter the synthesis of specific proteins by translational level regulation. Whether there is also selective and preferential synthesis of α - and β -MHC proteins will require further study (30, 39).

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