Myosin Types and Fiber Types in Cardiac Muscle.
II. Atrial Myocardium

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ABSTRACT Antibodies were produced against myosins isolated from the left atrial myocardium (anti-bAm) and the left ventricular myocardium (anti-bVm) of the bovine heart. Cross-reactive antibodies were removed by cross-absorption. Absorbed anti-bAm and anti-bVm were specific for the myosin heavy chains when tested by enzyme immunoassay combined with SDS gel electrophoresis. Indirect immunofluorescence was used to determine the reactivity of atrial muscle fibers to the two antibodies. Three populations of atrial muscle fibers were distinguished in the bovine heart: (a) fibers reactive with anti-bAm and unreactive with anti-bVm, like most fibers in the left atrium; (b) fibers reactive with both antibodies, especially numerous in the right atrium; (c) fibers reactive with anti-bVm and unreactive with anti-bAm, present only in the interatrial septum and in specific regions of the right atrium, such as the crista terminalis. These findings can be accounted for by postulating the existence of two distinct types of atrial myosin heavy chains, one of which is antigenically related to ventricular myosin. The tendency for fibers labeled by anti-bVm to occur frequently in bundles and their preferential distribution in the crista terminalis, namely along one of the main conduction pathways between the sinus node and the atrioventricular node, and in the interatrial septum, where different internodal tracts are known to converge, suggests that these fibers may be specialized for faster conduction.

Cardiac muscle is a heterogeneous tissue composed of distinct muscle cell populations. Ultrastructural studies have revealed the existence of significant differences between ventricular, atrial, and conduction fibers (see reference 36 for a review). However, a precise characterization of the cellular composition of the heart has been hampered by the lack of well-defined molecular markers for the different types of cardiac muscle cells. With the recent discovery that multiple myosin isoforms are present in cardiac muscle (14) and are differentially distributed among cardiac muscle cells (30), a new powerful tool for distinguishing cardiac muscle cells has become available. In previous immunofluorescence studies, we have analyzed the distribution of different isomyosins in the mammalian ventricular myocardium (9, 29). This study has now been extended to the atrial myocardium, where different muscle cell types have been identified by specific antiamyosin antibodies and immunofluorescence procedures.

Previous studies have shown that atrial myosin differs in structure and enzymatic activity from ventricular myosin. Ko-reeky and Michael (17) and Long et al. (19) showed that atrial myosin has a higher Ca$^{2+}$-activated ATPase activity than ventricular myosin and contains electrophoretically and immuno- logically distinct light chains. Differences in enzymatic activity, including actin-activated ATPase activity, and light chain pattern between atrial and ventricular myosin were subsequently observed in different mammalian species (37, 43). The structure of atrial myosin heavy chains was also found to differ from that of ventricular myosin heavy chains by polyprotein mapping after cyaogen bromide or proteolytic cleavage (6, 8, 42). Immunohistochemical studies with specific antimyosin antibodies have shown that the difference in atrial and ventricular myosin is a general feature of the vertebrate heart, being found also in birds (7, 30) as well as in amphibians and reptiles (our unpublished observations). These studies also showed that atrial myosin is antigenically related to fast skeletal myosin in birds and mammals (31). The different myosin composition of atrial and ventricular myosin appears to be of physiological significance. Atrial muscle contracts more rapidly than ventricular muscle (17, 40). In cardiac muscle, as in skeletal muscle, there seems to be a close correlation between Ca$^{2+}$- and actin-activated ATPase activity and speed of muscle shortening (32, 37, 43).

Multiple forms of ventricular myosin have been identified by electrophoresis of native myosin under nondenaturing con-
ditions (14) and by immunoaffinity chromatography (28). Ventricular isomyosins show a heterogeneous distribution in different regions of the ventricular myocardium (30) and their relative concentration can vary during development and in a variety of conditions (14, 17). Studies on the heterogeneity of atrial myosin are comparatively scanty. In different mammalian species, atrial myosin can be separated into two bands by pyrophosphate gel electrophoresis with relative mobilities different from those of ventricular myosins (4, 14). However, these two components have not yet been characterized biochemically and it is not known whether they differ in the heavy or light chains. Myosin light chains of ventricular type, in addition to atrial-type light chains, have been identified in human atrial muscle undergoing pressure overload hypertrophy (5, 26). In a previous immunofluorescence study of the chicken heart we described a number of atrial muscle fibers, some of which had features of Purkinje fibers, showing antmyosin immunoreactivity different from that of normal atrial fibers (30). In this study we have used two antmyosin antibodies with distinct specificities to investigate the cellular distribution of atrial myosin in the bovine heart. The functional significance of these findings will be discussed with particular reference to the problem of specialized conduction tracts.

MATERIALS AND METHODS

Antibodies

Antiserum to bovine atrial myosin (anti-bAm) has been described (29). The main points to recall here are that myosin was isolated from the left auricle, that specific IgG were obtained by affinity chromatography, and that cross-reactive antibodies were eliminated by absorption with insolubilized ventricular myosin.

Similar procedures were used for the preparation of antibodies to bovine ventricular myosin (anti-bVm). Myosin was isolated from the free wall of the left ventricle of adult bulls (heart weight ~3 kg) as described by Barany and Close (2) using ion-exchange chromatography as a final step (27). Myosin preparation was initiated within 1 h after death of the animal. Pepstatin (0.2 μg/ml) and PMSF (0.2 mM) were added during the first stages of the preparation. The use of protease inhibitors was found to reduce the amount of breakdown products during myosin preparation.

Four rabbits were injected subcutaneously at different sites with 0.5 mg of myosin in 0.4 M KCl-0.05 M Na-phosphate buffer, pH 7.4, emulsified with an equal volume of complete Freund's adjuvant. At two successive 14-d intervals the injections were repeated, and 7 d after the last injection the animals were bled for the first time. Subsequent boosts were given at various intervals.

The antisera were characterized by double immunodiffusion in Ouchterlony plates (20). Specific IgG were isolated by affinity chromatography on insolubilized ventricular myosin (29) from the antisera of two rabbits showing high titer
of antibody. These antibodies stained brightly ventricular muscle cells in indirect immunofluorescence but showed significant reactivity also with atrial muscle cells. Cross-reactive antibodies were eliminated by absorption with insolubilized myosin from the left atrium following previously described procedures (29). Specificity of absorbed anti-bVm was determined by enzyme immunoassay combined with SDS gel electrophoresis (21, 29). In brief, bovine ventricular myosin was run on a 10–15% polyacrylamide slab gel. One lane was cut transversely into ~1.5-mm slices that were incubated in polylysine tubes for 36 h to insure complete elution of the polypeptides and their attachment to the walls. A fixed amount of anti-bVm was then added to each well. Bound antimyosin was revealed by goat anti-rabbit IgG conjugated with alkaline phosphatase, enzyme concentration being determined with p-nitrophenylphosphate as substrate.

**Immunofluorescence**

Indirect immunofluorescence was performed on fresh-frozen cryostat sections of bovine atrial myocardium. Specimens were excised from different regions of the right and left atrium and interatrial septum. Samples from the crista terminals, Eustachian ridge, and border of coronary sinus were also examined.

Sections were first incubated with appropriate dilutions of anti-bAm or anti-bVm for 30 min at 37°C, then washed in phosphate-buffered saline and incubated with fluorescein-labeled goat anti-rabbit IgG (Miles Laboratories, Inc., Elkhart, IN) for 30 min at 37°C. Sections were then fixed in 2% formaldehyde, mounted in Elvanol, and examined with a Leitz microscope equipped with epifluorescence optics. Controls for immunofluorescence included sections stained with preimmune gamma globulin and with absorbed immune gamma globulin.

**RESULTS**

**Specificity of Antibodies**

The specificity of anti-bVm antibodies is illustrated in Figs. 1 and 2. When anti-bVm was tested on double immunodiffusion Ouchterlony plates a single precipitin line was formed against purified ventricular myosin or crude ventricular myosin extract, and no precipitate was generated with myosin isolated from left atrial myocardium (Fig. 1). When tested by enzyme immunoassay combined with SDS gel electrophoresis, anti-bVm reacted exclusively with ventricular myosin heavy chains, whereas myosin light chains and other components present in the crude myosin extract were unreactive (Fig. 2). The specificity of anti-bAm antibodies was described in a previous report (29); in brief, anti-bAm reacted with left atrial and not with left ventricular myosin and was specific for myosin heavy chains.

**Immunofluorescence Studies**

When applied to frozen sections of left atrial and ventricular myocardium anti-bVm was found to stain brightly all ventricular muscle fibers, whereas most atrial fibers were unlabeled (Fig. 3). A reversed staining pattern was obtained with anti-bAm, as previously described (29). However, a number of atrial...
muscle fibers did not conform with this pattern of reactivity and significant regional variations were observed in the distribution of different fiber types in the atrial tissue.

In the left atrial myocardium all muscle fibers without exception were stained by anti-bAm, and most of these fibers were unstained by anti-bVm. However, a minor proportion of atrial fibers were reactive with both antibodies. Fibers stained by anti-bVm were very rare in the auricular appendage and relatively more frequent in the atrial roof and towards the septum. They were usually interspersed among negative fibers (Fig. 4 a), and in longitudinally oriented bundles they appeared to be connected to negative fibers at their ends (Fig. 4 b). Labeled and unlabeled fibers could not be distinguished by size, position, or other morphological criteria. Occasionally, labeled fibers were grouped in clusters (Fig. 4 c). These clusters, however, were not generally segregated from the surrounding atrial myocardium, and a number of intermediate-type fibers showing varying degrees of staining intensity with anti-bVm were observed (Fig. 4 c); in contrast, no significant variation in reactivity with anti-bAm was seen (Fig. 4 d).

The pattern of reactivity of the right atrial myocardium was characterized by (a) a higher proportion of fibers labeled by anti-bVm, and (b) the presence of a limited number of fibers unlabeled by anti-bAm. The distribution of various fiber types was found to vary in different regions of the right atrium. Most fibers from the auricular appendage were labeled by anti-bAm and unlabeled by anti-bVm. In contrast, the proportion of doubly labeled fibers was considerable throughout the rest of the muscle. Sections through pectinate muscles incubated with anti-bVm showed a mosaic distribution of labeled and unlabeled fibers (Fig. 5 a). Homogeneous bundles of labeled fibers were frequently seen, both at the endocardial surface or deeper within the atrial muscle (Fig. 5 b–e). Some of the labeled fibers at the endocardial surface could be distinguished from the underlying myocardial cells by their larger size, strong PAS staining and, in some case, relative sparsity of the contractile material (Fig. 5 e). However, it should be stressed that these Purkinje-like cells were very rare in the atrial myocardium and

![Figure 5](https://example.com/figure5.jpg)

**Figure 5** Right atrial myocardium, anti-bVm. (a) Numerous labeled fibers distributed in a mosaic pattern. (b) A large cluster of labeled fibers in a pectinate muscle. (c) A bundle of labeled fibers, isolated from the surrounding myocardium. (d and e) Bundles of labeled fibers at the endocardial surface. Note in e two small bundles of tightly packed fibers stained with variable intensity by anti-bAm (arrowheads). These fibers, which are larger in size than neighboring atrial fibers, were found to be strongly PAS-positive in serial sections stained with PAS. a, b, and d: × 150; c and e: × 400.
that most fibers labeled by anti-bVm were morphologically indistinguishable from unlabeled neighboring fibers.

The proportion of fibers labeled by anti-bVm was even higher in the septum and in the region of the crista terminalis, Eustachian ridge, and coronary sinus (Figs. 6–8). Most fibers in these regions were reactive with both anti-bVm and anti-bAm. At those sites where pectinate muscles originate from the crista terminalis it was occasionally possible to visualize clear-cut transitions in the myosin composition of atrial fibers, bundles of fibers labeled by anti-bVm being often found beside bundles of unreactive fibers (Fig. 6). A distinguishing feature of these regions was also the finding of fibers labeled by anti-bVm and completely unlabeled by anti-bAm (Fig. 7). In serial sections processed for the histochemical demonstration of myosin ATPase activity, a number of fibers labeled by anti-bVm showed a reactivity different from that of surrounding unlabeled fibers. This was especially true for those fibers showing intense staining with anti-bVm and weaker reactivity with anti-bAm. The ATPase activity of these fibers was resistant to acid preincubation and sensitive to alkaline preincubation (Fig. 8). This response is similar to that of ventricular fibers and slow skeletal muscle fibers, whereas it stands in contrast to the response of atrial fibers unlabeled by anti-bVm, whose ATPase was inhibited by acid preincubation and resistant to alkaline preincubation, a response similar to that of fast skeletal fibers (see references 30, 31, and 38).

**DISCUSSION**

The results of the present study show that different myosin types and fiber types are present in the bovine atrial myocardium. Three main types of atrial muscle fibers can be distinguished by their reactivity with anti-bAm and anti-bVm antibodies: (a) muscle fibers reactive with anti-bAm and unreactive with anti-bVm, representing the major fiber population especially in the left atrium; (b) muscle fibers reactive with anti-bVm and unreactive with anti-bAm, very rare and present only in the interatrial septum and along the crista terminalis; (c) muscle fibers reactive with both anti-bVm and anti-bAm, representing a sizable population in the right atrium. In addition, fibers showing intermediate degrees of reactivity with one or both antibodies are present in the atrial myocardium, giving rise to what appears to be a whole spectrum of fiber types.

These findings can be accounted for by postulating that a minimum of two antigenic types of atrial myosin heavy chains are present in the bovine atrial myocardium: a major type, defined by its reactivity with anti-bAm, and a minor type, defined by its reactivity with anti-bVm. Following this interpretation, most left atrial fibers would contain only the major type of atrial myosin heavy chain, rare fibers in the right atrium only the minor type, whereas muscle fibers reacting with both anti-bAm and anti-bVm would contain the two myosin heavy chain types. The variable reactivity with one or the other antibody in doubly labeled cells could be accounted for by the presence of varying amounts of the two myosin heavy chains.

The relative prevalence of one or the other form of heavy chain is apparently correlated with differences in the enzymatic properties of atrial myosins. The histochemical reaction for myosin ATPase suggests that the ventricular-like atrial myosin shows a pattern of pH sensitivity similar to that of slow skeletal myosin, whereas the major type of atrial myosin is similar in this respect to fast skeletal myosin (see references 6, 30, 31, 38).
The relationship between these antigenic types of atrial myosin and the two bands that can be resolved upon pyrophosphate gel electrophoresis of atrial myosin (4, 14) remains to be determined. We are presently using immunoaffinity chromatography with insolubilized anti-βVM to separate the ventricularlike atrial isomyosin from the bovine right atrium in order to define its structure and properties.

The most interesting result of this study is the heterogeneous cellular distribution of different atrial myosin heavy chains. The functional significance of this finding is not clear. By analogy with the heterogeneous distribution of ventricular isomyosins (9, 29), one might assume that fibers containing predominantly one or the other type of myosin heavy chain represent different fiber types of the normal working myocardium. Alternatively, specific myosin heavy chains may be associated with specialized functions of certain atrial fibers as suggested by the striking regional variations in the distribution of atrial myosins. The fact that fibers labeled by anti-βVM are more numerous in the right atrium and are specially abundant along the crista terminalis and Eustachian ridge, namely along one of the main pathways where preferential conduction of the electric stimulus between the sinus node and atrioventricular node takes place, and in the interatrial septum, where different internodal tracts are known to converge, raises the possibility that these cells correspond to fibers specialized for faster conduction. The tendency for these fibers to occur frequently in bundles both in the right and in the left atrial myocardium would be consistent with this interpretation. It is still a moot point whether the spread of activation from the main pacemaker occurs preferentially along certain internodal pathways purely as a consequence of the geometrical arrangement of the atrial fiber bundles or as a consequence of the properties of specialized fiber types present in the internodal tracts (see references 12, 15, 16, 33). Morphological evidence for the presence of distinct fiber types in the atrial myocardium has been previously reported (see references 33 and 36), but this is to our knowledge the first report of a precise molecular marker that permits one to distinguish atrial fiber subpopulations. Electrophysiological studies with intracellular electrodes have shown that fiber types with different electrical characteristics are present in the atrial myocardium, fibers with longer action potential ("plateau fibers") being abundant in the crista terminalis (13, 22, 25, 41). On the basis of the similar location in the atrial myocardium, it is tempting to speculate that fibers with longer action potential correspond to fibers stained by anti-βVM, but this can only be established by direct studies combining antimyosin immunofluorescence with electrophysiology. One point that is clear by now is that fibers labeled by anti-βVM do not constitute insulated and discrete tracts but intermingle extensively with unlabeled muscle fibers. Therefore, if specialized conducting tracts do exist in the atrial myocardium and correspond to the fibers labeled by anti-βVM, they clearly differ from the ventricular Purkinje system and it may be not appropriate to draw a clearcut distinction in the atrial muscle between "normal working myocardium" and "conducting system". Indeed, it is possible that large portions of the right atrium consist of fibers that have faster conducting properties but in the same time give a significant contribution to the overall contractile performance of the right atrium and therefore should also be considered integral part of the working myocardium itself.

We wish to make a final comment on the relation between electric membrane properties, such as the configuration of the

FIGURE 7 Interatrial septum. (a) Muscle fiber heterogeneity after anti-βAm staining. (b and c) Serial sections stained with anti-βAm (b) and anti-βVM (c). Note the presence of fibers labeled by anti-βAm and unlabeled by anti-βVM, fibers labeled by anti-βVM and unlabeled by anti-βAm, and fibers reactive with both antibodies. a, x 150; b and c, x 400.
action potential, and the presence of particular myosin types in cardiac muscle. In the ventricular myocardium, variations in the duration of the action potential between right and left ventricle and between subendocardial and subepicardial regions (10, 35) appear to correlate with the distribution of different myosin types (9, 29), longer action potentials being associated with a higher concentration of the "slow" type of ventricular myosin. Prolongation of the action potential under conditions of pressure overload (1, 11, 39) is likewise related to the plateau phase of the action potential, which is associated with a higher concentration of the "slow" type of myosin (9, 29), longer action potentials being associated with a higher concentration of the "slow" type of myosin. We wish to thank Mr. Massimo Fabbri for excellent technical assistance.

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