Transitions in human atrial and ventricular myosin light-chain isoenzymes in response to cardiac-pressure-overload-induced hypertrophy

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1. The light-chain subunits of human atrial and ventricular cardiac muscle were examined by two-dimensional polyacrylamide-gel electrophoresis and limited proteolytic digestion. The light-chain patterns in the normal right and left atria were identical.
2. Myosin preparations isolated from right or left atria that had been subjected to cardiac-pressure-overload-induced hypertrophy also contained ventricular light-chain subunits. These were identified by peptide mapping in sodium dodecyl sulphate.
3. Ventricular light chain-2 was the major species in hypertrophied atria, although light chain-1 subsequently appeared in severe pressure-overload-hypertrophied cases. Evidence is presented for the existence of more than one form of ventricular light chain-2.
4. The transition from atrial to ventricular myosin light chains correlated with the degree of pressure-overload hypertrophy in 83 examples of surgically excised atria.
5. The adult atrial light chain-1 was shown to be homologous to the human foetal ventricular light chain-1 [Price, Littler & Cummins (1980) *Biochem. J.* **191**, 571–580] by peptide mapping.
6. A scheme of atrial/ventricular myosin light-chain isoenzyme transitions is discussed in relation to changing contractile properties in cardiac muscle, together with implications for the role of light-chain subunits.

Transitions in myosin isoenzymes in response to different stimuli and functional requirements have been investigated in a number of muscle types from different species and have involved both the heavy- and light-chain subunits. Such studies may throw light on both the mechanism of functional molecular response and the role of the different myosin subunits.

In skeletal muscle, fast and slow isoenzymes of myosin have been shown to change during neural cross-reinnervation (Buller *et al.*, 1969; Barany & Close, 1971), chronic stimulation (Sreter *et al.*, 1973) and alterations in thyroid status (Ianuzzo *et al.*, 1977). Myosin isoenzymes characteristic of embryonic, neonatal and adult tissues are produced sequentially during the development of skeletal muscle (Hoh & Yeoh, 1979; Whalen *et al.*, 1978, 1981) and may be related to changes in hormonal and neural influence. However, the precise role of these heavy- or light-chain isoenzymes is not clearly understood.

In the heart, transitions in heavy-chain isoenzymes of myosin have been studied after hypophysectomy (Hoh *et al.*, 1978), thyrotoxicosis (Hoh *et al.*, 1979), and in particular after acute and chronic, volume- and pressure-overload-induced hypertrophy (Lompre *et al.*, 1979; Klotz *et al.*, 1981). The con-

trasting increase and decrease in myofibrillar and myosin ATPase activities after thyrotoxicosis and chronic pressure overload respectively (Katagiri *et al.*, 1975; Yazaki & Raben, 1975; Alpert *et al.*, 1979) has been shown to be the result of changes in ventricular (V_1 , V_2 and V_3) isoenzymes of myosin that differ in heavy chain [$(HC_\alpha)_2$, $HC_\alpha HC_\beta$, and $(HC_\beta)_2$ respectively], but not, apparently, light chain (Hoh *et al.*, 1979), composition. Such changes have been observed in the rat (Klotz *et al.*, 1981) and rabbit (Hoh *et al.*, 1978), but not to date in man, where there is a predominance of only one (V_3) myosin heavy-chain isoenzyme (R. Janes & P. Cummins, unpublished work).

In addition to these different ventricular forms of myosin, the atrial and ventricular chambers of the heart have also been shown to contain distinct myosin isoenzymes that differ in heavy- and/or light-chain subunits in the dog (Long *et al.*, 1977), rabbit (Dalla Libera & Sartore, 1981), bovine (Flink *et al.*, 1978) and human (Cummins *et al.*, 1980a; Price *et al.*, 1980a) myocardium.

A characteristic feature of ventricular myosin in the mammalian foetal heart is the presence of a foetal or embryonic myosin light chain (Cummins *et al.*, 1980b; Price *et al.*, 1980a; Whalen & Sell, 1980) that has been likened in man to

an adult atrial light chain (Price *et al.*, 1980a). The disappearance of this foetal light chain during development and its gradual replacement by the adult ventricular form has led to the hypothesis (Price *et al.*, 1980a) that atrial/ventricular transitions in myosin type may play an important role in the functional adaptation of the myocardium, as in the response to changing pressure gradients characteristic of cardiac pressure-overload-induced hypertrophy. Subsequent evidence (Dalla Libera & Sartore, 1981 for ventricular-to-atrial-like heavy-chain transitions in the thyrotoxic rabbit ventricle have lent some support to this hypothesis. Although the different enzymic properties of atrial and ventricular myosin (Long *et al.*, 1977; Flink *et al.*, 1978; Syrový *et al.*, 1979) may correlate in part with the changing physiological demands of cardiac muscle in these conditions (Alpert *et al.*, 1979), possible structural differences in myosin isoenzyme molecules could also be an important factor in functional adaptation.

In the present investigation, transitions in myosin light-chain isoenzymes in response to cardiac-pressure-overload-induced hypertrophy were studied in the human atrium. It is demonstrated that, although only atrial myosin light chains are present in the normal adult right and left atria, these are replaced by ventricular myosin light chains (particularly ventricular light chain-2 and a related form of this light chain) when cardiac blood pressure, and presumably wall tension, increases and subsequent hypertrophy occurs. The degree of isoenzyme transition is shown to be related to the extent of cardiac pressure overload in a large number of individuals. Evidence is presented for the identification of these ventricular light chains, together with a comparison of the adult atrial light chain-1 and foetal ventricular light chain (Price *et al.*, 1980a). These latter two light chains are shown to be homologous, and possibly identical, and lend support to a unifying hypothesis of myosin light-chain transitions in the human myocardium in response to increased pressure gradients. The possible role of myosin light-chain subunits is also discussed.

Some aspects of this work have been briefly reported elsewhere (Cummins & Soars, 1981; Cummins, 1982).

Materials and methods

Tissue sources

Adult human myocardium was obtained either immediately after death from renal-transplant donors, with the informed consent of the relatives and coroner, or within 4 h *post mortem*. Donors were not known to be suffering from any cardiac abnormality, which was confirmed in the post mortem.

Human foetal myocardium was obtained as de-

scribed previously (Price *et al.*, 1980a). Surgically excised biopsy specimens from adult atria were kindly provided by Mr. D. B. Clarke and Mr. L. D. Abrams, Queen Elizabeth Hospital, Birmingham, U.K. Atrial appendages were excised primarily in order to facilitate the surgical cardiac by-pass procedures and not for any pathological abnormality *per se*. Right- and left-atrial biopsies were each classified into three groups, depending on the degree of cardiac pressure overload and hypertrophy. This was done on the basis of clinical condition, haemodynamic pressure data and surgical observation of degree of atrial hypertrophy (see the Appendix). The groups were as follows: (i) normal right atria ($n = 26$); (ii) normal left atria ($n = 4$); (iii) right atria with mild to moderate cardiac pressure overload and hypertrophy ($n = 20$); (iv) left atria with mild to moderate cardiac pressure overload and hypertrophy ($n = 12$); (v) right atria with moderate to severe cardiac pressure overload and hypertrophy ($n = 13$); (vi) left atria with moderate to severe cardiac pressure overload and hypertrophy ($n = 8$). All tissue samples were frozen, and stored at -25°C as soon as possible after excision, if not used immediately.

Preparation of myosin

Myosin was prepared as described previously (Price *et al.*, 1980a).

Preparation of crude myosin extracts

Crude myosin extracts from 250–500 mg of tissue were prepared as described by Price *et al.* (1980a), except that the final crude myosin pellet was dissolved in a minimum volume of 9 M-urea/0.75 M-2-mercaptoethanol and the solution was used directly for isoelectric focusing.

Electrophoresis

Isoelectric focusing was performed by the method of O'Farrell (1975) over the pH range 4–6 as described previously (Price *et al.*, 1980b).

Two-dimensional polyacrylamide-gel electrophoresis in the presence of 0.1% (w/v) sodium dodecyl sulphate was carried out in both 1.5 mm- and 3.0 mm-thick slab gels (Price *et al.*, 1980b). The thicker gels were used when protein spots, containing up to approx. 60 μg of protein, were required for proteolytic digestion.

Peptide mapping

Peptide mapping after limited proteolysis in the presence of sodium dodecyl sulphate was based on the method of Cleveland *et al.* (1977). Protein-containing spots from two-dimensional gels were cut out, after partial staining and destaining, agitated gently for 20 min in 0.125 M-Tris/HCl (pH 6.8)/0.1% sodium dodecyl sulphate/1 mM-EDTA, and

loaded on a 15% (w/v)-polyacrylamide gel incorporating a 3% (w/v) stacking gel. Samples for digestion were overlaid directly with a solution of *Staphylococcus aureus* V8 proteinase (Miles Laboratories, Slough, Berks., U.K.), at a proteinase/substrate molar ratio of 0.4. Electrophoresis was performed as described by Cleveland *et al.* (1977).

Gel-scanning densitometry

Isoelectric-focusing gels were densitometrically scanned with either a Gilford model 2400 recording spectrophotometer fitted with a Gilford model 2410 gel-scanning attachment or a Cecil Instruments model CE5095 spectrophotometer with gel-scanning attachment. Areas under the scanned peaks were quantified by using a Kontron image analyser.

Results

Myosin preparations from normal adult human right and left atria were obtained from two major

tissue sources. Large-scale preparations (up to 60mg of pure myosin from 10g of tissue) were prepared from fresh tissue obtained from renal-transplant donors. Small-scale preparations (between 50 and 150 μ g of pure myosin from 250–500mg of tissue) were obtained from biopsy specimens excised during cardiac surgery. These surgical atrial specimens were classified as normal on the basis of criteria outlined in the Materials and methods section. Myosin preparations from both large- and small-scale sources gave identical results.

When examined by isoelectric focusing in the pH range 4–6, right- and left-atrial myosin preparations gave identical electrophoretic patterns [Fig. 1a, gels (i) and (iv)]. Subsequent two-dimensional electrophoretic separation on the basis of molecular-weight differences (see below) enabled identification of light chain-1 and light chain-2 species. Both atrial light chain-1 (ALC-1) and the different components of atrial light chain-2 (ALC-2) could be clearly dis-

Fig. 1. Isoelectric focusing of pure and crude myosin preparations from right and left atria

Isoelectric focusing in 4.0% (w/v) polyacrylamide, in the presence of 9.2M-urea, in the pH range 4–6 was performed as described in the Materials and methods section. (a) Pure myosin preparations; (b) crude myosin extracts. (i) Normal right-atrial myosin; (ii) right-atrial myosin from patient with moderate to severe cardiac overload; (iii) left atrial myosin from patient with moderate to severe cardiac overload; (iv) normal left-atrial myosin.

tinguished. The latter light chain gave a similar pattern to that described previously for the ventricular light chain-2 (Price *et al.*, 1980a) when examined under identical conditions. The major components of ALC-2 were previously designated as ALC-2c, ALC-2d and ALC-2e in order of increasingly acidic pI, with the ALC-2d component often appearing as a very closely spaced doublet (Price *et al.*, 1980a). The doublet was not well resolved under these isoelectric-focusing conditions and required Ampholine carrier ampholytes of a narrower pH range for improved separation (P. Cummins, unpublished work). Phosphorylation and dephosphorylation of pure atrial myosin preparations (see the Materials and methods section) with a crude cardiac myosin light-chain kinase preparation (P. Cummins, unpublished work) indicated that ALC-2c and the more alkaline band in the ALC-2d doublet were dephosphorylated forms of the acidic band in the ALC-2d doublet and ALC-2e respectively. On this basis, and using the nomenclature proposed by Frearson & Perry (1975), species ALC-2c, ALC-2d doublet and ALC-2e have been designated ALC-2, ALC-2*, ALC-2P and ALC-2P* respectively as for ventricular myosin light chain-2 in this system (cf. Fig. 5) (Cummins *et al.*, 1980a; Price *et al.*, 1980b). The possible nature of the ALC-2* (and VLC-2*) components is discussed below.

In many instances, the size of specimen excised during cardiac surgery (less than 50 mg wet wt.) excluded the possibility of preparing pure myosin. In these cases, crude myosin extracts were prepared (see the Materials and methods section) and the resultant pellet was dissolved in 9 M-urea/0.75 M-2-mercaptoethanol for isoelectric focusing. Although the pattern was obviously more complicated, it was still possible to identify the ALC-1, ALC-2, ALC-2*, ALC-2P and ALC-2P* components in the normal right and left atria [Fig. 1b, gels (i) and (iv)], although the ALC-2P* component co-migrated near to one of the bands of tropomyosin. Identification of these atrial light-chain components in crude extracts was further aided by electrophoresis in the second dimension (see below).

When either crude or pure myosin preparations were examined from right- and left-atrial specimens derived from patients with moderate to severe atrial pressure overload and hypertrophy, additional components were seen [Fig. 1a, gels (ii) and (iii); Fig. 1b, gels (ii) and (iii)]. These were previously designated ALC-2a and ALC-2b on the basis of their relative migration positions on isoelectric-focusing gels (Price *et al.*, 1980a), but, for reasons discussed below, these will now be designated VLC-2 and VLC-2* respectively. The intensity of these additional atrial components, particularly VLC-2*, appeared to be significantly greater in atrial specimens

from the moderate to severe atrial overload group than in the mild to moderate overload group. This was especially noticeable in left-atrial specimens. Although VLC-2 was usually a faint band in the crude extracts, and was often masked by non-myosin components, it could be clearly seen in pure preparations, although in all cases it was the minor component relative to VLC-2*. Addition of proteinase inhibitors during preparation of both pure and crude myosin samples had no effect on the electrophoretic patterns.

VLC-2 and VLC-2* components from pressure-overloaded right- and left-atrial specimens co-migrated precisely with the corresponding ventricular myosin light-chain components isolated from normal ventricular myocardium, in both isoelectric-focusing gels (results not shown; cf. Price *et al.*, 1980a) and on two-dimensional electrophoresis (Fig. 2). Atrial and ventricular myosin preparations both give rise to two rows of horizontal components corresponding to the LC-1 and LC-2 light-chain subunits (Figs. 2a, 2b and 2c). The LC-2* and LC-2P components migrated close together in the second dimension, so that three spots were seen for LC-2 in normal right- (Fig. 2a) and left-atrial (Fig. 2b) and normal ventricular specimens (Fig. 2c). Co-migration of atrial and ventricular myosins clearly demonstrated the non-identical nature of their respective light chains (Fig. 2d). Myosin preparations from moderate-to-severe-pressure-overloaded left-atrial specimens were examined under the same conditions (Fig. 2e). In addition to ALC-1 and ALC-2 components, VLC-2* was seen to be a major component and VLC-2, VLC-2P and VLC-2P* were also present. Moreover, in those atrial preparations where VLC-2 and VLC-2* were major components, there appeared to be a corresponding decrease in the amount of ALC-2 relative to normal atrial myosin. Although VLC-2P and VLC-2P* were difficult to detect on isoelectric focusing of cardiac-overloaded atrial specimens, owing to the difficulty in resolving closely spaced bands, the phosphorylated forms could be clearly seen on second-dimension gels (Figs. 2e and 2f).

Confirmation of the relationship between ALC-2a/-2b and VLC-2/-2* was obtained by peptide mapping after limited proteolysis in sodium dodecyl sulphate (Cleveland *et al.*, 1977). The VLC-2* component from a second-dimension gel of severe-pressure-overloaded left-atrial myosin was cut out and compared with the VLC-2* spot from a normal ventricular myosin preparation. The protein components in the gel slices were digested directly by using an overlay solution of *Staphylococcus aureus* V8 proteinase at a proteinase/substrate molar ratio of 0.4 as described in the Materials and methods section. The resultant peptide maps are shown in Fig. 3(a). Both atrial VLC-2* [Fig. 3a, gel (ii)] and

Fig. 2. Two-dimensional polyacrylamide-gel electrophoresis of right- and left-atrial and -ventricular myosin. Isoelectric focusing and two-dimensional analysis of pure myosin preparations in the presence of 0.1% sodium dodecyl sulphate were performed as described in the Materials and methods section. (a) Normal right-atrial myosin; (b) normal left-atrial myosin; (c) normal ventricular myosin; (d) normal right- and left-atrial and -ventricular myosins; (e) left-atrial myosin from patient with moderate to severe pressure overload; (f) left-atrial myosin from patient with severe pressure overload and 56% transition in myosin light-chain type. For clarity, only the major light-chain species are labelled. The arrow indicates the VLC-2* component.

ventricular VLC-2* [Fig. 3a, gels (i), (iv)] gave identical peptide patterns, which were clearly different from that from ALC-2* [Fig. 3a, gel (iii)]. Atrial VLC-2 and ventricular VLC-2 also gave similar patterns to atrial and ventricular VLC-2* (results not shown), although, as only small amounts of the atrial VLC-2 were present, the peptide patterns were faint.

As these results were suggestive of an atrial-to-ventricular myosin light-chain transition in the pressure-overloaded atrial chamber, an attempt was made to quantify this transition in relation to the degree of overload. The amount of VLC-2 plus VLC-2* in both right- and left-atrial specimens was quantified by densitometric analysis of isoelectric-focusing gels and expressed as a percentage of total atrial plus ventricular LC-2 components. This was done for the three patient groups as outlined in the Materials and methods section. The results (Fig. 4 and Table 1) show that, in both normal right- and left-atrial specimens, either little or no transition had occurred. In cases of mild to moderate atrial overload, a 6.3% transition from atrial to ventricular LC-2 was seen in the left atria, but no marked

difference was seen in the right. However, in patients with moderate to severe overload, transitions were seen in both right and left atria, the transition being far greater in the left-atrial preparations (mean = 27.1%). The number of specimens excised from the right atria exceeded that from the left, for technical reasons associated with the clinical conditions and surgical procedures involved.

In the majority of the 83 patients studied, the biopsy specimens were excised as either a part or the whole of the atrial appendage and not the atrial free wall. Although the atrial chamber, and therefore pressures, are continuous throughout the lumen of the atrial appendage, the tensions in the appendage and free wall may well differ as a result of the anatomical shape of the structures (see the Discussion section). In one patient in the moderate-to-severe cardiac-overload group, specimens were obtained from both the left atrial appendage and left free atrial wall. The light-chain transition was higher (38.2%) in the free wall than in the appendage (22.9%). The highest transitions observed (35% and 56%) occurred in left-atrial specimens from two patients in the moderate-to-severe overload group. Electrophoretic

Fig. 3. *Peptide mapping of adult atrial and ventricular and foetal ventricular myosin light chains*
Peptide mapping of myosin light chains eluted from gel slices was performed as described by Cleveland *et al.* (1977), with *Staphylococcus aureus* V8 proteinase at a proteinase/substrate molar ratio of 0.4 (see the Materials and methods section). (a): Gels (i) and (iv), VLC-2* from normal adult ventricle; gel (ii), VLC-2* from cardiac-overloaded left atrium; gel (iii) ALC-2* from normal left atrium. (b): Gel (i) VLC-1 from normal adult ventricle; (ii) ALC-1 from normal adult right atrium; (iii) FLC-1 from normal foetal ventricle; (iv) ALC-1 from normal adult left atrium.

analysis of the light-chain composition in these patients (Fig. 2f) indicated not only the presence of VLC-2 and VLC-2* and decrease in ALC-2 components but also the appearance of a component co-migrating with VLC-1. In all patients studied, irrespective of the percentage transition, electrophoretic analysis gave approximately equimolar amounts of total LC-1 and LC-2, assuming equivalent molar dye uptake by both types of light chains.

This evidence for an atrial-to-ventricular transition in myosin light-chain type in response to increased pressure load, and therefore wall tension, was originally suggested on the basis of observations made in the developing human foetal ventricle (Price

Fig. 4. *Transition in myosin light-chain type in response to cardiac overload*

Transition from atrial to ventricular light chain is expressed as displayed in the legend to Table 1. Densitometric analysis of atrial myosin preparations was carried out as described in the Materials and methods section. (a), (b) and (c), right-atrial specimens; (d), (e) and (f), left-atrial specimens; (a) and (d), normal atria; (b) and (e), mild to moderate cardiac-overload atria; (c) and (f), moderate to severe cardiac-overload atria. For clarity, the values in columns (a) and (b) represent numbers of zero values.

Fig. 5. Schematic representation of isoelectric-focusing gels of adult right- and left-atrial and adult and foetal ventricular myosins (a) Normal foetal ventricular myosin; (b) normal adult ventricular myosin; (c) myosin from severely cardiac-overloaded adult, right or left atria; (d) normal adult right or left atria.

Table 1. Atrial-to-ventricular myosin light-chain transitions in response to cardiac overload
Results [means ± s.d. (n)] are expressed as percentages:

Transition (%) = 100 × $\frac{\text{VLC-2} + \text{VLC-2}^*}{\text{Total ALC-2} + \text{VLC-2}}$

Values were estimated by densitometric scanning of isoelectric-focusing gels.

Degree of cardiac overload	Right atria	Left atria
Normal	0.27 ± 0.9 (26)	0 ± 0 (4)
Mild to moderate	0.51 ± 1.0 (20)	6.3 ± 6.7 (12)
Moderate to severe	3.04 ± 4.1 (13)	27.1 ± 14.7 (8)

et al., 1980a). During development, a foetal ventricular myosin light chain (FLC-1) was observed, which gradually disappeared in the immediate post-natal period to be replaced by the adult ventricular light chain-1. The foetal light chain co-migrated on isoelectric focusing and two-dimensional electrophoresis with atrial LC-1, suggesting an atrial-type to ventricular myosin transition occurring as a result of changing cardiac pressure gradients.

Evidence that FLC-1 and ALC-1 are homologous and possibly identical was obtained by peptide analysis (Cleveland *et al.*, 1977) as described above (Fig. 3b). Both adult right or left ALC-1 [Fig. 3b, gels (ii) and (iv)] and foetal FLC-1 [Fig. 3b, gel (iii)]

gave identical patterns when digested with *Staphylococcus aureus* V8 proteinase at a proteinase/substrate molar ratio of 0.4. This pattern was clearly different from that obtained with adult VLC-1 [Fig. 3b, gel (i)], although there were similarities, as might be expected for related classes of myosin light chains.

On the basis of these results, a schematic representation of the atrial and ventricular myosin light-chain components on isoelectric-focusing gels is presented in Fig. 5.

Discussion

The present study indicates that a major factor responsible for the presence of ventricular myosin light chains in the human atria (Price *et al.*, 1980a) is the degree of pressure-overload-induced hypertrophy. The atrial-to-ventricular transition involved predominantly LC-2, even in severely hypertrophied atria, where both VLC-1 and VLC-2 were present. A differential transition rate was also apparent for the different forms of VLC-2. VLC-2* appeared first, whereas VLC-2 never appeared to exceed the amount of VLC-2* in hypertrophied atria.

The LC-2* (satellite) forms of ventricular LC-2 were originally thought to arise during preparation (Fearnson & Perry, 1975). By using two-dimensional analysis of fresh rabbit ventricular homogenates, Westwood & Perry (1981) have suggested that VLC-2* and VLC-2P* [P2 and P4 respectively in the Westwood & Perry (1981) nomenclature] may be different proteins from VLC-2 and VLC-2P (P1

and P3) light chains. Previous results (Price *et al.*, 1980b) and the early appearance of VLC-2* in hypertrophied atria in the present study lend support to the existence of two forms of phosphorylatable light chain. Chicken cardiac myosin has recently been shown to contain LC-2A and LC-2B light chains differing in primary sequence (Matsuda *et al.*, 1981). The similarity in pattern of atrial and ventricular human myosin light chains on isoelectric focusing suggests two forms of LC-2 are present in all four cardiac chambers.

Transitions in atrial and ventricular myosin isoenzymes due to changing cardiac pressure gradients, wall tension and therefore workload were originally proposed (Cummins *et al.*, 1980a; Price *et al.*, 1980a) on the basis of transitions in light chains in the developing human foetal ventricle. Evidence for atrial-like heavy-chain isoenzymes in thyrotoxic rabbit ventricle has been obtained by Dalla Libera & Sartore (1981) and Chizzonite *et al.* (1981). Although an oppositely directed ventricular-to-atrial heavy-chain transition could be occurring in the present study, several factors must be considered when comparing the thyrotoxic ventricle with the hypertrophied atria. The different direction of the transition in the two models is best explained by comparing the contractile properties of pressure-overloaded and thyrotoxic myocardium. Alpert *et al.* (1979) have elegantly analysed actin-myosin cross-bridge kinetics in terms of muscle mechanics and myosin ATPase activity and demonstrated that pressure-overload-hypertrophied muscle is adapted for slow, economical tension development. The molecular adaptation in thyrotoxic myocardium, conversely, adapts it for rapid, but less economical, tension development. As atrial actin-activated and myosin ATPase activities are significantly higher than ventricular activities (Yazaki *et al.*, 1979), an atrial-to-ventricular transition in pressure-overload hypertrophy and ventricular-to-atrial transition in thyrotoxic myocardium is compatible with the above analysis. Transitions could presumably occur in all cardiac chambers depending on the stimulus.

The involvement of heavy or light chains, or both, in a transition may also depend on the size of the animal. In small-animal ventricles (e.g. rat), three heavy-chain myosin isoenzymes exist (V_1 , V_2 , V_3 , in order of decreasing myosin Ca^{2+} -ATPase activity), the proportions changing in response to both pressure-overload hypertrophy and thyrotoxicosis (Lompre *et al.*, 1979; Hoh *et al.*, 1978). However, in larger mammals, (e.g. dog, cow, baboon and man), only the lowest-ATPase-activity form, V_3 , is present (R. Janes & P. Cummins, unpublished work). As ventricular myofibrillar ATPase activity is decreased, however, in patients with cardiac-overload hypertrophy (Alpert & Gordon, 1962), it seems difficult to explain this decrease in terms of

the current heavy-chain-isoenzyme hypothesis (Lompre *et al.*, 1979). Examination of both light- and heavy-chain transitions in larger animals may be necessary.

The light-chain response in the present study was greatest in the left atria, presumably owing to the high systemic pressures and wall tension compared with the lower pulmonary pressures on the right side of the heart. Moreover, the transition in the free atrial chamber wall was almost double that in the appendage. Although the lumens of the atrial appendage and atrial chamber are continuous, the wall tensions may be lower in the somewhat elongated appendage. This could explain the difference if the response is due to increased wall tension, possibly mediated via stretch receptors. The variability in transition in patient groups may be due to the difficulty in establishing the time of onset of the pathological condition. Other factors, such as normal anatomical variation and increasing peripheral resistance with age, could be involved (Price *et al.*, 1980a). New forms of myosin heavy chains in mechanically overloaded rat hearts are similar to those in normal hearts of older rats (Klotz *et al.*, 1981).

The atrial-to-ventricular transition in the atria appears similar to that occurring in the developing human foetal ventricle (Price *et al.*, 1980a; Cummins *et al.*, 1980a). Peptide analysis demonstrated that the foetal light chain-1, which is replaced by adult VLC-1, is homologous and may be identical with adult atrial LC-1. A foetal or embryonic light chain is also present in foetal skeletal and ventricular rat muscles and in the myogenic rat cell line L6 (Whalen *et al.*, 1978; Whalen & Sell, 1980). However, the existence of a foetal light-chain type in adult atrial muscle may be related to the suggestion (Rumyantsev, 1977) that phylogenetically the atria may be more primitive than the ventricles and other striated muscles in general (Cummins, 1982).

Whatever the sequence of events between development of pressure-overload hypertrophy and transitions observed in the present study, they may throw light on the possible role of the light-chain subunits. Although heavy-chain transitions cannot be excluded here, recent evidence (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982) suggests that light chains are not essential for actin binding or ATP hydrolysis in skeletal muscle, both sites being located on the heavy chains. Could the light chains be involved in a tension-developmental role, possibly by affecting the flexibility of the myosin hinge region? The present results would suggest that LC-2 light chains may play a more important role in this respect. This light-chain class is essential for control of tension development in scallop muscle fibres, although not for tension development *per se* (Simmons & Szent-Gyorgyi,

1980). One model proposed by Walliman & Szent-Gyorgyi (1981) locates the related classes of both light chains in scallop myosin in the hinge region. Further studies on the light-chain composition of chemically skinned muscle fibres in parallel with measurements of tension development are required to test this theory.

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APPENDIX

Criteria for establishing degree of cardiac pressure overload and hypertrophy of atrial biopsy specimens

Group I: normal atria

Patients in this group had normal left-ventricular function, with a pulmonary capillary wedge pressure (effective left atrial pressure) < 12 mmHg (normal left-atrial pressure = 4–12 mmHg), and a left-

ventricular end diastolic pressure < 12 mmHg (normal left-ventricular end diastolic pressure = 4–12 mmHg). Right-atrial pressures were less than 6 mmHg (normal right-atrial pressure = 0–6 mmHg). No signs of atrial hypertrophy were seen at surgery. The majority of patients in this group were under-

going surgery for coronary-artery disease and had no right- or left-atrial pathology.

Group II: mild to moderate atrial-pressure overload and hypertrophy

Atria in this group came from patients with effective left-atrial pressures of 12–24 mmHg and/or right-atrial pressures of 6–12 mmHg. Left-ventricular end diastolic pressures ranged between 14 and 16 mmHg. Mild to moderate enlargement of atrial appendages was observed at surgery. These patients had a variety of conditions, including mild aortic, mitral and tricuspid-valve disease.

Group III: moderate to severe atrial-pressure overload and hypertrophy

These atrial biopsies were excised from patients with effective left-atrial pressures greater than 24 mmHg at rest, right-atrial pressures in excess of 12 mmHg and left-ventricular end diastolic pressures greater than 16 mmHg at rest. Patients in this group had moderate to severe valve disease, including pulmonary hypertension and tricuspid stenosis, giving rise to severe right-atrial pressure overload, or severe mitral and aortic valve disease, which resulted in marked left-atrial pressure overload. All atrial appendages were grossly enlarged at surgery.