Myosin Transitions in the Bovine and Human Heart

A Developmental and Anatomical Study of Heavy and Light Chain Subunits in the Atrium and Ventricle

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SUMMARY Transitions in cardiac myosin isotypes occur in response to a variety of stimuli. These are well documented in small, but less so in larger, mammals where data on quantitative isotype composition of normal atria and ventricles are scarce. In this study, heavy and light chain isotypes were examined in fetal, neonatal, and adult bovine and human hearts. Defined anatomical areas of chambers were studied, and detailed mapping of isotypes was conducted on bovine atria. Heavy chain isotype quantification was carried out electrophoretically either after peptide mapping or by a new technique for separating whole cardiac α- and β-heavy chains capable of detecting 5% of either isotope. Atrial and ventricular light chains were resolved electrophoretically, and all isotypes were quantified densitometrically. β-Heavy chain was almost exclusively expressed (>95%) in the ventricles at all ages except in the bovine neonate. Ventricular light chains predominated in the adult ventricle, but atrial light chain 1 was present at mid-gestation. This was replaced by ventricular light chain 1 in the neonate with no transitions in light chain 2 isotypes. Transitions were more marked in the atria. Only α-heavy chains were detected at mid-gestation, but β-heavy chain increased toward birth. After a decline in the neonatal period, β-heavy chain levels usually increased in the adult. Atrial light chains were the main isotypes in the atria, but ventricular light chain 2 was the major adult bovine atrial isotype. Transitions in isotypes were significant at birth as in smaller mammals. No close relationship existed between heavy and light chain transitions. Significant anatomical variation in bovine atrial isotype expression was present, and may reflect functional demand in each area. (Circ Res 58: 846–858, 1986)

THE existence of isotypes of cardiac myosin which differ in both heavy and light chain subunit composition is now clearly established (Flink et al., 1978; Price et al., 1980; Lompre et al., 1981; Chizzonite et al., 1982). Studies in smaller mammals have clearly demonstrated a relationship between transitions in myosin heavy chain isotypes and a variety of stimuli, including hormonal (Hoh et al., 1978; Chizzonite and Zak, 1984), developmental (Lompre et al., 1981; Schwartz et al., 1982), and hemodynamic (Lompre et al., 1979; Mercadier et al., 1981; Rupp, 1981; Litten et al., 1982). Although the significance and underlying molecular interactions involved in these transitions are still unclear, studies on economy of force generation (Alpert and Mulieri, 1981, 1982), speed of myocardial contraction (Schwartz et al., 1981), and oxygen consumption (Kissling et al., 1982) suggest they constitute an adaptive response of the myocardium to changing functional requirements.

In smaller mammals, such as the mouse, rat, and rabbit, these changes are largely restricted to the ventricles (Bugaisky et al., 1983), although it is clear that both atrial and ventricular chambers may contain different isotypes of heavy and light chain subunits (Hoh et al., 1978; Dalla Libera and Sartore, 1981; Banerjee, 1983). Indeed, there is now compelling evidence for a minimum of at least two major heavy chain (α and β) (Sartore et al., 1981; Chizzonite et al., 1982; Gorza et al., 1982) and five light chain (two atrial and three ventricular) (Price et al., 1980; Sridhara et al., 1982; Klotz et al., 1982) isotypes in the mammalian heart which differ in primary sequence.

In view of the possible physiological significance of these transitions, several studies on myosin isotype expression have been conducted on the larger, mainly bovine and human heart (Whalen et al., 1982; Cummins, 1983; Bouvagnet et al., 1984). Although there is no doubt that comparable heavy and light chain subunit isotypes are present (Price et al., 1980; Lompre et al., 1981; Clark et al., 1982), it appears that isotype transitions in response to different stimuli are more limited. A lack of significant transitions in heavy chain isotypes in the hemodynamically overloaded left ventricle has been demonstrated in the adult human (Mercadier et al., 1983) and porcine (Wisenbaugh et al., 1983) heart, although changes in light chain (Cummins, 1982) and heavy chain (Gorza et al., 1984; Mercadier et al., 1985) expression have been observed in the overloaded human atrium. Transitions between atrial and ventricular light chain subunits are known to occur during development in the human (Price et
Most studies to date on myosin heavy chain expression in larger mammals have employed immunological techniques, using antibodies that react mainly with either α- or β-heavy chains. Immunofluorescence studies have been conducted by Sartore et al. (1981), Gorza et al. (1982, 1984), and Bouvagnet et al. (1984). Whereas this approach can be very sensitive in identifying specific isoforms, it is difficult to quantify, and may give only a general idea of the level of a particular isoform present in a given tissue. Radioimmunoassay (Clark et al., 1982; Chizzonite et al., 1983) and enzyme-linked immunosorbent assay (Mercadier et al., 1983) to distinguish whole myosin (V1, V2, and V3) isoenzymes (Hoh et al., 1978) have been used to provide a more quantitative approach to this problem, and there are limited data on larger mammals.

Electrophoretic resolution of closely related polypeptides is now a widely used method for examining different protein isoforms. However, major problems arise in accurately quantifying the relative amounts of individual myosin heavy chain isoforms in a given sample. This arises mainly as a result of the large molecular size, insolubility, and aggregability of myosin heavy chains which have homologous sequences. Electrophoretic resolution of whole native myosin isoenzymes containing both heavy and light chain subunits is possible (Hoh et al., 1976; D’Albis et al., 1979), but these may be resolved on the basis of differences in both heavy or light chain composition in ventricular (Hoh et al., 1979) and skeletal (Hoh, 1978) muscle, respectively, and uncertainty exists as to the precise composition of the different atrial isoenzymes resolved by this technique (Hoh et al., 1978; Chizzonite et al., 1982). The method does not readily lend itself to analysis of samples of unknown isotype composition. Consequently, it is difficult to examine combined transitions of heavy and light chain subunits. The initial aim of the present study therefore was to develop new techniques which would allow resolution, identification, and accurate quantification of myosin heavy chain isoforms in the heart. This would then allow the following questions to be answered: (1) To what extent do the human and bovine atria and ventricles express different heavy chain isoforms, both in the adult and during fetal and neonatal development, which could form the basis for transitional switches? (2) Does any quantitative or qualitative relationship exist between the expression of different heavy and light chain isoforms which could throw light on their developmental and functional regulation? and (3) How variable and heterogenous is heavy and light chain isotype composition in different anatomical regions of the myocardium and at different stages of development, and could this have any functional significance?

Some aspects of this work have been presented in abstract form elsewhere (Lambert et al., 1983; Cummins et al., 1984).

Methods

Tissue Sources

Bovine fetal and adult hearts were obtained from a local slaughterhouse. Fetal hearts were excised within 20 minutes of death and were deep frozen at −35°C until use. Adult hearts were collected immediately after death, chilled on ice, and dissected into different chambers and regions within 2 hours of death. Tissue was then flash frozen in liquid nitrogen and stored at −35°C.

Ages of bovine fetal hearts were determined either from measurements of fetal crown-rump length or from the heart weight. In the absence of information on conception date, the measurement of crown-rump length has been widely used to determine fetal age. Fifty-six hearts were obtained from fetuses with known crown-rump length, and fetal age was determined using the data of Evans and Sack (1973) These hearts, which covered gestation periods ranging from 10 weeks until term (39–43 weeks), were weighed and the data were used to construct a second graph of heart weight against fetal age (Lambert, 1984). This second graph was used to determine the fetal age of hearts for which crown-rump measurements were not available.

In the present investigation, studies were focused on the 24-, 32-, and 40-week gestation fetus, the 1-day and 3-month neonate, and 18- and 36-month adult. At least three hearts were studied, in detail, at each age point.

Human fetal hearts, ranging from 16 to 22 and 28 to 34 weeks of gestation, were obtained after therapeutic abortions and frozen at −35°C between 4 and 16 hours after death. Twenty-five hearts were studied.

Human neonatal hearts [2–6 months (n = 4) and 7 months to 2 years (n = 4)] were obtained within 12 hours after death. Subjects died as a result of "sudden infant death syndrome" or accidental choking, and there was no evidence of abnormal cardiac pathology.

Human young adult and adult hearts [2.5–6 years (n = 2), 6.5–12 years (n = 2), and 21–81 years (n = 11)] were obtained between 2 and 24 hours postmortem and were frozen at −35°C until use. Subjects died due to brain hemorrhage, renal failure, head injury, and other forms of accident. No evidence of cardiovascular disease was apparent at postmortem. All human tissue was obtained with the informed consent of the relatives and coroner, and in accordance with the local ethical research procedures.

Rat fast and slow skeletal muscles were obtained from fresh psosas and soleus muscles, respectively.

Rabbit thyrotoxic ventricle was obtained from adult animals given repeated injections of 3,5,3′,5′-tetraiodothyronine (Everett et al., 1983b). Neonatal hearts were excised from rabbits 2 weeks after birth.

Tissue Anatomy

All bovine hearts were dissected into defined anatomical regions. Ventricular chambers were divided into right and left free walls and interventricular septum. Atrial chambers were dissected into regions identified in...
Figure 1. Right atria were sectioned into atrial appendage (RAA), pectinate muscles (PC), crista terminalis (CT), and posterior wall (PW). Left atria were divided into atrial appendage (LAA) and free wall (LAFW), with the interatrial septum (IAS) treated separately. These seven atrial areas were dissected so as to account for most of the atrial musculature, and were used individually for myosin preparations. Human hearts were also sampled, where possible, at the above atrial and ventricular regions, although many hearts could not be sampled in all seven atrial and all three ventricular regions due to availability of tissue. No significant individual variation in results was obtained between different bovine or different human subjects on subsequent examination of myosin preparations (see Results).

Myosin Preparation

Myosin was prepared as described previously (Price et al., 1980). In some cases (particularly atrial myosin), actin contamination remained in the final preparation despite repeated ammonium sulfate fractionations. In these cases, actin was completely removed by gel filtration as described by Whalen et al. (1978), except that Sepharose 4B (Pharmacia Fine Chemicals) was used instead of Sepharose 2B. Whole myosin was stored either in 0.5 M NaCl, 50% (vol/vol) glycerol, or in 0.5 M NaCl, 0.5% (wt/vol) sodium dodecyl sulfate (SDS), both at −25°C, prior to electrophoretic examination. Myosin heavy and light chain subunits were dissociated as described previously (Weeds and Lowey, 1971) with the modifications that 8 M and not 4 M urea, and 15 mM 2-mercaptoethanol and not 5 mM dithiothreitol, were used. Myosin heavy and light chains were stored as for whole myosin preparations. Crude myosin extracts were prepared as described previously (Price et al., 1980).

Electrophoresis

Examination of myosin light chain composition was carried out by the SDS discontinuous buffer system of Laemmli (1970). Resolving gels contained 12.5% (wt/vol) acrylamide, 0.33% (wt/vol) N,N'-methylenebisacrylamide (bisacrylamide), and stacking gels contained 3% (wt/vol) acrylamide, 0.08% (wt/vol) bisacrylamide.

Partial proteolytic digestion of myosin heavy chains in the presence of SDS was carried out essentially as described previously (Cleveland et al., 1977; Whalen et al., 1979). Whole myosin or isolated heavy chains were dissolved in 0.5 M NaCl, 0.5% (wt/vol) SDS, 0.02 M sodium phosphate, 0.1 mM MgCl₂, pH 6.5, at 0.4 mg/ml, and were heated at 100°C for 2 minutes before digestion. Digestion was carried out using either α-chymotrypsin at protease:substrate molar ratios of 4.69:1 (bovine) and 12.5:1 (human), or papain at a protease:substrate molar ratio of 0.02:1 (bovine and human) for 30 minutes at 37°C. Digested samples were examined as described above (Laemmli, 1970), using a 15% (wt/vol) acrylamide, 0.087% (wt/vol) bisacrylamide resolving gel. Proteases were obtained from Sigma Chemical Company.

Electrophoretic resolution of whole cardiac myosin heavy chain isotypes was carried out by a technique based on the SDS-discontinuous buffer system (Laemmli, 1970), with several important modifications. The resolving gel contained 3.975% (wt/vol) acrylamide and 0.025% (wt/vol) bisacrylamide (T = 4%, C = 0.625%, where T = total acrylamide and C = bisacrylamide concentration as a percentage of total). The height of the stacking gel was reduced to 1 cm. Gels were cast to a thickness of 0.75 mm in glass plates previously silanized to prevent gel adherence. Sample wells were filled with stacking gel buffer at the same concentration as the stacking gel prior to sample loading, and a maximum of 2–4 μg of a 1 mg/ml protein sample was loaded. Electrophoresis was carried out at a constant 220 V for 3–3.5 hours in a LKB 2001 Vertical Electrophoresis Unit (LKB Producter) with cooling between 13 and 17°C and continuous stirring of bottom tank buffer. Gels were stained overnight in 0.125% (wt/vol) Coomassie brilliant blue, 50% (vol/vol) ethanol, 5% (vol/vol) acetic acid, and were destained in 40% (vol/vol) ethanol, 6% (vol/vol) acetic acid. After destaining, gels were dried down immediately onto glass plates to minimize handling difficulties. At no stage could gels be handled out of solution, prior to drying down, due to their poor mechanical strength.

Densitometric analysis was carried out at 600 nm with an LKB Ultrosan Laser Densitometer and LKB Gelscan program coupled to an Apple IIe microcomputer.
Protein Concentrations

Protein concentrations were determined either spectrophotometrically using an extinction coefficient (E1%/l cm) at 280 nm of 53 for myosin, or by Folin-Lowry assay.

Results

Myosin Heavy Chain Isotype Quantification

Two methods were used to quantify the relative amounts of different cardiac myosin heavy chain isotypes in preparations from defined anatomical regions and at specified developmental ages. These were based on comparison of peptide maps produced by partial proteolysis of heavy chains and electrophoretic separation of whole heavy chains.

Electrophoresis of pure heavy chains from the adult (36-month) bovine right or left ventricle or interventricular septum gave identical but characteristic peptide maps after digestion with chymotrypsin (Fig. 2) or papain (results not shown). Previous studies employing electrophoretic (Lompre et al., 1981; Whalen et al., 1982; Cummins, 1984) and immunological (Sartore et al., 1981; Clark et al., 1982) techniques have clearly indicated that adult bovine ventricular myosin consists almost exclusively of the V3 isoenzyme constituted of β-heavy chain dimers. The peptide patterns produced by these proteases were therefore considered to be characteristic of cardiac β-heavy chain. Digestion at identical protease substrate:molar ratios was then carried out with bovine atrial myosin heavy chain preparations from different anatomical regions and at different developmental ages. Although the peptide maps produced were in general very similar, a number of peptides were either decreased or increased in intensity, depending on the particular myosin preparation. However, peptide maps (with a given protease) of preparations from the same anatomical areas and ages were always highly reproducible, with no relative loss or gain of even minor peptides. It has been established by immunoochemical techniques (Sartore et al., 1981; Gorza et al., 1982) that the bovine atria contain considerable levels of α-heavy chain (the dimer of which constitutes the V1 isoenzyme); therefore, it was reasoned that the differences in peptide maps between ventricular and atrial preparations were due to the presence of variable levels of α- and β-heavy chains with the possibility of additional uncharacterized heavy chains.

Peptide maps of atrial myosins at specific ages revealed peptides of varying relative intensity, either present or completely absent in certain preparations. These changes occurred in a coordinated manner for all these particular peptides. Preparations from bovine atrial regions at 24 weeks of gestation were marked with respect to presence or absence of these peptides (Fig. 2). Those unique to ventricular or certain atrial preparations were used as characteristic markers of β- and α-heavy chains, respectively. We saw no evidence of other unique peptide sets, which suggested that there were varying proportions of only two different heavy chains. Consequently, seven β-heavy chain ("ventricular") and eight α-heavy chain ("atrial") chymotryptic peptides were selected as unique markers of each isotype (Fig. 2). Peptide maps were quantified densitometrically. The amounts of either α- or β-heavy chain were determined as the amount of each heavy chains' total unique peptides expressed as a percentage of the total number of unique α-plus β-peptides. Chymotryptic digests gave a more even spread of molecular size peptides (molecular weight range, 50,000–175,000) compared with other proteases (e.g., papain). Because high densitometric resolution is required, chymotrypsin was preferred. Both whole and crude myosin could be used as light chain subunits, actin or their digestion products having lower molecular weights did not interfere with analysis.

Comparable peptide mapping analysis was conducted on human myosin heavy chain preparations from the adult (37-year) ventricle and fetal (18–20 weeks gestation) atrium. Chymotryptic and papain digests of these preparations also revealed sets of unique peptides (results not shown) considered characteristic of α- and β-heavy chains, although the peptide maps and individual unique peptides selected were different.
To compare this technique with an independent method of quantifying heavy chain isotypes in myosin mixtures, attempts were made to resolve whole cardiac α- and β-heavy chains by SDS gel electrophoresis (Perrie et al., 1983; Carraro and Catani, 1983) and gel isoelectric focusing (John, 1980), techniques that have been used to examine skeletal and cardiac heavy chain components. None of these methods proved successful when carried out on a mixture of bovine adult ventricular and 24-week fetal atrial myosin heavy chains, or on equivalent human ventricular and atrial mixtures.

Satisfactory resolution of components was obtained with a SDS, discontinuous electrophoretic system, as outlined in Methods. Under these conditions, the above bovine mixture gave two closely spaced bands, the faster migrating being derived from the ventricular preparations, and the slower from the atrial preparations [Fig. 3 (1–3)]. Identical results also were obtained when human adult ventricular and fetal atrial myosin heavy chains were examined under these conditions [Fig. 3 (5–7)]. The technique was also capable of resolving heavy chain isotypes from skeletal muscles. Both rat fast (psosas) and slow (soleus) skeletal myosin heavy chains (Fig. 3, 8–10) and rabbit fast (1. dorsi) and slow (soleus) heavy chains (results not shown) were resolved to similar extents. In all cases, the cardiac ‘atrial’ and fast skeletal heavy chains co-migrated as did the cardiac ‘ventricular’ and slow skeletal heavy chains.

Confirmation that this method was resolving whole cardiac α- and β-heavy chains was obtained by comparing results from electrophoresis of myosin under nondissociating conditions (Hoh et al., 1976, 1978) with the denaturing conditions employed here. Myosin from a 2-week neonatal rabbit ventricle, which is known to contain significant levels of V1 (α-heavy chain dimer) and V3 (β-heavy chain dimer) (Everett et al., 1983b), was electrophoresed under nondissociating conditions in the presence of pyrophosphate buffer [Fig. 3b (2)]. All three whole myosin isoenzymes, including the V2 form (α-heavy chain, β-heavy chain heterodimer), were observed. The same preparation gave two components in the presence of SDS [Fig. 3a (12)]. When either normal adult or thyrotoxic adult rabbit ventricular myosin was electrophoresed under nondissociating conditions, pure V3 or V1 isoenzymes, respectively, were seen [Fig. 3b (1,3)]. This is consistent with previous observations (Everett et al., 1983a). The same preparations, when examined in the presence of SDS, gave the expected faster-migrating band for the β-heavy chain (from the V3 isoenzyme) and the slower-migrating band for the α-heavy chain (from the V1 isoenzyme) [Fig. 3a (11, 12)], confirming the identification of each isotype. Using this new method, we observed only two different electrophoretic whole heavy chain components in the different cardiac preparations examined in this study.

Although the technique required considerable care in handling of gels and careful monitoring of protein loadings, the subsequent densitometric analysis was much simplified, compared with the peptide mapping method. Validation of the two

**Figure 3.** Electrophoresis of whole myosin heavy chains and native myosin. Part a polyacrylamide gel electrophoresis of whole myosin heavy chains in the presence of SDS 1, bovine atrial (24-week gestation fetal), 2, bovine atrial and ventricular, 3, bovine ventricular (36-month adult), 4, bovine ventricular (3-month neonatal), 5, human atrial (18–20 week gestation fetal), 6, human atrial and ventricular, 7, human ventricular (37-year adult), 8, rat fast skeletal (psosas), 9, rat fast and slow skeletal, 10, rat slow skeletal (soleus), 11, rabbit adult ventricle, 12, rabbit neonatal ventricle, and 13, rabbit thyrotoxic adult ventricle. Part b polyacrylamide gel electrophoresis of whole, native myosin in the presence of pyrophosphate 1, rabbit adult ventricle, 2, rabbit neonatal ventricle, and 3, rabbit thyrotoxic adult ventricle.
methods for quantifying heavy chain proportions was carried out by mixing together variable, but known, amounts of pure α- and β-heavy chains from bovine fetal (24-week) atrial and adult ventricle (Table 1). Overall, there was good agreement between the two methods over a wide range of α:β-heavy chain ratios. In general, the whole heavy chain method gave the closest agreement with expected values, resulting mainly from difficulties in densitometrically resolving closely spaced peptides in mixtures with very low amounts of one heavy chain. The peptide mapping was somewhat less accurate at low proportions of β-heavy chain, with a standard deviation at 10% of 2.8, compared with 2.2 for whole heavy chain electrophoresis. For most of the human studies reported here, the latter was the preferred and more rapid method. The lowest level of isotype that could be consistently detected by whole heavy chain electrophoresis was 5% compared with 7% for peptide mapping. Both methods were used on all bovine preparations.

**Myosin Light Chain Isotype Quantification**

Bovine and human atrial (ALC-1, ALC-2) and ventricular (VLC-1, VLC-2) light chains 1 and 2 were resolved by the method of Laemmli (1970), as has been reported previously for the light chains in these species (Price et al., 1980; Whalen et al., 1982; Cummins, 1984). Under these conditions, the migration order of the bovine [Fig. 4 (1–3)] and human [Fig. 4 (6–8)] ALC-1 and VLC-1 isotypes is reversed. Densitometric analysis of relative amounts of cardiac light chain isotypes was carried out assuming similar relative molar dye uptake for the two classes of light chain (Fenner et al., 1975). No quantitative differences were observed when the analyses were conducted on isolated whole light chains, pure myosin, or crude myosin preparations. Densitometric analysis of light chain isotypes was conducted by the method described by Price et al. (1980). The amounts of either atrial or ventricular total light chains 1 and 2 were expressed as a percentage of the total atrial plus ventricular light chains.

**Myosin Transitions in the Ventricle**

In the bovine ventricle at all fetal periods, and in the adult, identical peptide maps were obtained with both chymotrypsin and papain, and only a single β-heavy chain was detectable (Fig. 5a). No evidence of heterogeneity was observed in the right and left free walls or interventricular septum. However, in the 3-month neonatal ventricle, both peptide mapping and whole heavy chain electrophoresis indicated small quantities of α-heavy chain [Fig. 3a (4)], amounting to 14% of the total and with no significant regional differences. No α-heavy chain was detected in the 1-day neonatal ventricle.

Light chain composition was also variable, but this was confined to the fetal period. VLC-1 was partially replaced by ALC-1 at mid-gestation (Fig. 5a), and the highest levels of ALC-1 (16% of total light chains) were present in the free right and left walls. Only trace amounts of ALC-1 were detected in the interventricular septum at mid-gestation. This was the only evidence of regional myosin isotype heterogeneity in the bovine ventricle. ALC-1 levels declined in the latter half of gestation to disappear near term. Low levels reappeared immediately after birth, but were absent thereafter. No changes were seen in LC-2 expression; only VLC-2 was present at all stages. The total LC-1:LC-2 molar ratio was constant, and not significantly different from unity in all preparations.

In the human ventricle, no change was seen at any fetal, neonatal, or adult stage by peptide mapping or whole heavy chain electrophoresis in any of the human specimens studied (Fig. 5b). Only a single β-heavy chain was detectable in all preparations. Light chain transitions displayed a trend similar to that seen in the bovine ventricle, once again affecting only the LC-1 class (Fig. 5b). However, the magnitude of the changes was greater. ALC-1 accounted for almost half the total LC-1 at mid-gestation, declining throughout gestation, but not disappearing until the 6.5–12.0 year age range. Small amounts reappeared in the adult, but no significant regional differences were observed at any age. No transitions in LC-2 were observed; only VLC-2 was detected throughout, and total LC-1:LC-2 molar ratios remained at unity in all preparations.

**Myosin Transitions in the Atria**

Transitions in both heavy and light chain isotypes were particularly marked in the bovine atria, both regionally and developmentally. Regional differences were apparent even when myosin prepara-

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**Table 1**

Comparison of Myosin Heavy Chain Isotype Quantification Methods

<table>
<thead>
<tr>
<th>% β-Myosin heavy chain in α-β-heavy chain mixture</th>
<th>% β-Myosin heavy chain by peptide mapping</th>
<th>% β-Myosin heavy chain by whole heavy chain electrophoresis</th>
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<tr>
<td>10</td>
<td>7.2.8 (11)</td>
<td>11.2.2 (15)</td>
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<tr>
<td>20</td>
<td>16.2.6 (14)</td>
<td>20.1.5 (14)</td>
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<tr>
<td>40</td>
<td>39.2.2 (12)</td>
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<td>50</td>
<td>54.4.3 (15)</td>
<td>56.2.9 (16)</td>
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<td>63.3.8 (13)</td>
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<tr>
<td>90</td>
<td>95.3.4 (14)</td>
<td>92.1.5 (14)</td>
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</table>

α-myosin heavy chains from bovine 24-week fetal atrial and β-myosin heavy chains from 36-month adult ventricles were mixed in varying proportions, and the relative amounts of each isotype were determined by densitometric gel scanning of selected peptides or whole heavy chains as outlined in Methods and in Results. Amounts of β-heavy chain are expressed as a percentage of total α-plus β-heavy chain. Determinations were carried out on three different preparations with the number of estimations (n) indicated for each method and proportion. Results are expressed as mean ± SD.
FIGURE 4. Electrophoresis of myosin light chains. Electrophoresis of crude myosin extracts was carried out in the presence of SDS (Laemmli, 1970). 1, bovine ventricle (18-month adult); 2, bovine atrium (32-week fetal); 3, bovine atrium and ventricle; 4, bovine atrium (24-week gestation fetal crista terminalis); 5, bovine atrium (36-month adult crista terminalis); 6, human ventricle (46-year adult); 7, human atrium (64-year adult); 8, human ventricle and atrium.

ions from whole right and whole left atria were compared (Fig. 5c). At 24 weeks of gestation, only α-heavy chain was detectable in whole atrial chambers, but β-heavy chain appeared during gestation, and levels increased rapidly to peak around birth. Thereafter, β-heavy chain levels declined but increased subsequently in the later adult period, with the greatest increases apparent in the right atria. At 36 months, almost half the total right atrial heavy chain composition was of the β-heavy chain ("ventricular") type.

Similar trends were observed in atrial light chain expression, but in contrast to the ventricles, only the LC-2 class was involved in transitions (Fig. 5c). ALC-2 constituted almost all the LC-2 expressed in total right and left atria at mid-gestation, but VLC-2 levels increased rapidly toward term, reaching a peak just before birth. Thereafter, as with the β-heavy chain, VLC-2 levels declined in the immediate neonatal period, but later increased, again reaching a maximum in the total right atria, where VLC-2 almost completely replaced ALC-2.

The differences in both heavy and light chain transitions between total right and left bovine atria were reflected by even greater regional variations when defined anatomical regions in each chamber and in the interatrial septum were studied (Fig. 6). At mid-gestation, only α-heavy chain was present in all regions (Fig. 6, a and c). After increasing during gestation, β-heavy chain levels peaked before or after birth, depending on the region, with levels in the crista terminalis in the right, and atrial appendage in the left, atria being highest. Between birth and 18 months, β-heavy chain levels declined (or, in the case of the interatrial septum, remained constant), with the exception of the right posterior wall where levels increased from an initially low level. In the 36-month adult, β-heavy chain expression was also extremely variable, with the highest levels (ranging from 45% to 75% of total) appearing in the right posterior wall, crista terminalis, interatrial septum, and left atrial free wall.

Regional atrial light chain expression was not as variable in magnitude as for the heavy chains, although differences in transitions were apparent in the fetus and neonate (Fig. 6, b and c). Although, as expected from the examination of whole atrial chamber preparations, ALC-2 was the only LC-2 present in most regions at mid-gestation, significant levels (up to 23%) of VLC-2 were already being expressed in the interatrial septum, left free wall, and right posterior wall at this stage. Moreover, in the case of the right posterior wall and pectinate muscles, there was no obvious peaking of VLC-2 levels at birth. In all areas, with the exception of the left free wall, VLC-2 expression in the 3-year adult was 80–100% of the total LC-2. This complete transition from ALC-2 to VLC-2 synthesis can be seen clearly when samples from the 24-week fetal and 36-month adult crista terminalis are compared (Figs. 4 and 6b). Once again, no transitions in LC-1 types were observed in any atrial region, and total LC-1:LC-2 molar ratios remained at or near unity throughout.

Parallel comparison of heavy and light chain expression in each of the bovine atrial areas (Figs. 7 and 8) revealed that, although there were, in general, similar developmental trends in transition, there was no close link between the relative amounts of β-heavy chain (ventricular) and VLC isotypes
FIGURE 5. Myosin heavy and light chain transitions in human and bovine ventricle and total right and left atria (parts a and c, bovine, parts b and d, human) Parts a and b: ventricular transitions are expressed as α-heavy chain or total atrial light chains as a percentage of total α-plus β-heavy chains or total atrial plus ventricular light chains, respectively. (Only atrial light chain 1 appears to be involved in ventricular light chain transitions.) Parts c and d: atrial transitions are expressed as β-heavy chain or total ventricular light chains as a percentage of total α-plus β-heavy chains or total atrial plus ventricular light chains, respectively. (Only ventricular light chain 2 appears to be involved in atrial light chain transitions.) Arrow indicates birth. Bovine value immediately after arrow is 1 day neonate. □ □ heavy chains; ▲ ▲ light chains HC, heavy chains; LC, light chains, RA, right atria, LA, left atria.

expressed. Indeed, this possibility was excluded, as only LC-2 and LC-1 classes were exclusively involved in transitions in the atria and ventricles respectively. This lack of any obvious relationship between heavy chain and light chain expression was most marked in the neonatal and young adult period, when the direction of the trends in heavy and light chain isotypes was often opposed.

The magnitude of transitions in heavy and light chain isotype expression in the human atrial chambers was far less than that observed in the bovine heart (Fig. 5d). At mid-gestation, only low amounts of β-heavy chain were detected in the atria (8% in the total left atrium and 5% in the total right atrium). Thereafter, there was a gradual increase in β-heavy chain levels during gestation to reach a maximum of 26% soon after birth. This level then was maintained in the adult, with no significant differences between right and left atria. Moreover, although it was not possible to carry out a detailed regional study on all human specimens as in the bovine hearts, there was far less evidence for any marked differences in transitions in either heavy or light chain isotypes in different regions. As in the bovine atria, no obvious link between heavy and light chain isotype transitions was observed. At all developmental ages and in all regions, ALC-1 and ALC-2 light chains were almost exclusively expressed, with significant but low levels of VLC-2 appearing mainly in the adult left atria (Fig. 5d).

Discussion

Previous attempts to resolve cardiac whole heavy chain isotypes electrophoretically have been largely unsuccessful (D’Albis et al., 1979; John, 1980). Cardiac α-heavy chains from the different species examined in this study all migrated with slower velocity than β-heavy chains, and these, in turn, co-migrated with the fast and slow skeletal muscle forms, respectively. As recent studies have indicated the identity of the cardiac β-heavy chain and slow skeletal heavy chain cDNA (Lompre et al., 1984), the findings here lend further support to the general
Figure 6. Regional heavy and light chain transitions in bovine right and left atria. Parts a and c, heavy chains; parts b and d, light chains; parts a and b, right atria; parts c and d, left atria. Abbreviations of atrial regions as in Figure 1.

indications of a relationship between atrial and fast skeletal myosin isotypes on the one hand and slow skeletal and ventricular isotypes on the other (Cummins, 1983).

The results from peptide mapping and whole heavy chain electrophoresis were consistent with the presence of only two major forms of myosin heavy chain in the bovine and human heart. Recently, evidence has been obtained for two molecular variants of myosin in the human atria, differing in heavy but not light chain composition, and with the same in vitro adenosine triphosphatase (ATPase) activity (Dechesne et al., 1985). One of these forms is immunologically close to ventricular myosin, but differs in respect to heavy and light chains and enzymic activity. The close electrophoretic migration of whole α- and β-heavy chains might well exclude resolution of closely related homologous forms, but it seems less likely that detailed peptide mapping with different proteases would not identify major new variants. As small amounts of α-heavy chain have been detected in adult human ventricle (Mercadier et al., 1983), and as Dechesne et al. (1985) used anti-ventricular myosin antibodies, it is possible that the second form detected by them is the β-heavy chain, which the present study indicates is present in significant quantities in the adult human atria. Studies on atrial myosin under nondissociating conditions reveal two isoenzymes in rat, rabbit, guinea pig, dog, pig, rhesus monkey, baboon, bovine, and human atrium (Hoh et al., 1978; Clark et al., 1982; Cummins, 1984), but only one in human atrium by Klotz et al. (1983). These may or may not co-migrate with ventricular forms and vary in reactivity with antibodies to V1 and V3 isoenzymes, depending on species (Clark et al., 1982). The heavy or light chain composition of these isoenzymes is not clear, because isoenzymes in different muscles may resolve due to differences in heavy and/or light chain content. One brief report (Chizzonite et al., 1981) suggests that atrial isoenzymes in the rabbit differ in light chain composition. A minimum of two heavy and three light chain isoatypes was seen in the bovine and human atria in this study. If no restrictions on interactions between isoatypes exist (see below), the potential for different combinations of heavy and light chains in the myosin dimer resulting in different whole isoenzymes is considerable.

The ability of the ventricle to express multiple heavy chains was severely limited, in support of
recent studies indicating that V3 (β-heavy chain dimer) is almost exclusively expressed in larger mammalian species (Lompre et al., 1981; Clark et al., 1982; Lambert et al., 1983; Cummins, 1984). Low levels of VI isoenzyme (α-heavy chain dimer) have been observed in the normal adult human left ventricular free wall, by enzyme immunoassay (Mercadier et al., 1983). These ranged from 0.9% to 2.2% in four individuals, with increased levels of 5.7 and 14.2 in two others. Although no α-heavy...
chain was detected in either the right or left ventricle or interventricular septum in the 13 adult human hearts used in this study, these findings are not necessarily inconsistent. They could result either from differences in sensitivity of the two techniques, the limited number of patients in each study, and heterogeneity of expression (both inter- and intra-individual), of the low levels of a-heavy chain in the ventricle (Mercadier et al., 1983; Gorza et al., 1984). With regard to the latter aspect, as either the whole right or left ventricle or interventricular septum tissue was used for preparations in the current study, we can only say that the level of a-heavy chain did not exceed 5% of the total (by whole heavy chain electrophoresis) in these overall areas in the patients examined here. It does not exclude the possibility of higher levels in smaller localized regions. A level of 14–24% ventricular a-heavy chain has been reported in one patient (Gorza et al., 1984), although most samples were estimated to contain “less than 10%.” No obvious regional variation in distribution of ventricular fibers containing a-heavy chain was found in the human heart (Gorza et al., 1984).

The transitory appearance of a-heavy chain in the bovine neonatal ventricle parallels that in mouse, rat, and rabbit heart (Lompre et al., 1981; Schwartz et al., 1982), although its retention as the major adult isoform occurs only in small mammals. This neonatal expression of a-heavy chain also occurs at low levels in the pig ventricle (Lompre et al., 1981), and is reflected in increased myosin Ca++ ATPase in the 4-day neonate (Syrovy, 1982). Although no a-heavy chain was detected in the human neonatal ventricle in this study, other studies (Everett, 1985) using immunofluorescence with a “fast myosin type” monoclonal antibody have reported minor amounts in some individuals. This appearance of a-heavy chain in the larger neonatal ventricle may reflect changing circulating thyroid hormone levels. This induces synthesis and plays a permissive role for continued synthesis of a-HC in fetal and neonatal rat and rabbit ventricle (Everett et al., 1983b; Chizzonite and Zak, 1984). Studies of thyroid hormone on myosin transitions in larger mammals are rare. The ATPase of myosin subfragment-1 from thyrotropic bovine ventricle is 2.5 times higher than normal (Flamig and Cusanovich, 1983), whereas studies of thyroid influence on adult pig cardiac myosin (Wiegand et al., 1985), confirm a β- to α-heavy chain transition, indicating that similar hormonal mechanisms exist. Certainly marked transitions occurred in the late fetal and neonatal period in the present study, particularly in the bovine atria affecting both heavy and light chains. Although an initial increase in atrial β-heavy chain occurred during mid- to late gestation, this was often reversed for a period soon after birth, suggesting that the relative increase in a-heavy chain expression at this time may be subject in part to thyroidal influence throughout the heart. If thyroid hormone is involved, the marked regional variation in transitions near birth could imply a variation in receptor tissue sensitivity.

The quantitative potential for transitions in both subunits probably is higher in the atria, given the greater heterogeneity of heavy and light chain isoforms. Hemodynamic overload causes atrial-to-ventricular light chain transitions in human right and left atria, in line with degree of pressure overload hypertrophy (Cummins, 1982), and corresponding α- to β-heavy chain switches take place in overloaded and hypertrophied human left atrium (Gorza et al., 1984; Mercadier et al., 1985). Although no comparable hemodynamic studies have been conducted in bovine atria, the potential for atrial-to-ventricular transitions is presumably greater in the human heart, as significant levels of both β-heavy chain and VLC-2 are present in normal adult bovine atria.

In the current study, different light chain classes were involved in transitions in atria and ventricles. Although, in severe atrial hemodynamic overload, ALC-1 to VLC-1 transitions may occur (Cummins, 1982), it appeared that light chains 1 and 2 were independently involved in developmental transitions in the ventricles and atria, respectively. Moreover, although heavy and light chain transitions displayed similar developmental trends, at most stages they were not temporally closely linked. This implies a lack of isotype-specific interactions between heavy and light chains, with no simple relationship between the different populations expressed. Indeed, the available evidence suggests that different light chains can combine with different heavy chains. On this basis, it may not be helpful to think strictly in terms of atrial and ventricular myosin isoforms in larger mammals, but, rather, of the variety of mixed isoforms potentially available.

The regional heterogeneity in isotype expression in bovine and, to a lesser degree, human myocardium supports immunohistochemical findings (Sartore et al., 1981; Gorza et al., 1982; Thornell and Forsgren, 1982; Gorza et al., 1984; Bouvagnet et al., 1984) and places quantitative values on these. Gorza et al. (1982), using indirect immunofluorescence and polyclonal antibodies to β-heavy chain, found increased localization in bovine right atrium and, in particular, crista terminalis and interatrial septum. Highest adult levels (ranging from 40% to 65% of total) of β-heavy chain in this study were found in the same areas, in addition to right atrial posterior and left atrial free wall. Similar increased localization in these areas was found in the human heart (Gorza et al., 1984). Discrepancies between findings may reflect not only different technique sensitivities, but also the possible effect of light chains on binding of heavy chain antibodies to the myosin in situ.

The reasons for this regional variation in myosin expression in the atria are not clear. It has been suggested that β-heavy chain is concentrated in fibers specialized for faster conduction, because immunohistochemical staining is marked in those areas...
implicated as favored regions of intermodal conduction of atrial depolarization (Gorza et al., 1982). If isotype composition modulates fiber conduction velocity, then the high levels of \( \beta \)-heavy chain (and possibly VLC-2) seen in areas of the bovine atria, which in total constituted the bulk of atrial musculature, would argue against defined conduction pathways. The recent discovery of a specific nodal myosin heavy chain (Schiaffino et al., 1985) may allow analysis of this hypothesis. Other factors may also be involved in the different regional atrial expression. We have noted a possible relationship between architectural structure and myosin composition. In areas that are relatively muscular, thick-walled, and trabeculated, i.e., the left and right atrial appendage and right pectinate muscles, the highest adult levels of \( \alpha \)-heavy chain were present, whereas, in smoother, relatively thinner-walled areas (crista terminalis, right posterior wall, left free wall, and atrial septum), we found the highest levels of \( \beta \)-heavy chain. If myosin transitions that occur as a result of hemodynamic overload are adaptive, then it may be that a similar relationship occurs within the normal atria to compensate for architectural heterogeneity. Perhaps a stimulus to increased \( \beta \)-heavy chain synthesis exists, which becomes increasingly predominant in the adult, in those areas that are less suited in design to increased tension development. A suggestion that regional variations in myosin distribution could be related to the tension of the different cardiac walls has been made by Bouvagnet et al. (1984). Embryological origin of the different parts of the atrial chambers may also be involved, although the lack of significant \( \beta \)-heavy chain expression at mid-gestation in any atrial area would argue against this hypothesis, and we could find no relationship between origin and isotype composition.

Whatever the reasons for this extraordinary heterogeneity of myosin isotype expression, it is to be hoped that further quantitative studies on these transitions in response to different stimuli will give clues to these important questions.

This work was supported by grants from the British Heart Foundation.

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Received October 28, 1985, accepted for publication March 13, 1986

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INDEX TERMS Myosin • Heavy chains • Light chains
Myosin transitions in the bovine and human heart. A developmental and anatomical study of heavy and light chain subunits in the atrium and ventricle.

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_Circ Res._ 1986;58:846-858
doi: 10.1161/01.RES.58.6.846

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/58/6/846

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