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Research Article

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Structural and Enzymatic Comparison of Human Cardiac Muscle Myosins Isolated from Infants, Adults, and Patients with Hypertrophic Cardiomyopathy

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ABSTRACT Human cardiac ventricular myosins were prepared from autopsy samples from nine adults, seven infants, and from surgical specimens from seven patients undergoing left ventricular septal myectomy for obstructive hypertrophic cardiomyopathy. Infant myosin differed from adult myosin in two important characteristics: (*a*) ~30% of the 27,000-dalton myosin light chain is replaced by a 28,000-dalton light chain, and (*b*) the actin-activated myosin MgATPase activity of infant myosin is significantly lower than that of adult myosin (64 nmol phosphate released/mg myosin per min vs. 124 nmol/mg per min at 37°C). The K^+-EDTA ATPase activity of the myosin measured in 0.5M KCl is also lower in infants (1,210 nmol/mg per min vs. 620 nmol/mg per min at 37°C), but the Ca^{++}-activated ATPase is not significantly different. There were no differences in enzymatic activity between the normal adult and cardiomyopathic myosins.

A detailed study was performed to investigate possible variations in the structure of the myosin heavy chain in infant, adult, and cardiomyopathic samples. There were no significant differences between infant and normal adult, or between normal adult and cardiomyopathic myosins seen in pyrophosphate polyacrylamide gel electrophoresis, or peptide mapping using alpha-chymotrypsin, papain, or cyanogen bromide to generate peptides.

These results suggest that isoenzymes of human ventricular myosin do not exist for the myosin heavy chain in the specimens examined from infants, adults, and patients with obstructive hypertrophic cardiomyopathy. The decreased actin-activated MgATPase activity found for infant myosin appears to be due solely to a partial replacement of the 27,000-dalton light chain of myosin with a 28,000-dalton light chain.

INTRODUCTION

The existence of isoenzymes of cardiac myosin has been postulated to explain the correlation between in vitro myosin ATPase activity and altered myocardial contractile properties of disease states (1–3). Recently, new myosins have been demonstrated in animal models of cardiac hypertrophy that differ from controls in primary structure. The myosin molecule is dimeric, being composed of two 200,000-dalton heavy chains and two pairs of light chains, which in ventricular myocardium are ~20,000 and 27,000 daltons. Isoenzyme variants, differing in either the heavy or light chains (or both), may account for the altered enzymatic activities seen in the myosins from hypertrophied myocardium (3) in man.

Flink and Morkin (4), using cyanogen bromide to cleave the polypeptide chains at methionine residues, showed the presence of a new myosin heavy chain isoenzyme in thyrotoxic rabbit hearts, which correlated with an increased actin-activated myosin MgATPase activity (5–8). Hoh (9), using a technique for polyacrylamide gel electrophoresis (PAGE)[1] of intact native myosin in a glycerol-pyrophosphate buffer system, was able to separate three different ventricular myosin heavy chain isoenzymes (V1, V2, and V3, in order of decreasing velocity of migration) in rat heart (10). These isoenzymes differ in Ca^{++}-activated ATPase activity with V1 showing the highest, and V3 the low-

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[1] *Abbreviations used in this paper:* DTT, dithiothreitol; MOPS, 4-morpholino-propane sulfonic acid; PAGE, polyacrylamide gel electrophoresis.

est activity. Manipulations of the thyroid status (hypophysectomy and thyroxine replacement) were accompanied by appropriate shifts in isoenzyme distribution: the hypothyroid rats showed mostly V3, and thyroxine-replaced rats showed mostly V1 myosin. The isoenzyme patterns also varied with age, being completely V1 at 3 wk of age and showing increasing amounts of the V3 isoenzyme up through 12 wk of age. Conditions were thus available in which only one of the three isoenzymes would be present in the myocardium in a given animal: the hypothyroid rat shows only V3, and the weanling rat (3 wk of age) shows only V1 myosin. That these electrophoretically different myosins are heavy chain isoenzyme variants is substantiated by differences in two-dimensional peptide maps performed following cleavage with cyanogen bromide. Differences in the methionine and arginine content, and in Ca^{++}-activated ATPases (11, 12) are also reported for these two isoenzymes. There were no differences seen in myosin light chains in V1 and V3 cardiac myosins by sodium dodecyl sulfate (SDS) PAGE.

Microcomplement fixation and pyrophosphate PAGE (13–16) have also been used to demonstrate a change in distribution of the myosin heavy chain isoenzymes in rat hearts undergoing hypertrophy due to mechanical overload caused by aortic stenosis and regurgitation. With increasing hypertrophy there was a decrease in the proportion of V1 myosin and an increase in that of V3 myosin along with an accompanying decrease in myosin Ca^{++}-activated ATPase activity.

Marked changes in the distribution of these cardiac myosin heavy chain isoenzymes have also been seen in fetal and early postnatal development in the rat, rabbit, mouse, guinea pig, dog, pig, and beef hearts (17). In each species, the slow migrating isoenzyme, V3, is the predominant form during fetal development. The V1 isoenzyme appears during the perinatal period. In the rat and mouse V1 becomes predominant and remains so throughout adult life. In the rabbit and pig, on the other hand, V1 myosin appears only transiently, V3 myosin being the dominant isoenzyme in adulthood.

In addition to isoenzymes of the myosin heavy chain, the existence of a unique human fetal cardiac muscle myosin light chain isoenzyme has recently been demonstrated by Cummins (18). This light chain of myosin (M_r, 28,000) is detectable in decreasing amounts from birth to 8 mo of age. It appears to migrate electrophoretically with an R_f identical to adult atrial myosin light chain and a development shift from atrial to ventricular myosin gene expression in the newborn period has been suggested.

In view of these recent advances in the study of cardiac myosin isoenzymes in animal models, the role of cardiac myosin isoenzyme variation in man was considered. An earlier paper from this laboratory (19) reported on purified myosin isolated from the ventricles of subjects with hypertrophic obstructive cardiomyopathy. These studies showed no differences between the myosins from patients with hypertrophic cardiomyopathy and normal hearts with respect to K^+-EDTA or Ca^{++}-activated ATPase activity measured in 0.5 M KCl, in SDS-PAGE, and in bipolar filament formation. The present study reports a comparison of myosins isolated from adults, infants, and patients with hypertrophic cardiomyopathy incorporating peptide maps, pyrophosphate gel electrophoresis, as well as actin-activated MgATPase determinations in an attempt to identify human cardiac myosin isoenzymes and their role in human development and disease.

METHODS

Tissue sources. Autopsy samples of ventricular myocardium were generously provided by Dr. Walter Lawrinson, Department of Pathology, Washington Hospital Center; by Dr. Roma Chandra, Department of Anatomical Pathology, Children's Hospital of District of Columbia; by Dr. Donald Kerwin, Department of Pathology, Georgetown University Hospital; and by the Pathological Anatomy Service of the National Institutes of Health Clinical Center. Table I lists pertinent clinical information along with assay results for each tissue specimen individually.

The adult cardiac myosins were prepared from the left ventricular myocardium of eight adults and one juvenile (12 yr). The adult heart weights, recorded from the autopsy report, varied from 360 to 600 g. The ages ranged from 12 to 80 yr. Only three patients (patients 2, 6, and 9) had medical histories of hypertension and only one of these (patient 2) had clinical congestive heart failure. The remaining patients had no evidence of heart disease at autopsy.

There were specimens from five newborn premature infants expiring of pulmonary complications in the 1st wk of life, one from a full-term infant with lethal noncardiac congenital anomalies, and one from a 4 mo old with cerebral palsy. None had evident cardiac pathology. In each instance all atrial tissue was removed and the myocardium from both ventricles was pooled for myosin extraction. All autopsy specimens were frozen at the time of necropsy (<24 h post mortem) and stored at −30°C for a period of <3 mo before extraction.

Surgical specimens were obtained from seven patients with hypertrophic obstructive cardiomyopathy who underwent left ventricular septal myotomy and myectomy by Dr. A. G. Morrow (National Institutes of Health, Bethesda, MD) during this study (20). All but one of these patients had resting left ventricular outflow tract obstruction (mean gradient 100 mm Hg, range 75–120 mm Hg); two had significant left ventricular free-wall thickening by echocardiogram. Operative samples were frozen and stored in a similar fashion and kept for an interval of <18 mo before extraction.

For technical controls for peptide maps and pyrophosphate polyacrylamide gel electrophoresis, myosin was also prepared from rat hearts at different development stages. These hearts are known to contain different mixtures of heavy chain isoenzymes (see above). Cardiac ventricular samples were obtained from Sprague-Dawley rats at 18-d

TABLE I
Myosin ATPase Specific Activities

	K⁺-EDTA	Ca^{++}	Mg^{++}	Actin-activated	Comments		
					Age	Diagnosis	Heart weight
		μmol P_i/mg/min			*yr*		*g*
Adults							
1	1.053±0.055	0.262±0.030	0.004±0.001	0.109±0.006	12	Wilms' tumor	
2	1.165±0.034	0.350±0.060	0.011±0.002	0.101±0.005	60	CHF HBP	600
3	1.480±0.110	0.458±0.034	0.005±0.001	0.131±0.016	62	met. ca.	460
4	1.314±0.160	0.411±0.032	0.011±0.002	0.186±0.012	56	met. ca.	420
5	1.270±0.010	0.232±0.007	0.006±0.001	0.113±0.025	39	seminoma	380
6	—	0.224±0.006	0.013±0.001	0.143±0.002	58	HBP CVA	495
7	1.373±0.057	0.309±0.017	0.011±0.001	0.086±0.001	72	renal ca.	450
8	0.966±0.008	0.509±0.010	—	—	69	met. ca. HBP	360
9	1.028±0.013	0.301±0.020	—	—	80	CRF HBP	420
Infants							
10	0.733±0.049	0.276±0.008	—	—	Full term VATER syndrome		
11	0.624±0.046	0.506±0.020	—	—	4 mo cerebral palsy		
12	0.653±0.017	0.197±0.027	0.003±0.002	0.064±0.006	33 wk gest. RDS		
13	0.848±0.041	0.204±0.006	0.014±0.001	0.061±0.002	28 wk gest. RDS		
14	0.557±0.030	0.226±0.023	0.004±0.001	0.064±0.002	32 wk gest. RDS		
15	0.502±0.020	0.236±0.009	—	—	26 wk gest. RDS		
16	0.452±0.011	0.168±0.050	—	—	27 wk gest. RDS		
Hypertrophic cardiomyopathy							
17	1.303±0.121	0.440±0.013	0.010±0.001	0.156±0.018	26 yr LVOT 120, IVS 40/FW 2		
18	0.955±0.045	0.317±0.030	—	—	52 yr LVOT 0, IVS 35/FW 18		
19	0.835±0.050	0.350±0.040	—	—	36 yr LVOT 120, IVS 23/FW 1		
20	1.530±0.040	0.264±0.009	0.005±0.001	0.153±0.002	42 yr LVOT 110, IVS 28/FW 1		
21	1.105±0.090	0.226±0.013	0.006±0.001	0.090±0.001	51 yr LVOT 75, IVS 20/FW 10		
22	1.049±0.022	0.265±0.004	0.009±0.002	0.127±0.012	58 yr LVOT 80, IVS 20/FW 13		
23	0.972±0.045	0.333±0.020	—	—	34 yr LVOT 100, IVS 25/FW 122		
Adult rat							
	1.32±0.050	2.140±0.110	0.0014±0.001	0.256±0.006			
Weanling rat (3 wk)							
	1.11±0.100	1.760±0.030	0.0043±0.001	0.223±0.020			
Newborn rat							
	0.245±0.020	0.370±0.015	0.0013±0.001	0.133±0.010			

Values are mean±standard error.
CHF, congestive heart failure; CRF, chronic renal failure; CVA, cerebrovascular accident; FW, left ventricular free wall thickness (mm) by echocardiogram; gest., gestation; HBP, hypertension; IVS, interventricular septal thickness (mm) by echocardiogram; LVOT, left ventricular outflow tract gradient in mm Hg; met. ca., metastatic carcinoma; RDS, respiratory distress syndrome.

gestation (fetal, 60 rats), 1 d of life (newborn, 20 rats), 3 wk postnatal (weanling, 12 rats), and at 12 wk (adults, 6 rats). Assay results are listed also in Table I, for comparison. Two separate preparations were made for each group.

Preparation of myosin. All procedures were performed at 2–5°C, using deionized water unless otherwise noted. Myosin was prepared essentially by the method of Focant and Huriaux (21) as modified by Chantler and Szent-Györgyi (22) differing in the use of 4-morpholino-propane sulfonic acid (MOPS) rather than phosphate buffer. Between 1 and 5 g of muscle tissue was homogenized with a Virtis Omni-mixer (VirTis Co., Inc., Gardiner, NY) in 5 vol of the fol-

lowing buffer: 40 mM NaCl, 10 mM MOPS (pH 7.5), 1 mM $MgCl_2$, 5 mM EGTA, 1 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonylfluoride. The homogenate was sedimented at 24,000*g* for 5 min, the supernate discarded, and the precipitate washed an additional two to four times in the same buffer until the supernate became clear. The washed precipitate, essentially myofibrils, was resuspended in 40 mM NaCl, 10 mM MOPS (pH 7.5), 5 mM EGTA, 1 mM DTT, 1 mM phenylmethylsulfonylfluoride by homogenization in the Virtis Omnimixer. Next 5 M NaCl and 100 mM ATP were added to a final concentration of 0.6 M NaCl and 5 mM ATP, pH 7.5. The mixture was stirred for 20 min to extract actomyosin, and then sedimented at 24,000*g* for 15 min. The precipitate was discarded and 0.5 M $MgSO_4$ and 100 mM ATP were added to the supernate to a final concentration of 20 mM and 10 mM, repectively, pH 7.5. Saturated $(NH_4)_2SO_4$ in 10 mM EDTA was added to 40% saturation while stirring on ice for 30 min, and the resulting precipitate sedimented at 24,000*g* for 20 min. The precipitate was discarded; the supernate was brought to 55% saturation by the further addition of saturated $(NH_4)_2SO_4$. The 40–55% ammonium sulfate fraction was collected at 24,000*g* for 20 min, redissolved in 20 mM NaCl, 10 mM MOPS (pH 7.5), 5 mM EGTA, 1 mM DTT, and then dialyzed against two changes of 1 liter of the same buffer. The low salt precipitate was dissolved in 0.6 M NaCl, 10 mM MOPS (pH 7.5), 5 mM EGTA, 1 mM DTT to a final concentration of 20–25 mg/ml. The state of purity of the protein was confirmed by SDS-PAGE, and the concentration of myosin was quantitated by UV spectrophotometry using an extinction coefficient, $E^{1\%}_{280nm} = 5.60$. The ratio E_{280}/E_{290} of 1.58 was assumed in correcting for residual ATP (12). The yield of myosin was 15–20 mg/g of wet muscle tissue.

Myosin ATPase assays. The myosin ATPase activities (K^+-EDTA and Ca^{++}-activated in 0.5 M KCl) were assayed by a modification of the radioactive method of Pollard and Korn (23). Measurements were performed at 37°C in 0.5 ml of a final reaction mixture of 20 mM Tris-HCl (pH 7.0), 0.5 M KCl, 2 mM ([γ-^{32}P]ATP added to a sp act of 12.5 mCi/mmol), 1 mg/ml myosin and either 2 mM EDTA or 10 mM $CaCl_2$. The reaction was started by addition of ATP with 0.075-ml aliquots removed at 1, 2, 3, 4, and 5 min. Phosphate liberation was measured by the method of Martin and Doty (24) except that ^{32}P radioactivity in the organic phase phosphomolybdate extract was measured rather than using the colorimetric procedure. The time-course of each assay, performed in triplicate, was linear.

Actin-activated myosin MgATPase activity was determined by a modification of the method previously described (25). Human or rat cardiac myosin and rabbit skeletal muscle F-actin were mixed with 2 M KCl added to a final concentration of 0.5 M at 4°C. With gentle vortexing the mixture was diluted with cold water and a buffer of 12.5 mM MOPS, 2.5 mM $MgCl_2$, 0.25 mM $CaCl_2$ to the final reaction conditions of 30 mM KCl, 10 mM MOPS (pH 7.0, 37°C), 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.1 mg/ml myosin, 0.5 mg/ml rabbit skeletal muscle actin, and 1 mM ATP. The reaction was started by the addition of ATP, and the time-course of ^{32}P liberation was measured as above. The time-course of the reaction was generally nonlinear over 5 min, but using 30-s assay intervals over 2.5 min, linear reaction rates could be measured (Fig. 1). Actin activation was determined comparing the assay with actin to that replacing actin with an equal volume of water. Each of these assays was performed in triplicate. Statistical comparison between the three groups was performed by an analysis of variance using the General Linear Models procedure of SAS (Statistical Analysis Systems, SAS Institute, Inc., Raleigh, NC). Rabbit skeletal muscle actin was the generous gift of Dr. Evan Eisenberg (National Institutes of Health).

Proteolytic digestions. Alpha-chymotryptic digestions were performed on undenatured myosin, 1.2 mg/ml, at 37°C in 0.5M NaCl, 10 mM MOPS (pH 7.5), 5 mM EGTA, 1 mM DTT. Chymotrypsin was added at 1 mg/5 mg myosin to initiate the digestion and aliquots were sampled every 2 min from 0 to 10 min. The reaction was stopped by boiling at 100°C for 3 min, and the sample divided into aliquots for gel electrophoresis. Papain digests were performed similarly at 37°C on native myosin (0.02 U/mg) in the same buffer system. Papain was activated with DTT at 25°C for 30 min in the following reaction mixture: 0.1 M NaCl, 10 mM MOPS (pH 7.0), 2 mM EDTA, 5 mM DTT, and papain 0.72 mg/ml (28 U/mg papain). The digestion was started with the addition of activated papain. Timed aliquots were sampled at 0, 2, 5, 10, and 15 min and the reaction stopped by the addition of iodoacetic acid to a concentration of 1 mM. Papain and alpha-chymotrypsin were obtained from Worthington Chemical Corp., Freehold, NJ.

For cyanogen bromide cleavage, lyophilized myosin was dissolved in 70% formic acid and treated with solid cyanogen bromide in 200-fold excess over methionine residues for 17 h at 25°C. The cyanogen bromide peptides were diluted with water, lyophilized, and dissolved in 8 M urea for gel electrophoresis.

PAGE. One-dimensional 0.1%SDS-12.5% PAGE of myosins and myosin digests was performed using the microslab apparatus of Matsudaira and Burgess (26) using the Laemmli (27) buffer system. One-dimensional slab urea-PAGE was performed by the method of Perrie and Perry (28). Pyrophosphate-PAGE was performed by a modification of the method of Hoh (9). Gels were 5 × 110 mm, 3.88% acrylamide, 0.12% bis-acrylamide, 10% glycerol, 40 mM $Na_4P_2O_7$, pH 8.5, run at 50 V for 24–36 h at 2–5°C. The upper and lower buffer solutions were 40 mM $Na_4P_2O_7$ (pH 8.5), 8.5% glycerol, and were recirculated to maintain equal pH and temperature. Two-dimensional PAGE was performed by the method of O'Farrell (29); the first dimension was isoelectric focusing (pH 3.5–10), the second dimension SDS-PAGE in a 20% acrylamide slab gel. Gels were stained with Coomassie Brilliant Blue and when appropriate were scanned using a Quick Scan (Helena Laboratories, Beaumont, TX).

RESULTS

Myosin ATPase activities and gel electrophoresis. A summary of the ATPase determinations for adult, infant, and hypertrophic cardiomyopathic cardiac myosins is shown in Table II. Whereas cardiomyopathic myosin did not differ significantly from adult myosin in K^+-EDTA or Ca^{++}-activated ATPase activity in 0.5 M KCl or in actin-activated MgATPase activity in 0.01M KCl, the infant cardiac myosin showed significantly lower actin-activated MgATPase (Fig. 1) and K^+-EDTA ATPase activities. The Ca^{++}-activated ATPase activity was lower in infant myosin as well, but did not reach statistical significance.

Infant myosin consistently differed from adult in showing three different light chains, rather than two. In addition to the adult 20,000- and 27,000-dalton light chains, there was a third peptide of slightly greater

TABLE II
Comparison of Myosin ATPase Activities

	HCM	P_1	Normal adult	P_2	Infant	P_3
K^+-EDTA	1.11±0.07	NS	1.206±0.067	0.001	0.624±0.072	0.001
Ca^{++}	0.314±0.037	NS	0.340±0.032	NS	0.263±0.040	NS
Mg^{++}	0.007±0.002	NS	0.009±0.002	NS	0.009±0.003	NS
Actin-activated	0.132±0.021	NS	0.124±0.016	0.02	0.0637±0.017	0.02

P_1, probability of equal mean values comparing hypertrophic cardiomyopathy (HCM) and normal adult samples.
P_2, probability of equal means value comparing normal adult with infant myosins.
P_3, probability of equal mean values comparing infant and hypertrophic cardiomyopathy samples.

molecular mass (~28,000 daltons), which is consistent with the fetal light chain described by Cummins et al. (18). The purity of these preparations as well as the presence of the fetal light chain can be seen in the zero time points of the chymotryptic and papain digests shown in Figs. 2 and 3. Fetal light chain comprised ~30% of the 27,000-dalton light chain by scanning of stained SDS-polyacrylamide gels.

Proteolytic digests. One-dimensional peptide maps of alpha-chymotryptic and papain digests of myosins from seven adults (patients 1 through 7), four infants (patients 10, 12, 13, and 14) and four patients with hypertrophic cardiomyopathy (patients 17, 20, 21, 22) were performed. To identify clearly the differences in heavy chain isoenzymes, weanling and fetal rat heart myosin digests were run as controls. Those peptides between 30,000 and 35,000 daltons, particularly in the 6- and 8-min samples, are consistently different between fetal, weanling, and adult rat myosin (Fig. 4). To visualize a variety of peptides, time-courses for each digestion were run rather than comparing limit digests. Fig. 2 illustrates a typical chymotryptic digestion time-course analyzed by SDS-PAGE for adult (patient 1), infant (patient 14), and hypertrophic cardiomyopathy (patient 22) myosin. There are no consistent differences between samples other than the

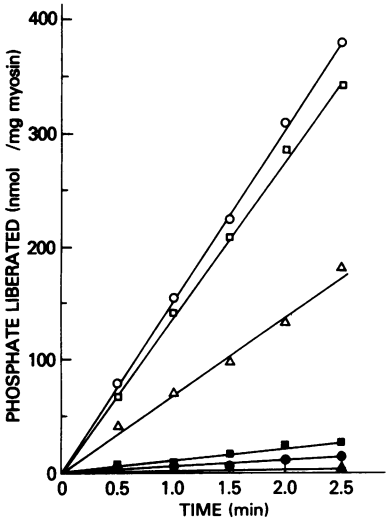

FIGURE 1 Representative assay time-courses for actin-activated MgATPase determinations. Human myosins were incubated at 37°C with ^{32}P-labeled ATP in the following reaction conditions: 30 mM KCl, 10 mM MOPS (pH 7.0), 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.1 mg/ml myosin, 1 mM ATP, either with (open symbols) or without (filled symbols) added actin 0.5 mg/ml. Aliquots were removed for determination of ^{32}P liberation at the time shown. Samples were assayed with and without actin as follows: (○, ●) adult; (□, ■) hypertrophic cardiomyopathy; and (△, ▲) infant myosins. The MgATPase activities were determined using the appropriate slopes.

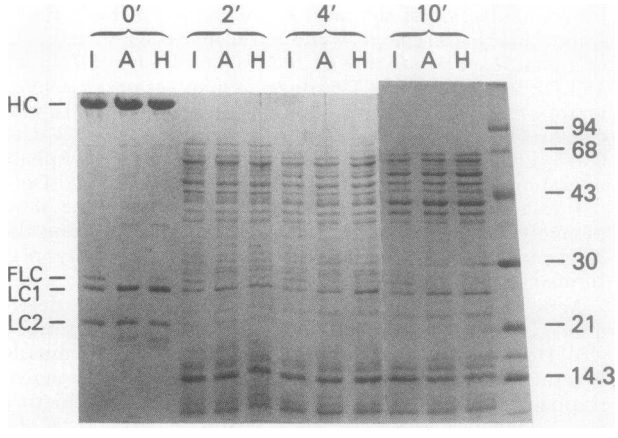

FIGURE 2 SDS-PAGE of alpha-chymotryptic digests of human cardiac myosins. Human cardiac myosins were digested with chymotrypsin, 1 mg/5 mg myosin at 37°C in the following buffer: 0.5 M NaCl, 10 mM MOPS (pH 7.0), 5 mM EGTA, 1 mM DTT. The digest time-course shown was sampled at 0, 2, 4, and 10 min. The samples shown are as follows: I, infant; A, adult; and H, hypertrophic cardiomyopathic myosins. The standards are shown on the right. The myosin heavy chain (HC) and adult light chains (LC_1 and LC_2) as well as the fetal light chain (FLC) are identified for the 0 time point. In the 0 time for samples A and H a number of extra bands can be seen particularly below LC_2. These are due to a small amount of proteolysis of the 20,000-dalton light chain in the original samples.

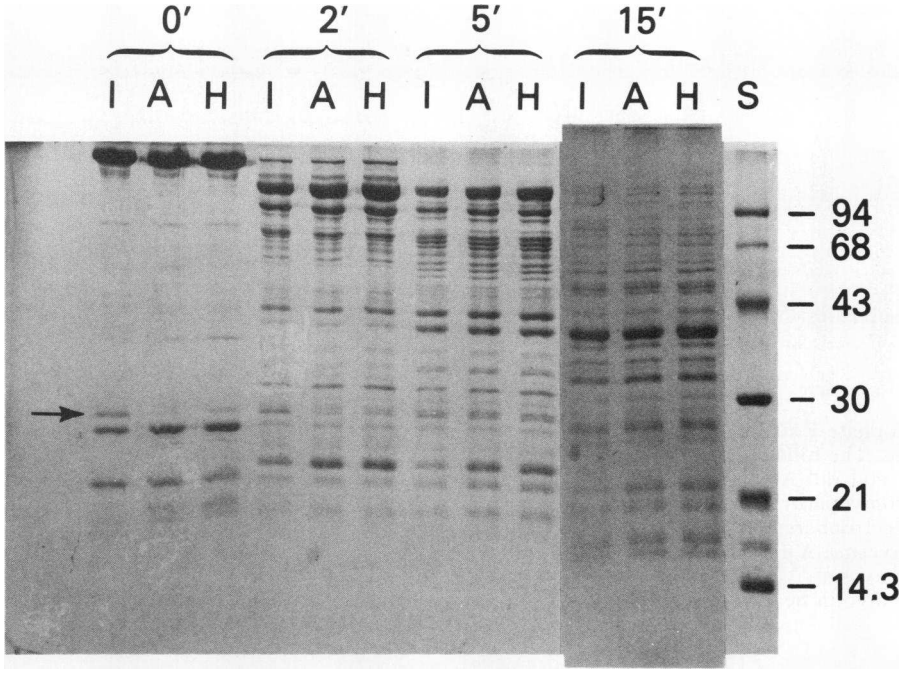

FIGURE 3 SDS-PAGE of papain digests of human cardiac myosins. Human cardiac myosins were digested with papain, (0.02 U/mg myosin) at 37°C, in the following buffer: 0.5 M NaCl, 10 mM MOPS (pH 7.0), 5 mM EGTA, 1 mM DTT. Digest samples were taken at 0, 2, 5 and 15 min. Myosin samples include: I, infant; A, adult; H, hypertrophic cardiomyopathy. The standards are shown on the right. The arrow identifies the fetal light chain.

persistent band at 28,000 daltons, presumed to be the fetal light chain and apparently resistant to proteolytic attack. Chymotryptic digests were also analyzed in the urea-polyacrylamide gel system, which separates peptides by charge. Again, no consistent differences were seen (data not shown). A typical papain digest time-course for the same patients is illustrated in Fig. 3 with no evident differences. These results suggest that there is only a difference in light chains between infant and adult myosins, with no evident heavy chain difference.

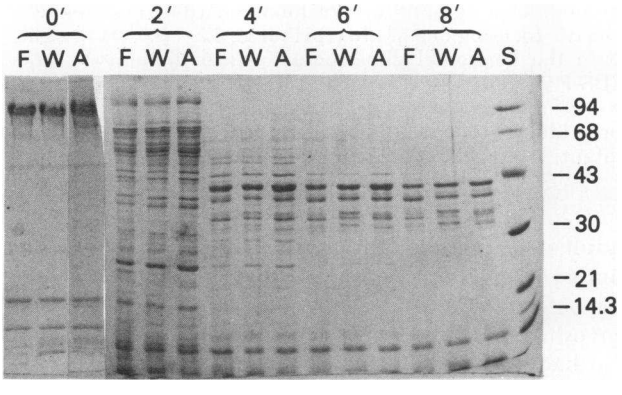

FIGURE 4 SDS-PAGE of chymotryptic digests of rat ventricular cardiac myosins. Samples run are: F, fetal rat; W, weanling rat; A, adult rat. Digest sampled at 0, 2, 4, 6, and 8 min. SDS-PAGE molecular weight standards (S), are run in the well on the extreme right.

Pyrophosphate-PAGE. None of the human cardiac myosins studied demonstrated multiple isoenzyme bands on pyrophosphate gel electrophoresis. Furthermore, coelectrophoresis demonstrated no differences between normal and cardiomyopathic myosins, or between adult and infant myosins. As controls, samples of rat ventricular myosin, known to show a mixture of heavy chain isoenzymes, were run along with the human samples. Adult rat cardiac myosin separated into three distinct bands. Representative pyrophosphate polyacrylamide gels are shown in Fig. 5.

Two-dimensional PAGE. To further analyze for possible heavy chain differences, cyanogen bromide cleavage maps of myosins from two normal adults, two patients with hypertrophic cardiomyopathy, and two infants were prepared. O'Farrell two-dimensional PAGE of these cyanogen bromide peptides (Fig. 6) showed no apparent qualitative differences. The benefit of the two-dimensional peptide mapping in the study of rat and rabbit cardiac myosin isoenzymes came from the discovery of conditions in which there was only a single isoenzyme expressed, such as hypothyroid rat for V1 and weanling rat for V3 (Introduction). The two-dimensional comparison of those

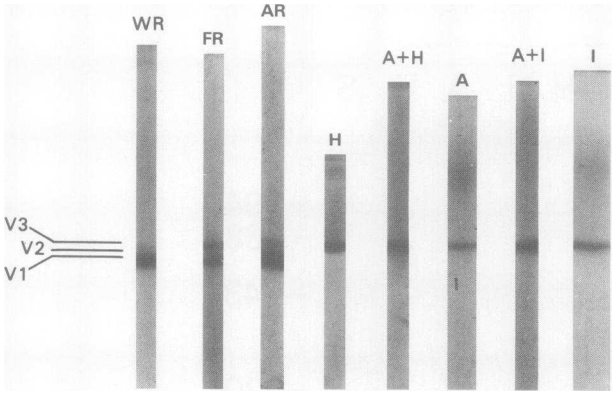

FIGURE 5 Pyrophosphate-PAGE of rat and human ventricular cardiac myosins. The following samples were run: WR, weanling rat; FR, fetal rat; AR, adult rat; H, human with hypertrophic cardiomyopathy; A, adult human; I, human infant, A + H, coelectrophoresis of adult and hypertrophic cardiomyopathic myosins; A + I, coelectrophoresis of adult and infant cardiac myosins. V1, V2, and V3 denote the rat cardiac ventricular myosin heavy chain isoenzymes.

specimens showed clear qualitative differences. Without information from pyrophosphate gel separation, it would not be likely to detect a mixture of isoenzymes in human tissue by this qualitative method. On the other hand, Flink and Morkin (4) and Hoh (11) had documented significant primary structure differences using only one-dimensional peptide maps, which lend themselves to quantitative scanning. One-dimensional cyanogen bromide peptide maps of cardiac muscle myosin from these patients also showed no differences (Fig. 7).

DISCUSSION

The perinatal period in rat, rabbit, and other species is one of very rapid alterations in cardiac myosin heavy chain isoenzyme composition (17), though the exact significance of this is unknown. Human newborn and infant tissue was examined with this in mind. The myosin actin-activated MgATPase activity and K^+-EDTA ATPase activity in 0.5 M KCl are shown to be significantly lower in human newborn cardiac myosin (Table II) than in the adult. Heavy chain peptide maps using cyanogen bromide cleavage and alpha-chymotryptic or papain digests, however, show no differences that would suggest the presence of a human fetal myosin heavy chain isoenzyme. Furthermore, the human infant and adult ventricular cardiac myosins were not separable by pyrophosphate-PAGE. Infant myosin did differ from adult by the presence of a new light chain isoenzyme of molecular mass ~28,000 daltons. This fetal light chain was initially described by Price, et al. (30) to coelectrophorese with the 28,000-dalton

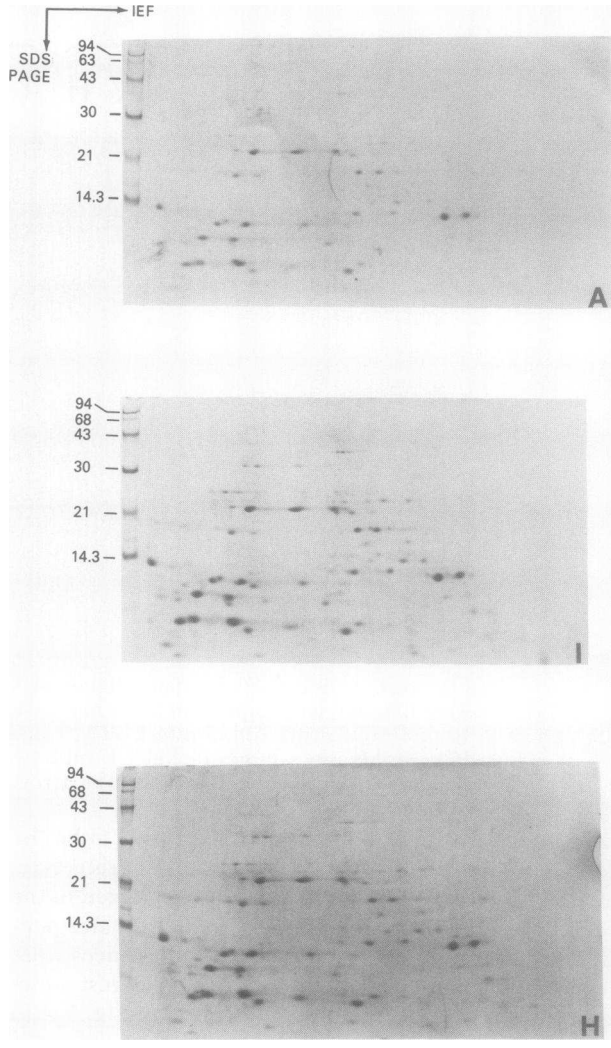

FIGURE 6 Two-dimensional gel electrophoreses of cyanogen bromide cleavage products of human cardiac myosins. Isoelectric focusing in 8 M urea (pH range 3.5—10, left to right) is in the horizontal dimension. Vertical dimension shows SDS-PAGE, with low molecular weight standards in daltons $\times 10^{-3}$ run on the left hand side. The following cyanogen bromide peptide maps of myosin are shown: A, adult; I, infant; H, hypertrophic cardiomyopathy.

adult atrial myosin light chain. A similar 26,000-dalton embryonic light chain was previously described in embryonic rat (31) and rabbit (32, 33) skeletal muscle myosins. Price et al. (30) have suggested that human cardiac fetal light chain corresponds to the embryonic fetal light chain seen in human skeletal muscle.

The pattern of the 27,000- and 28,000-dalton myosin light chains shown at 0 time in Fig. 2 was also observed when SDS-polyacrylamide gels were run throughout the purification procedure (data not shown). Thus the

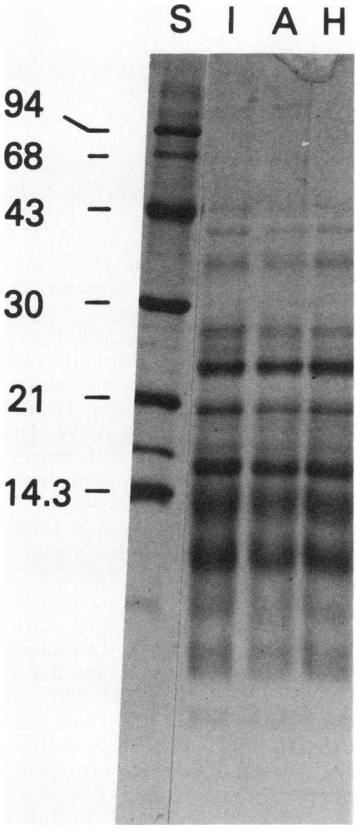

FIGURE 7 One-dimensional SDS-PAGE of cyanogen bromide peptides of human ventricular cardiac myosins. Samples are: I, infant; A, adult; H, hypertrophic cardiomyopathy. SDS-PAGE low molecular weight standards, in daltons $\times 10^{-3}$ are shown on the left.

same distribution of myosin light chains, for each of the three myosins, was seen following preparation of the myofibrils and extraction of the actomyosin. This indicates, in agreement with others (18, 30), that the fetal light chain is not a proteolytic product of the myosin heavy chain. Our study shows that the fetal light chain appears to be relatively resistant to proteolysis, since it appears to persist following 10 min of digestion with chymotrypsin (Fig. 2) and at least 2 min of digestion with papain (Fig. 3).

That human infant myosin heavy chain would not differ from adult might have been predicted. Lompre et al. (17) have shown a marked species variation in myosin isoenzyme expression during development (see above). By pyrophosphate electrophoresis they demonstrated that the adult rat and mouse express mostly the V1 form of myosin, which has the greater electrophoretic mobility and Ca^{++}-activated ATPase activity. Adult rabbits and pigs, on the other hand, express the V3 myosin isoenzyme only. If, as Lompre et al. speculate, all mammalian species show V3 for the fetal myosin, then larger mammals with V3 as the adult myosin would not show a dramatic shift in the myosin heavy chain composition postnatally. Lompre et al. had observed, in addition, that human adult cardiac myosin migrates with an electrophoretic mobility similar to rat and rabbit V3 in the pyrophosphate-PAGE system (17).

If our deduction is correct and there is no difference in the heavy chain of myosin in human adults and infants, how then could the replacement of only 30% of the adult light chain by a fetal light chain result in a 50% decrease in actin-activated MgATPase activity? This is possible because myosin is a dimer containing two 27,000-dalton light chains. If the fetal myosin isoenzyme were a heterodimer, containing one 27,000-dalton adult light chain and one 28,000-dalton fetal light chain, then replacement of 30% of the 27,000-dalton light chains with the 28,000-dalton fetal light chain would amount to replacing 60% of the adult myosin isoenzyme molecules with fetal isoenzyme molecules (heterodimers). The finding of a 50% decrease in enzymatic activity would furthermore imply that there is an interaction between the two heads of infant myosin, at least with respect to the actin-activated MgATPase activity, similar to the recent findings with scallop myosin (34).

It is of note that the myosin ATPase activities, peptide maps, and pyrophosphate gel electrophoreses were not different comparing normal human cardiac myosins to those myosins isolated from patients with hypertrophic cardiomyopathy. Maron et al. (19) reported no differences comparing these two groups with regard to K^{+}-EDTA and Ca^{++}-activated ATPase activities (in 0.5 M KCl) and SDS-PAGE. The present study, though confirming our earlier negative results, reports a comparison of hypertrophic cardiomyopathy with normal myosin incorporating techniques that have been useful in detecting myosin heavy chain structural differences in animal models of cardiac hypertrophy. The fact that all but one of the patients with hypertrophic cardiomyopathy had resting left ventricular outflow tract obstruction and pressure overload might have suggested that a different myosin species would be present in these hearts, as an adaptive mechanism similar to the rat pressure overload model (16). It may be equally significant that there were no differences in actin-activated MgATPase activity or peptide maps within the adult group of myosins despite such a wide range in heart weights and ages. One patient in fact had congestive heart failure and cardiomegaly (heart weight 600 g) and yet was not distinguishable from the remaining adults by these tests. Swynghedauw et al. (35) and Leclerq et al. (36), on the other hand, have observed a negative correlation between myofibrillar ATPase activity and heart weight

in a series of 70 human autopsy hearts. The ATPase activity decreased markedly with heart weights >500 g.

There is not yet a biochemical explanation for the decreased myosin ATPase activity observed in human cardiac hypertrophy and failure. From the observation of Lompre et al. (17) that the adult man has predominantly a V3 myosin (i.e., one which is electrophoretically similar to the V3 myosin isoenzyme of rat and rabbit) it is unlikely that the same form of isoenzyme shift seen in rat with chronic cardiac overload would appear in man, because adult human myosin, differing from the rat, is already in the V3 form. For this reason cardiac hypertrophy in the rat may not be a suitable model for the study of myocardial hypertrophy in man. These interspecies variations underscore the difficulties in extrapolating from animal investigations of cardiac structure and function to the clinical setting.

In summary, human infant cardiac myosin contains a mixture of adult and fetal myosin light chain isoenzymes, has significantly lower actin-activated MgATPase and K^+-EDTA ATPase activities than the adult, and has no evident differences in myosin heavy chain primary structure identifiable by peptide digest maps or pyrophosphate-PAGE. These findings suggest that the fetal 28,000-dalton light chain per se is responsible for the difference in enzymatic activities, and further that this peptide may be a useful probe in studies to further elucidate the role of the 27,000-dalton light chain in cardiac myosin function.

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