Local Diversity of Myosin Expression in Mammalian Atrial Muscles

Variations Depending on Age and Thyroid State in the Rat and the Rabbit

Claude A. Dechesne, Jocelyne Léger, Patrice Bouvagnet, Helga Mairhofer, and Jean J. Léger

From the Institut National de la Sante et de la Recherche Médicale, Unité 249, Laboratoire de Pathologie Moléculaire du Muscle, Institut de Biologie, Montpellier, France

SUMMARY. Rat, rabbit, pig, and bovine atrial myocardia were investigated with anti-α and anti-β myosin heavy chain monoclonal antibodies. Analysis of atrial fibers by indirect immunofluorescence and assay of myosin heavy chains in tissue micro samples by immunoaffinity chromatography revealed both heterogeneity and plasticity in the atrial myosin heavy chains, undetected by electrophoresis of native atrial myosins under nondenaturing conditions. We found both α- and β-like myosin heavy chains to be expressed in rat and rabbit, as they are in pig and bovine atrial myocardia. They were regionally distributed within atrial muscles. The β-like myosin heavy chains were present at much lower levels in rat and rabbit atria than in pig and bovine atria. Young rat atrial myosin was composed of only α-like heavy chains. In the rat and the rabbit, hyperthyroidism induced a β- to α-like myosin heavy chain transition, which was considerable in the right atria and complete in the left atria. In the rat, thyroidectomy induced a moderate α- to β-like myosin heavy chain transition, visible in the left atria. The significance of this atrial myosin heavy chain polymorphism is discussed in relation to the existence of anatomical localizations of the two myosin variants. (Circ Res 57: 767-775, 1985)

TWO molecular variants of myosin heavy chains are found within the atria of bovine (Gorza et al., 1982) and human hearts (Gorza et al., 1984; Bouvagnet et al., 1984; Tsuchimochi et al., 1984; Yazaki et al., 1984). Both molecular variants of myosin heavy chains identified in human atria have been separated and some of their biochemical features characterized (Dechesne et al., 1985). On the basis of immunological and structural analogies with the rat ventricular α- and β-myosin heavy chains (Hoh et al., 1979), the two myosin variants appeared to be composed of α- and β-like heavy chains, respectively. The most striking result of the immunofluorescence studies of bovine and human atria is that atrial fibers contain variable proportions of α- and β-myosin heavy chains, and that the fibers containing only either α- or β-myosin heavy chains are preferentially found in certain regions of normal atria. This heterogeneous local myosin distribution has been found to vary in response to mechanical overloading in human hearts (Gorza et al., 1984; Tsuchimochi et al., 1984; Yazaki et al., 1984; Bouvagnet et al., 1985); thus it appears to be of great functional significance in bovine and human atria, and should be taken into consideration in further studies of myosin expression and the environmental factors controlling it.

The presence of only α- or of both α- and β-myosin heavy chains in the atrial cells of small animals is still under discussion, probably because of the three following points. First, the α-form of atrial myosin heavy chains has long been recognized as being predominant, if not unique, in all small hearts observed (Dalla Libera and Sartore, 1981; Clark et al., 1982). Second, all studies of the atria of small animals have been done on myosins prepared from minced entire atria, and were thus unable to detect any possible subtle local variation in the myosin distribution. Third, according to Chizzonite et al. (1984), the A1 and A2 bands of rat and rabbit atrial myosins separated by native gel electrophoresis in fact result from heterogeneity in atrial myosin light chains and not from different atrial α- or β-myosin heavy chain contents. Thus, the absence of modifications, with age and thyroxine treatment, found by native gel electrophoresis of rat and rabbit atrial myosins (Hoh et al., 1978; Clark et al., 1982; Banerjee, 1983), does not provide any indication about myosin heavy chains in these situations. In spite of these experimental limitations, some studies have shown changes in atrial myosin heavy chain synthesis after thyroxine treatment of rabbits (Everett et al., 1983) and in atrial myosin adenosine triphosphatase (ATPase) activities in rats during postnatal development (Syrový, 1984). In addition, recent recombinant DNA experiments have revealed the presence of small amounts of β-myosin heavy chain messenger ribonucleic acids (mRNA) in preparations of entire atria from rats (Lompré et al., 1984) and rabbits (Sinha et al., 1984).
In this study, we used six different antimyosin monoclonal antibodies: three of them were previously found to be specific for the \( \alpha \)-myosin heavy chains in human atria and in rat ventricles; the three others were previously found to be specific for the \( \beta \)-myosin heavy chain in the same tissues. We looked for myosin diversity in different (possibly isolatable) regions of rat, rabbit, pig, and bovine atria, and for the influence of age or thyroid state on the myosin composition of rat and rabbit atria. Immunofluorescence studies were more suitable for qualitatively evaluating myosin content in atrial fibers, whereas immunoaffinity chromatography of small atrial blocks adjacent to the immunofluorescence sections allowed a more quantitative evaluation of the local amounts of each atrial myosin variant. The atria of all the mammals studied here contained both \( \alpha \)- and \( \beta \)-myosin heavy chains; the amounts of atrial \( \beta \)-myosin heavy chains were visibly much smaller in the small animals than in the large ones. Our results confirm and extend the idea that the analysis of local myosin expression is a prerequisite for the accurate description of differences among animal species and of variations in myosin types according to age or thyroid state.

**Methods**

**Animals and Tissues**

Atrial muscles were obtained from two small and two large animal species. Three-, 6-, and 12-week-old rats (Wistar males) and 1-year-old rabbits (New Zealand White males) were used in the present work. Pig and bovine hearts were obtained from slaughterhouses and were about 5 months and 4 years old, respectively. A hyperthyroid state was induced in 12-week-old rats and 1-year-old rabbits by daily injections of \( \alpha \)-thyroxine (300 microg/kg, intramuscularly) for 21 days. A hypothyroid state was induced in 12-week-old rats, which were killed 3 months after surgery. The efficiency of hyperthyroid and hypothyroid treatments was verified on rat ventricle, more than 95% of myosin heavy chains was retained on the column (unpublished observations).

**Monoclonal Antibodies**

Monoclonal antibodies (Mab) were prepared against two different human myosins, according to the method of Köhler and Milstein (1975). The three Mab that are specific for \( \beta \)-myosin heavy chains were prepared from a mouse immunized with myosin extracted from the hypertrophic left ventricle of a 53-year-old woman (Bouvagnet et al., 1984). The three Mab that are specific for \( \alpha \)-myosin heavy chains were prepared from a mouse immunized with \( \alpha \)-like atrial myosin; this myosin was purified from the left atrium of a 69-year-old man, on an immunoaffinity chromatographic column using an anti-ventricular-myosin Mab (Dechesne et al., 1985). Immunization procedures, screenings of culture wells by radioimmunoassay, cloning of hybridoma, Mab production, specificity tests of Mab by immunoblotting and electron microscopic localization of Mab epitopes have been described previously (Bouvagnet et al., 1984, 1985; Dechesne et al., 1985).

**Immunofluorescence**

Indirect immunofluorescence was performed as follows. The cryostat sections (4-6 \( \mu \)m) of frozen atrial muscles were incubated for 30 minutes at 37°C, with two or three dilutions of Mab solutions (Gorza et al., 1981). After being washed with phosphate-buffered saline, the sections were incubated for 30 minutes at 37°C, with fluorescein-labeled rabbit anti-mouse immunoglobulins (Nordic Laboratoires). All immunoglobulins were diluted in phosphate-buffered saline. Controls were performed by omitting successive steps in the procedure. The sections were examined under a Leitz Orthoplan microscope with epifluorescent optics.

**Imunoaffinity Chromatography**

Imunoaffinity chromatography using Mab has been performed as previously described (Dechesne et al., 1985), except for the following micro procedures. Atrial extracts were prepared from very small pieces of tissue, about 10 mg, adjacent to the cryostat sections used for immunofluorescence. The extracts were made in 40 \( \mu \)l of column buffer: 0.5 M sodium chloride, 20 mM sodium pyrophosphate, 50 mM Tris, 1 mM EDTA, 1 mM dithiothreitol, HQ, pH 7.5. About 10 \( \mu \)l of atrial extracts were applied on micro columns, consisting of disposable polycrylonitrile tips containing 25 \( \mu \)l of anti-\( \beta \)-Mab-coupled immunoaffinity gel. We verified that these conditions (1 \( \mu \)g of myosin/ \( \mu \)l of immunoaffinity gel) do not saturate the column. The unretracted fraction was collected in 200 \( \mu \)l of column buffer. The retained myosin fraction was eluted with 200 \( \mu \)l of 8 M urea. The unretracted fraction was either dialyzed against 20 mM sodium pyrophosphate, 50% glycerol (vol/ vol), HCl, pH 8.8, for electrophoresis under non-denaturing conditions, or was treated with 8 M urea. In the retained and 8 M-urea-unretracted fractions, both 2% sodium dodecyl sulfate and 10% 2-mercaptoethanol were added for further sodium dodecyl sulfate electrophoretic analysis.

Each immunoaffinity experiment was carried out with three to five different atria. The efficacy of the separation of two myosin types by immunoaffinity columns, made with highly specific antimyosin Mab, has been demonstrated (Dechesne et al., 1985). The efficiency of the present anti-\( \beta \)-myosin heavy chain immunoaffinity micro columns can be estimated from two different situations: with pure \( \beta \)-myosin heavy chains, extracted from thyroidectomized rat ventricle, more than 95% of myosin heavy chains was retained on the column (unpublished observation); with pure \( \alpha \)-like myosin heavy chains, extracted from thyroxine-treated rat atrium, no significant amounts of myosin heavy chains were retained on the column (see Results).
Electrophoretic Procedures

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out on 12% polyacrylamide slab gels according to the method of Laemmli (1970), and the proteins were stained with Coomassie blue. The intensities of the myosin heavy chain bands were determined by densitometric scanning at 565 nm: each value represents a mean value of three densitometric scans, and the standard deviation of this measurement is within 5%. The linearity of the densitometric scanning technique was verified between 0.5 and 12 μg of myosin heavy chains in the type of gel used. Electrophoresis of native myosins under nondenaturing conditions was performed essentially as described by Hoh et al. (1978) and D'Albis et al. (1979), except for the following modifications: the pyrophosphate buffer contained 20 mM sodium pyrophosphate, 2 mM cysteine, 1 mM EDTA, 10% glycerol (vol/vol), HCl, pH 8.8, and polyacrylamide gels were prepared with 3.88% acrylamide, 0.12% bisacrylamide in pyrophosphate buffer without cysteine or EDTA.

Results

Six monoclonal antibodies (Mab), specifically reacting with one or the other of the two myosin heavy chain variants present in human atria (Bouvagnet et al., 1984, 1985; Dechesne et al., 1985), were used for studying fiber and myosin types in the rat, rabbit, pig, and bovine atria. These Mab were selected from two larger Mab populations, obtained from mice injected either with adult human ventricular myosin or with one of the adult human atrial myosins purified by immunoaffinity chromatography using an anti-human ventricular myosin Mab. Three Mab are referred to as anti-α Mab, since they specifically reacted with the rat ventricular α-myosin heavy chains (Hoh et al., 1979); the three other Mab are referred to as anti-β Mab, since they specifically reacted with the rat ventricular β-myosin heavy chains. According to immunoblot and immunoelectron microscopic experiments, the six Mab are specific for different epitopes along the rod portion of the myosin heavy chains. The three different Mab in the same specificity set always reacted in the same way in each animal species and in each physiological or pathophysiological situation under study.

Fiber and Myosin Types in Bovine Atria

Figure 1 shows the immunofluorescence pattern of two serial sections from the bovine right auricle, treated with one anti-α Mab (Fig. 1a) and one anti-β Mab (Fig. 1b). Both sections exhibited reactive, unreactive, and intermediate fibers with either of the Mab. Some fibers stained by the anti-α Mab were totally unstained by the anti-β Mab and vice versa (see horizontal and vertical arrows in Fig. 1). This Mab complementarity in fiber staining confirms the strong specificity of each Mab set for each myosin heavy chain variant present in beef atria, and suggests concomitantly the presence of α- and β-like myosin heavy chain variants in bovine atria.

The detection of other bovine fibers variably labeled by both the anti-α and anti-β Mab (see arrowheads in Fig. 1) indicates the possible presence of variable amounts of α- and β-like myosin heavy chains within these fibers.

A quantitative evaluation, by anti-β immunoaffinity chromatography, of the amounts of myosin heavy chains not retained, i.e., composed of only α-like heavy chains, and retained on the column, i.e., composed of at least one β-like heavy chain per myosin molecule, was performed on tissue micro quantities, obtained from atrial tissue blocks adjacent to the cryostat sections used for the immunofluorescence experiments (see Methods). Figure 2 shows an example of the electrophoretic and densitometric analyses of fractions of bovine atrial myosins, separated by such a column. About 20% of the total myosin content from the bovine left auricle extracts was retained by the anti-α Mab. Horizontal arrows: a fiber reactive only with the anti-α Mab. Vertical arrows: a fiber reactive only with the anti-β Mab. Arrowheads: a fiber reactive with both the anti-α and anti-β Mab. Bar: 40 μm.
FIGURE 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and densitometric patterns of myosin heavy chain content in bovine atrial myocardium. Myosin was extracted from tissue blocks of the left auricle (panel a) and crista terminalis (panel b). For each preparation, myosin was separated in unretained (first lane) and retained (second lane) fractions on an anti-β immunoaffinity column. The retained fractions represent 20 ± 5% of the total myosin heavy chain content in the left auricle and 80 ± 5% in the crista terminalis.

Chain types: the sections from the left auricles contained small amounts of stained fibers, whereas those from the crista terminalis essentially contained only strongly stained fibers. Apart from this β-like myosin-rich region of the crista terminalis, most of the blocks from the bovine right atria were characterized by a nearly balanced mixture of myosin heavy chain amounts retained and not retained by the anti-β immunoaffinity columns and/or of fibers that were unreactive or reactive with the anti-β Mab. Bovine left atria appeared to be mainly an α-like myosin-rich region (about 65%), whereas the interatrial septum predominantly contained myosin with β-like heavy chains (60%).

Atrial Fiber and Myosin Types According to Species

Atrial myocardia from normal adult pigs, rats, and rabbits were also analyzed by the two Mab sets. Figure 3 illustrates the most representative fiber patterns obtained with one anti-β Mab applied to pig atrial sections: Figure 3a shows a cryostat section from the roof of the left atrium and Figure 3b a section from the posterior face of the right atrium. Heterogeneous staining patterns resulting from a variable mixture of reactive, unreactive, and intermediate fibers were observed, suggesting the presence of α- and β-like myosin heavy chains in the pig atria. The amount of reactive fibers was smaller in the roof of the left atrium than in the posterior face of the right atrium. The assays of myosin heavy chain types after anti-β immunoaffinity chromatography indicated a large amount of α-like myosin heavy chains in the pig left auricle (about 85%) and a large amount of myosin with β-like heavy chains in the crista terminalis of the right atrium (80%). All immunohistochemical and densitometric determinations made from eight different atrial regions within the pig atria indicate the same regional variations in terms of fiber and/or myosin contents as...
were previously observed in bovine atria. In addition, about 40% of the myosin contents in total extracts made from entire bovine or pig atrial muscles were retained on these anti-β columns.

The regional analysis of fiber and/or myosin contents in 12-week-old rat and in 1-year-old rabbit atria was limited to cryostat sections or tissue blocks prepared from entire left and right atria (Fig. 3, c and d, and Fig. 4, respectively). Since rat and rabbit fiber diameters are obviously smaller than those of pig and bovine fibers, these sections each represent a large portion of the atrial muscle, including roof and auricle. All the sections of rat and rabbit atria exhibited a mixture of reactive, unreactive, and intermediate fibers with each of the anti-β Mab (Fig. 3, c and d, and Fig. 4b), suggesting the presence of two different types of atrial myosin heavy chain. In all rat and rabbit atria analyzed, the left atrial sections had a majority of unstained fibers with a few strongly stained fibers; the right atrial sections were characterized by larger amounts of reactive fibers. In addition, the reactive and unreactive fibers were nonhomogeneously distributed within the left and right atrial sections (Fig. 3, c and d, and Fig. 4b). Figure 4a shows a cryostat section of rabbit left atrium, serially close to that shown in Figure 4b, but treated with an anti-α Mab; only strongly stained fibers were observed here. None of the rabbit or rat atrial fibers were unreactive with the anti-β Mab in any particular local situation, regardless of what dilution of anti-α Mab was used. A few fibers were, however, equally reactive with the anti-α and the anti-β Mab (see arrowheads in Fig. 4, a and b).

According to affinity chromatography experiments made on tissue blocks adjacent to these rat and rabbit sections, about 5% of the total myosin content of the left atria and about 12% of that of the right atria were retained by the anti-β columns. These values agreed with the 10% of retained myosin when extracts from entire rat or rabbit atrial muscles were applied on anti-β affinity columns.

**Atrial Fiber and Myosin Types According to Age**

Serial cryostat sections of 3- and 6-week-old rats were treated either with the anti-α or with the anti-β Mab (for 12-week-old rat atria, see Fig. 3, c and d). The left atrial sections of the 3-week-old rats were uniformly stained by the anti-α Mab (Fig. 5a) and totally unstained by the anti-β Mab (Fig. 5b); the same results were observed on the corresponding right atrial sections. None of the myosin heavy chain content in the extracts from the 3-week-old rat left atria fixed to the anti-β columns (Fig. 5c). The left atrial sections of the 6-week-old rats were also uniformly stained by the anti-α Mab (Fig. 5d), but were heterogeneously stained by the anti-β Mab (Fig. 5e). The amount of anti-β reactive fibers so detected was small but identical in all 6-week-old rat left atria analyzed; about 5% of the total myosin heavy chain content in the extracts from 6-week-old rat left atria was retained by the anti-β immunoaffinity columns (Fig. 5f). Similar results were obtained with the corresponding right atrial sections; they contained visibly more anti-β reactive fibers than the left atria. However, no significant differences between these small β-like myosin heavy chain contents could be detected by immunoaffinity chromatography of 6-week-old rat left and right atrial myosins.

**Atrial Fiber and Myosin Types According to Thyroid State**

The cryostat sections of the left atria of 12-week-old thyroidectomized rats contained a nearly balanced mixture of reactive and unreactive fibers with the anti-β Mab (Fig. 6a), indicating a clear increase in the population of reactive fibers compared to those observed in the normal rat left atria (Fig. 3c). The densitometric evaluations of the atrial myosin fractions separated by anti-β Mab affinity columns indicated that about 10% of the myosin heavy chains in the 12-week-old thyroidectomized rat left atria was bound to the columns (Fig. 6b). This increase, compared to the 5% amounts detected in the
normal rat left atria, is at the limit of statistical significance but it was found in all four left atria of the thyroidectomized rats analyzed. No change in the amounts of \( \alpha \)-reactive fibers or myosin with \( \beta \)-like heavy chains was detected in the right atria of 12-week-old thyroidectomized rats compared to those of similarly aged normal rats. All the atrial fibers in thyroidectomized rats were uniformly stained by the anti-\( \alpha \) Mab, as in normal rats (Fig. 5a).

Figure 6, c and d, show the immunofluorescence and densitometric analyses of the roof area of the left atrium from a thyroxine-treated adult rabbit, using an anti-\( \beta \) Mab: no reactive fibers were detected and no myosin heavy chains were fixed on the anti-\( \beta \) Mab column (for comparison, see, in Fig. 4b, the left atrium of a normal adult rabbit treated with an anti-\( \beta \) Mab). A visible decrease in the amounts of \( \beta \)-reactive fibers or of \( \beta \)-like myosin heavy chains was also observed in the right atria of the same thyroxine-treated rabbits. However, some rare \( \beta \)-reactive fibers could still be seen in the right atria. After fixation on an anti-\( \beta \) Mab column, the myosin heavy chain amounts were densitometrically evaluated to be less than 2% (not shown). The three anti-\( \alpha \) Mab homogeneously stained the left and right atria of the treated rabbits. Twelve-week-old rats were also treated with \( \alpha \)-thyroxine and compared to normal rats; their atria exhibited exactly the same changes in terms of fiber and myosin content as those described in the atria of normal and thyroxine-treated rabbits.

**Electrophoretic Analysis of Native Atrial Myosins**

Myosins prepared from whole atria of normal adult rabbits and pigs were applied on anti-\( \beta \) affinity columns. The electrophoretic mobilities under non-denaturating conditions and the respective proportions of A1 and A2 bands (Hoh et al., 1978) were unchanged when the rat or pig native total myosins were compared to the corresponding myosin fractions not retained by the columns (Fig. 7, lanes a and b for the rat, and lanes c and d for the pig). The denaturation of retained fractions (eluted from affinity columns by 8 M urea) prevented their analyses by this electrophoretic procedure. Native myosins from whole normal adult rabbit and bovine atria are shown as controls (Fig. 7, lanes e and f). No difference in the mobilities or in the proportions of the bands could be detected between myosins prepared from the different regions of pig or beef atria.

**Discussion**

These results show the concomitant expression of two immunologically (and presumably structurally) different forms of atrial myosin heavy chain in rat, rabbit, pig, and bovine hearts. The two atrial myosin heavy chain variants seen here can be designated as \( \alpha \)-like and \( \beta \)-like myosin heavy chains, since they are distinguished by two sets of three Mab specific for rat ventricular \( \alpha \) or \( \beta \)-myosin heavy chains (Bouvagnet et al., 1984, 1985). These atrial \( \alpha \)- or \( \beta \)-myosin heavy chains are expressed in a complementary but variable way in the different regions of the atria; this local diversity in myosin expression depends on the age and/or on the thyroid state of the animal. The total amounts of atrial myosin heavy chains retained on the anti-\( \beta \) immunoaffinity columns varied between 0 and 12 ± 5% in the small animal atria and between 20 ± 5% and 80 ± 5% in the large animals. However, this very sensitive quantitative approach cannot distinguish the proportions of \( \beta \beta \) homodimers and \( \alpha \beta \) heterodimers in the retained myosin heavy chains: further investigations are needed to quantitate the heterodimer amounts.
Our observations extend to small mammalian hearts previous works demonstrating the expression of two different atrial myosin heavy chain variants in beef and human hearts (Gorza et al., 1982, 1984; Tsuchimochi et al., 1984; Yazaki et al., 1984; Bouvagnet et al., 1984, 1985; Dechesne et al., 1985). The small amounts of β-myosin heavy chains detected here in rat and rabbit atria obviously correspond to the small amounts of β-myosin heavy chain mRNA recently detected in these tissues (Lompré et al., 1984; Sinha et al., 1984). The expression of other molecular variants of the atrial myosin heavy chain is of course not ruled out by any of these experiments. However, the Mab or nucleic acid probes currently available indicate that an additional myosin variant such as this would be immunologically or structurally very similar to the atrial α- or β-myosins defined at present. Because of our immunofluorescence and immunoaffinity experiments, our electrophoretic analyses of the different native atrial myosins and of some of their fractions not retained by Mab affinity columns (Fig. 7) show that the two bands of rat, rabbit, and pig atrial myosins detected by nondenaturating gel electrophoreses (Hoh et al., 1978; Clark et al., 1982; Banerjee, 1983) are not separated on the basis of their heavy chain heterogeneity. These results confirm recent observations by Chizzonite et al. (1984), and show the inadequacy of this electrophoretic technique for studying the diversity of atrial myosin heavy chains.

The similarities between the immunological reactivities observed with the three different Mab of the anti-α set and with those of the anti-β set in the atria of the four animals studied here and in humans (Bouvagnet et al., 1985), suggest that the three regions on atrial myosin heavy chain rods labeled by either Mab set constitute sites or domains that structurally determine the α- or β-character of the corresponding atrial myosins. The persistence of the immunological reactivities of the same six Mab within the atria of five different species indicates that the amino acid sequences of these six different myosin domains are largely similar in these animal species. The absence of fibers containing only β-like myosin heavy chains in rat and rabbit atria must be
due to the non-expression of β-myosin heavy chain genes in these fibers, rather than to the existence of an immunological cross-reactivity of the anti-α Mab with the atrial α- and β-myosin heavy chains in rat and rabbit hearts. The detection of fibers containing only α-like myosin heavy chains in these atria confirms this point of view, as well as the high specificity of the anti-β Mab. The existence of structural domains that are typical of α- and β-myosin heavy chains has already been suggested in the ventricles of the same animals by immunological approaches (Clark et al., 1982) or recombinant DNA techniques (Mahdavi et al., 1982). However, the present study provides no new information concerning the structural similarities between the ventricular and atrial forms of α- and β-myosin heavy chains present in the hearts of these animals.

Although large quantitative differences exist between the total amounts of atrial β-myosin heavy chains present in the atria of large and small mammals, the same environmental factors apparently influence the expression of the α- and β-myosin heavy chain variants in the atria of these animals. In rat and rabbit hearts, aging, hypothyroidism, mechanical cardiac overloading, and heart size favor the expression of β-myosin heavy chains, whereas hyperthyroidism favors the expression of atrial α-myosin heavy chains. Atria of very young rats and rabbits and human fetuses (P. Bouvagnet, unpublished results) contain only atrial α-myosin heavy chains. In addition, our study confirms the fact that, regardless of the myosin variant present in any of the animals studied, the two myosin variants are not randomly distributed between or within the atria (Gorza et al., 1982; Bouvagnet et al., 1984). This regional diversity in myosin expression indicates that the environmental factors controlling this expression could act differently at the level of each fiber or of a limited fiber group, depending on the location of the fibers in the atria. On the other hand, if the two atrial myosin variants present in a fiber have different physicochemical properties, such as their ATPase activity or stiffness, any change in the myosin expression could significantly modulate the contractile properties of the fiber concerned and consequently affect its immediate environment. The high density of atrial β-reactive fibers found in bovine, near the preferential conduction pathways of atrial electric stimulation (Gorza et al., 1982), and in the atria of human hearts subjected to local hemo-dynamic pressure overload (Tsuchimochi et al., 1984; Bouvagnet et al., 1985), suggest a probable multi-functional role for this regional myosin heterogeneity in atria. In any case, the present work again emphasizes the need to use very small and well-located (if possible) fragments of atrial tissues in further studies of myosin expression. The use of minced entire atria and the small amounts of atrial β-myosin heavy chains present in rabbit atria probably explain the discrepancies between our results and those recently published, which conclude that only α-myosin heavy chains are expressed in rabbit atria, which is not affected by the thyroid state of the animal (Chizzonite et al., 1984).

Age, hypo- or hyperthyroidism, mechanical over-loading (and heart size) affect the expression of the atrial α- and β-myosin heavy chains in the same way as previously observed in the expression of the ventricular α- and β-myosin heavy chains in the same five animals (for recent reviews, see Schiaffino et al., 1983; Chizzonite et al., 1984; Lompré et al., 1984; Mercadier et al., 1984). Although regional diversities in the expression of the two different myosin variants do exist in the atria as well as in the ventricles of all mammals (Gorza et al., 1981; Sartore et al., 1981; Léger et al., 1984), a curious quantitative dichotomy appears in the expression levels of each myosin type in the ventricles and the atria, and in small and large animals. Quantitatively large variations occur in the amounts of each myosin type within the ventricles of the small animals and within the atria of the large animals, but only small percentages of atrial β- or of ventricular α-myosin heavy chains are expressed in the atria of the small animals or in the ventricles of the large animals, respectively. Furthermore, the ventricles of normal adult rats and rabbits mainly contain either ventric-ular α- or β-myosin heavy chains respectively, whereas their atria contain mainly atrial α-myosin heavy chains. Finally, irrespective of the animal studied, a large difference exists between the mechanisms controlling the expression of α-myosin heavy chains and those controlling the expression of β-myosin heavy chains in the atria and in the ventricles. A comparison of the metabolic environment of myosin in atrial and ventricular fibers (Rupp et al., 1984) is essential for understanding the physiological meaning of these differences.

We thank Dr. B. Pau, who allowed us to produce monoclonal antibodies in the Service Immunodiagnostic of the firm Clin Mab, and Dr. T. Srihari for helpful discussions. We are also grateful to Dr. D. Hillaire for the protein assay of electrophoresis gels by densitometric scanning and to M. Anoal for her excellent technical assistance with the hybridoma cultures.

Address for reprints: C.A. Dechesne, INSERM U249, LPM2, Institut de Biologie, Boulevard Henri IV, 34000 Montpellier, France. Received March 18, 1985; accepted for publication August 20, 1985.

References

Circulation Research/ Vol. 57, No. 5, November 1985
Dechesne et al. / Myosin Variants in Mammalian Atria

of myosin heavy chains in atria and ventricles from hyperthyroid, hypothyroid, and euthyroid rabbits. J Biol Chem 259: 15564–15571


INDEX TERMS: Atrium • Immunofluorescence • Monoclonal antibodies • Myosin
Local diversity of myosin expression in mammalian atrial muscles. Variations depending on age and thyroid state in the rat and the rabbit.
C A Dechesne, J Léger, P Bouvagnet, H Mairhofer and J J Léger

Circ Res. 1985;57:767-775
doi: 10.1161/01.RES.57.5.767

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
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/57/5/767

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/