Human atrial and ventricular myosin light-chain subunits in the adult and during development

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1. Myosin was isolated from human right- and left-atrial and -ventricular myocardium, and examined both in adult subjects and at different stages during pre- and post-natal development. 2. The myosin light-chain subunits in the atria and ventricles were different when characterized by isoelectric focusing and subsequent two-dimensional polyacrylamide-gel electrophoresis. 3. No differences were observed between the light-chain subunits in the right and left ventricle at any stage of development. 4. The foetal ventricle contained a characteristic light chain that was a major component throughout the latter half of gestation. This foetal light chain, which disappeared in the postnatal period, could not be distinguished from adult atrial light chain 1 on two-dimensional electrophoresis. 5. Myosin in the adult atria, particularly the left, contained components similar to ventricular light-chain components. 6. The possible stimuli for the observed changes in myosin light-chain expression are discussed in relation to the known physiological changes occurring during development.

The presence of myosin isoenzymes that differ in heavy-chain (Trayer *et al.*, 1968; Hoh *et al.*, 1976, 1978) and light-chain subunit composition (Lowey & Risby, 1971; Sarkar *et al.*, 1971) has been demonstrated in both cardiac muscle and fast and slow skeletal muscles. The labile nature of the myosin isoenzyme population expressed in a particular muscle has been elegantly demonstrated in experiments involving the transformation of fast- into slow-skeletal-muscle myosin by cross-innervation of a fast muscle with a slow-muscle nerve (Buller *et al.*, 1969; Barany & Close, 1971). Fast-twitch muscle subjected to chronic low-frequency stimulation has also been shown to acquire myosin light and heavy chains similar to those of slow-twitch-muscle myosin (Sreter *et al.*, 1975b). Changes in myosin isoenzyme population have been reported in the heart after hypophysectomy and thyroxine replacement (Hoh *et al.*, 1978) and chronic heart overload (Lompre *et al.*, 1979).

The type of myosin gene expressed in a particular muscle also changes during normal development. Hoh & Yeoh (1979) and Whalen *et al.* (1979) have reported changes in the heavy chains of myosin during the development of rat skeletal muscles and demonstrated the existence of a foetal myosin isoenzyme. Slow- and fast-muscle isoenzymes of myosin have been reported to coexist in all the fibres of a fast-twitch mammalian muscle during early

development (Gauthier *et al.*, 1978), the synthesis of slow-muscle myosin being 'switched off' during the normal course of differentiation, perhaps in response to withdrawal of multiple innervation of the fibres.

Several reports (John, 1976; Pelloni-Mueller *et al.*, 1976a; Dabrowska *et al.*, 1977) have suggested that during foetal development of a prospective slow skeletal muscle, only light chains similar to or identical with those of adult fast-skeletal-muscle myosin are present, there being a gradual transition to the slow-muscle myosin isoenzyme form as the muscle differentiates.

The situation with regard to the development of cardiac muscle is not clear. Pelloni-Mueller *et al.* (1976b) reported that cardiac-muscle myosin of rabbits at the age of 9–3 days before birth contained a mixture of light chains as found in adult cardiac and fast skeletal muscle. Although most studies on cardiac-muscle myosin have focused on the ventricle, different myosin light-chain isoenzymes have also been reported in the atria and ventricles of the dog (Long *et al.*, 1977) and rat (Hoh *et al.*, 1978). However, no detailed studies have been carried out on the myosin genes expressed in the different chambers of the human heart.

The present investigation describes the characterization of human myosin light-chain subunit isoenzymes expressed in the atria and ventricles in

the adult and during foetal development. It is demonstrated that the adult atrial and ventricular light chains differ electrophoretically. Although no differences are shown between the right and left ventricle at any stage of development, the light-chain subunits differ in the adult right and left atria. The adult left atrium in particular is shown to contain components that are absent in the foetal atria and that may be related to one of the ventricular myosin light chains. A foetal myosin light chain is described in the foetal ventricle that is indistinguishable electrophoretically from one of the atrial light chains. The implications and possible mechanisms of these changes are discussed.

Some aspects of this work have been briefly reported elsewhere (Price *et al.*, 1979; Cummins *et al.*, 1979).

Materials and methods

Tissue sources

Human foetal myocardium was deep-frozen at -30°C as soon as possible after removal and was kindly provided by Dr. J. H. Marston, Department of Anatomy, University of Birmingham.

Adult human myocardium was obtained either up to 6 h *post mortem* or immediately after death from renal transplant donors, with the informed consent of the relatives and coroner, from the Renal Unit, Queen Elizabeth Hospital, University of Birmingham. Patients of both sexes ranged in age from 35 to 71 years and were not known to suffer from any abnormal cardiovascular condition. Subsequent examination *post mortem* confirmed the absence of any pathological cardiac abnormality. Tissue was frozen in liquid N_2 after excision and stored at -30°C if not used immediately.

Preparation of myosin

The preparation of myosin was based on the methods of Awan *et al.* (1972) and Wikman-Coffelt *et al.* (1973). All preparative procedures were carried out at 4°C . Muscle was homogenized for 1 min in 4 vol. of 40 mM-imidazole/5 mM-EDTA/1 mM-dithiothreitol, adjusted to pH 7.2 with 1 M-HCl and centrifuged at 2600 g for 10 min. The pellet was washed a further two times in the same buffer. Myosin was then extracted for 20 min with constant stirring with 3 vol. of 40 mM-imidazole/0.6 M-KCl/10 mM-tetrasodium pyrophosphate/4 mM- MgCl_2 /1 mM-dithiothreitol, adjusted to pH 7.2 with 1 M-HCl. The extract was centrifuged at 2500 g for 20 min. After filtering through glass wool, the supernatant was slowly diluted 10 times with 1 mM-dithiothreitol and the precipitate was collected by centrifugation at 2500 g for 20 min. The precipitate was dissolved in the minimum volume of 0.5 M-KCl and diluted 10 times with 1 mM-dithiothreitol/2 mM-EDTA. After

centrifugation at 10000 g for 10 min, the pellet was suspended in 50 mM-tetrasodium pyrophosphate/1 mM-EDTA/1 mM-dithiothreitol, adjusted to pH 7.5 with 1 M-HCl, to a protein concentration of 2 mg/ml. The solution was then made 2 mM in ATP by the addition of 50 mM-sodium ATP, adjusted to pH 7.5 with 5 M-NaOH. The protein solution was stirred for 10 min, then centrifuged at 40000 g for 20 min and the pellet was discarded. Saturated $(\text{NH}_4)_2\text{SO}_4$, adjusted to pH 6.8 with 1 M- NH_3 , was then added to the supernatant to 35% (v/v) saturation. The solution was centrifuged at 10000 g for 15 min and the precipitate was discarded. Myosin was precipitated between 35 and 42% (v/v) saturation with $(\text{NH}_4)_2\text{SO}_4$ and collected by centrifugation at 5000 g for 15 min. The myosin pellet was dissolved in the minimum volume of 50 mM-Tris/1 mM-dithiothreitol/0.5 M-KCl, adjusted to pH 7.5 with 11.65 M-HCl and dialysed against the same buffer for 16 h. The myosin solution was then centrifuged at 40000 g for 15 min, the pellet discarded and the myosin supernatant was retained.

Preparation of crude myosin extracts

Crude myosin extracts for electrophoretic analysis were prepared from 50–200 mg of myocardium. Tissue was cut up and homogenized in 1.5 ml of 40 mM-imidazole/5 mM-EDTA/5 mM-2-mercaptoethanol, adjusted to pH 7.2 with 1 M-HCl, in an Ultra-Turrax homogenizer (Sartorius Instruments, Belmont, Surrey, U.K.) and centrifuged at 2600 g for 10 min. After washing once with the same buffer, the pellet was extracted for 15 min with 1 ml of 40 mM-imidazole/0.6 M-KCl/10 mM-tetrasodium pyrophosphate/5 mM-2-mercaptoethanol, adjusted to pH 7.2 with 1 M-HCl. After centrifugation at 10000 g for 10 min the supernatant was diluted 10 times with 5 mM-2-mercaptoethanol and the precipitate was collected by centrifugation at 5000 g for 15 min. The pellet was dissolved in the minimum volume of 0.5 M-KCl and solid urea was added to give a final concentration of 9 M before electrophoresis.

Whole-muscle extracts

Whole-muscle extracts were prepared by homogenizing 2–30 mg of tissue in 9 M-urea/0.75 M-2-mercaptoethanol (60 μl /mg of tissue). The extract was centrifuged at 1000 g for 10 min at 20°C and the supernatant was made 2% (v/v) in Ampholine Carrier Ampholytes, pH range 4 to 6, before isoelectric focusing.

Electrophoresis

Electrophoresis was carried out in vertical slab assemblies (Cummins & Perry, 1973; Perrie *et al.*, 1973) under the following conditions. (1) In 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.1%

(w/v) sodium dodecyl sulphate as described by Weber & Osborn (1969) in 10% (w/v) polyacrylamide gels. (2) In 6M-urea/25 mM-Tris/80 mM-glycine buffer (pH 8.5)/15 mM-2-mercaptoethanol in 10.0% (w/v) polyacrylamide gels as described by Schaub & Perry (1969).

Isoelectric focusing was carried out as described by O'Farrell (1975) with a stick gel apparatus from Shandon Southern Products, Runcorn, Cheshire, U.K. Gels of 4% (w/v) polyacrylamide contained 2% (v/v) Ampholine Carrier Ampholytes, consisting of 1.6% (v/v) of pH range 4–6 and 0.4% (v/v) of pH range 3.5–10.0. Gels were polymerized by using 0.05% (w/v) ammonium persulphate and were electrophoresed at 300V for 18h followed by 400V for 2h. Ampholine Carrier Ampholytes were obtained from LKB, South Croydon, Surrey, U.K.

Two-dimensional polyacrylamide-gel electrophoresis in the presence of 0.1% (w/v) sodium dodecyl sulphate was carried out as described by O'Farrell (1975) with the following modifications. A 12.5% (w/v) polyacrylamide separating gel was employed and 3 mm slab gels were electrophoresed at a constant current of 50 mA for 7h. A vertical slab-gel electrophoresis apparatus, model SAE-2790, from Shandon Southern Products, was used.

Densitometric analysis

Densitometric scanning of the gels was carried out with a Gilford model 2400 recording spectrophotometer fitted with a Gilford model 2410 gel-scanning attachment.

Results

Adult myosin

Myosin was isolated from the free right- and left-ventricular wall and right- and left-atrial myocardium and examined by electrophoresis at pH 7.0 in the presence of 0.1% (w/v) sodium dodecyl sulphate (Fig. 1a). Under these conditions the myosin heavy chains remained at the origin and the two light chains of cardiac-muscle myosin (Sarkar *et al.*, 1971; Malhotra *et al.*, 1977) were clearly resolved. The larger atrial and ventricular light chains (termed ALC-1 and VLC-1 respectively) migrated with a molecular weight corresponding to approx. 26000 by comparison with standards of known molecular weight. The smaller light chains (termed ALC-2 and VLC-2) migrated with an apparent mol.wt. of 19000. No evidence of proteolysis was observed in any preparations as would be

Fig. 1. Polyacrylamide-gel electrophoresis of adult atrial and ventricular myosins

(a) 10% (w/v) polyacrylamide/0.1% (w/v) sodium dodecyl sulphate (pH 7.0); (b) 10% (w/v) polyacrylamide/6M-urea (pH 8.5); (c) isoelectric focusing, 4% (w/v) polyacrylamide/2% (v/v) Ampholine Carrier Ampholytes (pH 4–6); (i), (iv) and (vi), right-atrial myosin (70 μ g); (ii), (v) and (viii), left-ventricular myosin (70 μ g); (iii) and (vii), a mixture of right-atrial and left-ventricular myosin (100 μ g).

evidenced by components of lower molecular weight. When atrial and ventricular myosin were co-electrophoresed [Fig. 1a(iii)], ALC-2 and VLC-2 co-migrated, but ALC-1 appeared to possess a slightly higher molecular weight than VLC-1, although they were not well resolved. No differences were observed between either atrial or ventricular myosin in the right and left side of the heart.

In the presence of 6M-urea at pH8.5 (Fig. 1b), ALC-1 and VLC-1 co-migrated, but ALC-2 and VLC-2 did not co-migrate and were both resolved into more than one component. The different components of VLC-2 have been previously identified in the rabbit ventricle (Frearson & Perry, 1975) as the dephosphorylated and phosphorylated forms of this light chain (termed VLC-2 and VLC-2P respectively). In addition, a component of altered charge of these two forms (termed VLC-2* and VLC-2P* respectively) was also identified under the same electrophoretic conditions by these authors, although the nature of the modification involved is not clear. In the present study, two major components were observed, VLC-2 and VLC-2* [Fig. 1b(v)], but smaller amounts of the phosphorylated forms (VLC-2P and VLC-2P*) were also seen in some preparations. Although the different components of ALC-2 have not been completely identified and were not well resolved under these conditions, they did not appear to co-migrate with the VLC-2 components. Preparations from the right and left atria and ventricles were identical under these electrophoretic conditions.

The differences between atrial and ventricular myosin light chains were more clearly resolved by using isoelectric focusing between pH4 and 6 (Fig. 1c). Six components were identified in ventricular myosin preparations [Fig. 1c(viii)]. These are also represented schematically in Fig. 2(e). Two methods were used to identify the components. Isoelectric-focusing gels were electrophoresed in the second dimension in the presence of 0.1% (w/v) sodium dodecyl sulphate, as described in the Materials and methods section, to resolve those components that were related on the basis of the same molecular weight to either VLC-1 or VLC-2 (cf. Fig. 3a). Components were also identified by the cutting out and elution of the different bands from a pH8.5 6M-urea gel and subsequent co-electrophoresis of these components by isoelectric focusing with added ventricular myosin (K. M. Price & P. Cummins, unpublished work). After co-electrophoresis, gels were scanned densitometrically to identify the individually added bands.

When examined by isoelectric focusing, the five components seen at pH8.5 in the presence of 6M-urea migrated in the same order, with VLC-1 being the most alkaline, and VLC-2, VLC-2*, VLC-2P and VLC-2P* focusing with increasingly

Fig. 2. Schematic representation of isoelectric-focusing gels of atrial and ventricular myosin

Isoelectric focusing was carried out in 4% (w/v) polyacrylamide gel/2% (v/v) Ampholine Carrier Ampholytes (pH4–6) as described in the Materials and methods section. (a) Foetal atrial myosin (18–21 weeks gestation); (b) adult right-atrial myosin; (c) adult left-atrial myosin; (d) foetal ventricular myosin (18–21 weeks gestation); (e) adult ventricular myosin.

acidic isoelectric points [Fig. 1c(viii)]. In addition to these components, VLC-1 also gave rise to a derivative of altered charge designated VLC-1* [Figs. 1c(viii) and 2e]. The nature of the modification involved is not clear, but similar derivatives have been observed in the dog (Siemankowski & Dreizen, 1978). Its presence did not appear to be related to the freshness of the tissue sample, so it seems unlikely that proteolysis is involved. Its presence may be related to the state of the electrophoresis sample, but the possibility that this form is present *in vivo* cannot be excluded. When isoelectric-focusing gels of right- and left-ventricular myosin were quantified by densitometry no differences in type or relative amounts of light chains were observed. In both the right and the left ventricle, the total amounts of VLC-1- and VLC-2-related components were almost identical (Table 1).

When ventricular myosin was examined by two-dimensional electrophoresis (Fig. 3a), VLC-2 and VLC-2P* were resolved, but VLC-2* and VLC-2P and also VLC-1 and VLC-1* often tended to migrate as single spots.

Examination of atrial myosin by isoelectric focusing and subsequent two-dimensional electrophoresis revealed consistent differences in the light chains not only between atrial and ventricular myosin but also between preparations from the right and left atria. Right-atrial myosin was resolved into seven components on isoelectric focusing [Figs. 1c(vi) and 2b]. Components related to either ALC-1 or ALC-2 were identified by two-dimensional electrophoresis (Fig. 4a). The two major components in the

Table 1. *Myosin light-chain composition of adult and foetal human atria and ventricles*

Isoelectric-focusing gels were quantified by gel-scanning densitometry as described in the Materials and methods section. No allowance was made for any possible difference in dye uptake by components. The amounts of VLC-1 and ALC-1a were corrected to 100 and the amounts of the other light-chain derivatives were expressed relative to this value. VLC-2* and VLC-2P and likewise ALC-1b and ALC-2a were not well enough resolved to allow accurate quantification; the ratios of ALC-1/ALC-2 could not therefore be determined in the adult. In the adult right and left atria, both ALC-2 and the component co-migrating with VLC-2P* (i.e. ALC-2x) contributed towards the total ALC-2c content. Abbreviations used: LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium.

Source	Age	VLC-2*						Total LC-1	Total VLC-2	LC-1/ VLC-2
		VLC-1	FLC-1	VLC-1*	VLC-2	+ VLC-2P	VLC-2P*			
LV, RV	18–21 weeks gestation	100	87	—	122	87	—	187	209	0.89
LV, RV	28–34 weeks gestation	100	64	—	123	26	—	164	149	1.10
LV, RV	8 months postnatal	100	6	16	68	31	—	122	99	1.23
LV	Adult	100	—	12	49	57	10	112	116	0.97
RV	Adult	100	—	13	55	59	12	113	126	0.90
ALC-1b										
		+							ALC-1/ ALC-2	
		ALC-1a	ALC-2a	ALC-1c	ALC-2b	ALC-2c	ALC-2d	ALC-2e		
RA	Adult	100	42	8	22	65	24	—	—	
LA	Adult	100	60	28	85	76	26	—	—	
RA, LA	18–21 weeks gestation	100	—	—	—	28	34	46	0.93

Fig. 3. *Two-dimensional polyacrylamide-gel electrophoresis of adult and foetal atrial and ventricular myosin*
Isoelectric focusing and two-dimensional analysis in the presence of 0.1% (w/v) sodium dodecyl sulphate carried out as described in the Materials and methods section. (a) Adult left-ventricular myosin (70 µg); (b) adult right-atrial and left-ventricular myosin (100 µg); (c) foetal ventricular myosin (18–21 weeks gestation) (70 µg); (d) foetal ventricular myosin (18–21 weeks gestation) plus adult right-atrial myosin (100 µg); (e) foetal ventricular (18–21 weeks gestation) whole-muscle extract. Only the major molecular-weight species are labelled in Fig. 3(b).

Fig. 4. *Two-dimensional polyacrylamide-gel electrophoresis of adult and foetal atrial myosin*

Isoelectric focusing and two-dimensional analysis in the presence of 0.1% (w/v) sodium dodecyl sulphate carried out as described in the Materials and methods section. (a) Adult right-atrial myosin (70 μ g); (b) adult left-atrial myosin (70 μ g); (c) foetal right-atrial myosin (70 μ g); (d) adult right atrium (crude myosin extract); (e) adult left atrium (crude myosin extract). Abbreviations used: A, actin; Tm, tropomyosin; Tn-C, troponin C.

right atrium, which were designated ALC-1a and ALC-2c, did not co-migrate with any of the ventricular components [Fig. 1c(vii)]. ALC-1b and ALC-1c appeared to be charged derivatives of the major band ALC-1a. This appeared to involve a sequential modification of ALC-1a, as can be seen in Fig. 4(a), and may be similar to that observed with VLC-1*. The remaining components, ALC-2a, ALC-2b and ALC-2d, were present in smaller amounts in the right atrium (Fig. 2b and Table 1) and ALC-2d was occasionally present in the form of a doublet. ALC-2a and ALC-2b co-migrated on isoelectric focusing with VLC-2 and VLC-2* respectively [Fig. 1c(vii)]. In the left atrium, the same seven components were observed on isoelectric focusing, but although ALC-1a was a major component in both right and left atria (Fig. 2), ALC-2b was the major component in the left. This was confirmed by subsequent densitometric analysis (Table 1).

The differences between ventricular and right- and left-atrial myosin were more clearly seen when preparations were examined by two-dimensional electrophoresis. Although ventricular myosin gave only two molecular-weight species in the second dimension (Fig. 3a), right-, and in particular,

left-atrial myosin light chains gave rise to three molecular-weight species. ALC-1a, ALC-1b and ALC-1c have been described above. ALC-2c and ALC-2d migrated with the same apparent molecular weight, which was slightly higher than ALC-2a and ALC-2b (Figs. 4a and 4b). The differences in relative amounts of these components in the right and left atria seen on isoelectric focusing were clearly evident in the second dimension. ALC-2a and ALC-2b were only minor components in the right atrium when compared with the left atrium.

When atrial and ventricular myosin were co-electrophoresed in the second dimension, ALC-1 components and VLC-1 components were clearly resolved, the former possessing an apparently higher molecular weight (Fig. 3b). ALC-2c and ALC-2d also possessed higher molecular weights than the VLC-2 components; however ALC-2a and ALC-2b co-migrated exactly with VLC-2 and VLC-2* respectively as in the first dimension. In addition, a component (designated ALC-2x) that possessed a similar isoelectric point to ALC-2c, but that co-migrated with VLC-2P* in the second dimension was apparent in left-atrial myosin preparations (Fig. 4b).

The atrial components that co-migrated with

those present in the ventricle, i.e. ALC-2a, ALC-2b and ALC-2x, were always present in much greater amounts in the left atrium than in the right atrium (cf. Figs. 4a and 4b) and their presence was a constant feature in all preparations of pure myosin studied. The absence of any VLC-1-related components in atrial myosin preparations suggested that ventricular muscle contamination was not responsible for the presence of these 'ventricular-like' components. The possibility that ALC-2a, ALC-2b and ALC-2x occurred as a result of modification or proteolytic degradation of a major atrial light chain during preparation was examined by using a shortened technique to produce crude myosin extracts (see the Materials and methods section). Two-dimensional analysis of these extracts (Figs. 4d and 4e) from the right and left atria revealed the presence of ALC-2a, ALC-2b and ALC-2x and the same difference between right and left as was seen with pure myosin. Actin, tropomyosin and troponin-C were also identified in crude myosin extracts.

Foetal and neonatal myosin

Myosin was isolated from the free right- and left-ventricular wall of human foetal myocardium at 18–21 weeks gestation and examined by isoelectric focusing (Figs. 2d and 3c). No difference was observed between right- and left-ventricular preparations. The major components present, which co-migrated with the corresponding adult ventricular light-chain components, were VLC-1, VLC-2 and VLC-2P. The latter two components were present in approximately equal amounts and this was a constant feature of foetal ventricular myosin preparations of this age. There were also smaller amounts of VLC-2* and VLC-2P* and a major new component, designated FLC-1, which did not co-migrate with any adult ventricular components, and which was present in all foetal preparations studied. FLC-1 appeared to migrate in the same position on two-dimensional analysis as adult

ALC-1a and this was confirmed by subsequent co-electrophoresis of adult right-atrial myosin and foetal ventricular myosin (Fig. 3d). No traces of components related to ALC-2 were seen on isoelectric focusing or two-dimensional analysis of either pure myosin or crude myosin extracts of foetal ventricle. However, close examination of 9M-urea/0.75 M-2-mercaptoethanol extracts of whole muscle (see the Materials and methods section) in the second dimension (Fig. 3e) revealed the presence of a minor component co-migrating with ALC-2c.

To determine the time course of disappearance of the FLC-1 component, crude myosin extracts of ventricular tissue (see the Materials and methods section) of different pre- and post-natal ages were examined (Fig. 5). At 28 weeks and 33–34 weeks of gestation near term, FLC-1 was still a major component (Fig. 5b). However, 8 months after birth only small amounts were still present and it was absent in the 7-year-old ventricle (Figs. 5c and 5d).

The relative amounts of FLC-1, VLC-1 and VLC-2 in the adult and foetal ventricle were quantified by densitometric scanning of isoelectric-focusing gels (Table 1). Although no allowance was made for possible unequal dye uptake by components, it can be seen that the total contents of VLC-1 and VLC-2 in the adult were the same. However, there was a deficit of VLC-1 in the foetal ventricle and this appeared to be compensated for by the presence of FLC-1. At 18–21 weeks gestation, FLC-1 constituted almost half the total LC-1 population in the ventricle, dropping to about one-third of the total at term. No change was observed in the amount of VLC-2 components during this period, the ratio of total LC-1 to total VLC-2 indicating approximately equimolar amounts at all ages (Table 1).

The right and left atria of foetal hearts were only large enough to permit the preparation of crude myosin extracts. Similar results were obtained from the right and left atria on isoelectric focusing and

Fig. 5. *Isoelectric focusing of crude myosin extracts from human ventricular myocardium of different ages* Crude myosin extracts were prepared as described in the Materials and methods section. Isoelectric focusing was carried out in 4% (w/v) polyacrylamide gels/2% (v/v) Ampholine Carrier Ampholytes (pH range 4–6). (a) 18–21 weeks-gestation ventricular myosin; (b) 34 weeks-gestation ventricular myosin; (c) 8 months-postnatal ventricular myosin; (d) 7 years-postnatal ventricular myosin.

two-dimensional analysis throughout gestation and in the immediate post-natal period examined (Figs. 2a and 4c). Foetal ALC-1a co-migrated in both dimensions with adult ALC-1a. Three bands of approximately equal intensity were observed for foetal atrial LC-2. Foetal ALC-2a and ALC-2d co-migrated with adult ALC-2c and ALC-2d respectively. In addition, a component with the same molecular weight, designated ALC-2e, was observed. Preliminary experiments (K. M. Price & P. Cummins, unpublished work) suggest that foetal ALC-2d and ALC-2e are phosphorylated forms of ALC-2c. Little change was observed in this pattern throughout foetal development. Densitometric quantification of foetal atrial myosin (Table 1) indicated approximately equal amounts of total ALC-1 and ALC-2. No traces of ALC-2a, ALC-2b or ALC-2x were seen in foetal atrial preparations.

Discussion

The results presented here demonstrate significant differences between the light-chain subunits of myosin in the human atria and ventricles not only in the adult but also during prenatal development. Atrial and ventricular myosin light chains have also been shown to differ electrophoretically in the adult dog myocardium (Long *et al.*, 1977), although in the chicken, Libera *et al.* (1979) have reported that the light chains appear identical on two-dimensional electrophoresis. However, in an immunohistochemical study of the chicken heart, muscle cells from atrial, ventricular and conduction tissue were found to contain antigenically distinct myosins (Sartore *et al.*, 1978) by using antisera that were probably directed against the heavy-chain subunits of myosin. The peptides from CNBr treatment of bovine atrial and ventricular myosin heavy chains have indeed been shown to differ on two-dimensional electrophoresis (Flink *et al.*, 1978).

Controversy has existed concerning the enzymic and physicochemical properties of myosin in the right and left ventricle. Wikman-Coffelt *et al.* (1975) found higher Ca^{2+} - and K^{+} /EDTA-activated ATPase activities in the canine left ventricle and differences in the stoichiometry of light and heavy chains between the right and left side. However, subsequent examination of ATPase activities and electrophoretic properties by using isoelectric focusing have indicated no differences (Siemankowski & Dreizen, 1978).

Although we found no difference in electrophoretic properties of myosin light chains from the human right and left ventricle at any stage in the present study, and previous findings from this laboratory have shown that ventricular myosin Ca^{2+} -activated ATPase activities are the same

(Cummins *et al.*, 1979), right- and left-atrial light chains that did not differ before birth showed consistent electrophoretic differences in the adult. Syrový *et al.* (1979) have reported that right- and left-atrial and -ventricular myosin are identical both electrophoretically and enzymically in seven mammalian species. However, no previous studies have been carried out in man. It is possible that limited proteolytic degradation of myosin might account for the differences in right- and left-atrial myosin observed in man. This would, however, seem unlikely as similar results were obtained from all adult myocardium studied and the apparently lower molecular-weight species, ALC-2a, ALC-2b and ALC-2x, were present in tissue excised at different times after death. Proteolysis would also probably affect both sides of the heart to a similar extent. Although no proteinase inhibitors were used in the present study, similar electrophoretic results were obtained from pure myosin preparations and from whole muscle subjected to immediate electrophoretic analysis in 9M-urea, where the risk of proteolysis would be minimal.

Owing to the nature of the myocardial tissue available in the present study it was not possible to control the extent of phosphorylation of myosin LC-2 and this could also have given rise to some variability between preparations. However, phosphorylation of an atrial light chain is unlikely to produce a species with a significantly different molecular weight in the second dimension as evidenced by the co-migration of phosphorylated and dephosphorylated VLC-2 here. Consequently, although the possibility that phosphorylation may affect right- and left-atrial and -ventricular myosin to differing extents cannot be excluded, it seems unlikely to account for the difference between the right and left atria observed here. The formation of species of altered charge, as in the case of VLC-2* and VLC-2P*, may account for some of the ALC-2 components observed on isoelectric focusing. The precise nature of the modifications involved is not clear. If deamination of glutamine or asparagine residues is responsible, these also would not result in species with significantly different molecular weights. The possibility that disulphide bond formation is involved cannot be excluded, although the addition of fresh dithiothreitol and 2-mercaptoethanol to electrophoresis samples does not affect the number of components seen on two-dimensional electrophoresis of atrial or ventricular myosin. The relationship of the atrial to the VLC-2 components that co-migrate in the second dimension is currently being investigated by using right- and left-atrial appendages obtained at cardiac surgery. A possible clue to their presence may be gained by examining the nature of the foetal ventricular light chain (FLC-1) reported here.

FLC-1 was present in reproducible amounts in all foetal cardiac specimens and constituted almost half the total LC-1 content during the latter half of gestation. This light chain could be a unique foetal subunit or could be closely related to, if not identical with, an existing adult light chain. The existence of a foetal or embryonic form of myosin was proposed by several workers (Trayer *et al.*, 1968; Huszar, 1972; Sreter *et al.*, 1975a) and unique heavy (Hoh & Yeoh, 1979; Whalen *et al.*, 1979) and light chains (Whalen *et al.*, 1978) reported. The embryonic myosin light chain (LC1_{emb}) described by Whalen *et al.* (1978) has been identified in developing rat skeletal muscles and also in embryonic skeletal and cardiac tissue of mouse, rabbit and calf and has been proposed as a very specific marker of striated-muscle development. Moreover, we have identified a component in crude myosin extracts of mixed human foetal skeletal muscle of 18–21 weeks gestation that co-migrates on two-dimensional electrophoresis with FLC-1 (P. Cummins & K. M. Price, unpublished work). It does not co-migrate with adult skeletal-muscle myosin LC-1 subunits and is absent in adult skeletal muscle. This human foetal skeletal-muscle component may correspond to the LC1_{emb} of Whalen, although only trace amounts are present at a time when ventricular FLC-1 is a major component. If the pattern of neural innervation is a major factor in the differentiation of striated muscle, the presence in cardiac when compared with skeletal muscle of relatively larger amounts of a foetal light chain is unusual. Cardiac muscle differentiates earlier than skeletal muscle (Holtzer *et al.*, 1957), the primitive cardiac chambers appearing in the first few weeks of gestation (Dawes, 1968) and functioning in the absence of neural control. It may be that other factors, such as the different pressure gradients in the developing cardiac chambers, may also play a role in determining the type of myosin light chain synthesized.

In the present study, FLC-1 could not be distinguished from the adult ALC-1. Although ALC-2 could not be detected in foetal ventricular myosin, a component co-migrating with ALC-2c, the major adult ALC-2 component, was present in foetal whole ventricular myocardium. This may resemble the situation observed by Rubinstein *et al.* (1977) during the development of slow skeletal muscle, where a transition from the synthesis of fast to slow skeletal muscle was observed. During this period, fast-muscle myosin light chains were lost at different rates, with fast-muscle LC-1 being the last to disappear. It was also noted that when both fast- and slow-muscle myosin light chains were present, it was the combined and not the individual LC-1 and LC-2 populations that were equivalent. This is in agreement with the results obtained here for foetal ventricular myosin, where the sum of VLC-1 and

FLC-1 equalled the total VLC-2. Moreover, there may be a close relationship between atrial and fast-skeletal-muscle myosin on the one hand and ventricular and slow skeletal muscle on the other (Libera *et al.*, 1979). This has been suggested by the work of Sartore *et al.* (1978), where cross-reaction of chicken fast-skeletal-muscle myosin antibodies with atrial but not ventricular myosin was demonstrated. Atrial and fast-skeletal-muscle myosin are both characterized by the possession of higher Ca²⁺-activated ATPase activities relative to ventricular and slow-skeletal-muscle myosin respectively (Long *et al.*, 1977; Dreizen & Richards, 1972). Whalen *et al.* (1978) have also shown that the LC1_s and LC2_s light chains of slow skeletal muscle co-migrate exactly with the two cardiac, presumably ventricular, myosin light chains on two-dimensional gel electrophoresis.

Further support for the suggestion that FLC-1 and ALC-1 may be closely related comes from the studies on adult atrial light-chain composition. The presence of components, particularly in the left atrium, that could not be distinguished from VLC-2 components when considered together with the changes seen during development, suggests a possible scheme of myosin gene expression. During the very early stages of development, the primitive cardiac tube may synthesize predominantly only one type of myosin, possibly an atrial-related type. Under the stimulus of increasing pressures, wall tension and therefore workload experienced by the newly developing ventricular chambers, ventricular myosin might be synthesized. Although the stimulus is unlikely to be neural, as complete development of cardiac innervation in man occurs between the fourth and sixth postnatal months (Hirsch, 1970), an influence of circulating hormones on cardiac differentiation cannot be excluded. Certainly, marked changes in myosin heavy-chain isoenzymes are seen in adult animals under the influence of thyroid hormone (Hoh & Egerton, 1979) and neural influences may be involved in the disappearance of FLC-1 in the postnatal period.

If a transition from an atrial to a ventricular myosin type occurs during development, it is possible that, given the correct stimulus, similar changes could occur in the adult. This could be the explanation for the apparent appearance of 'ventricular-like' LC-2 components in the adult atria. Moreover these were more prominent in the left atrium and might be the result of the generally increasing peripheral resistance and arterial pressures experienced with age (Bakerman, 1969). Changes in myosin gene expression in later life coupled with altered mechanical properties have been observed in the slow skeletal muscles of senescent rats (Syrový & Gutmann, 1977). Sartore *et al.* (1978) have also reported that a minor

proportion of muscle cells in the adult chicken atria react not only with anti-(fast-skeletal-muscle myosin), but also with anti-(ventricular myosin) antisera. The precise nature of these 'ventricular-like' components in the atria and the question of whether they are present throughout adult life or are related to a later physiological change must await further work.

Whatever the precise time course and stimuli of the above changes, the physiological consequences of changes in myosin gene expression in the atria and ventricles must play an important role in the functional adaptation of the myocardium.

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