A NEW CARDIAC MYOSIN CHARACTERIZED FROM THE CANINE ATRIA

Lawrence Long, Ferenc Fabian*, Dean T. Mason, and Joan Wikman-Coffelt

Section of Cardiovascular Medicine, Department of Medicine
University of California, Davis School of Medicine, and
*Dept. of Biochemistry, Lorand Eötvös Univ. of Budapest

Received February 28, 1977

SUMMARY Canine atrial myosin light chains were electrophoretically distinct from myosins of canine ventricles on 5-20% polyacrylamide gradient slab gels (SDS), giving molecular weights of 26,000 and 21,000 as compared to 28,000 and 18,500 for ventricular myosin light chains. While atrial myosin heavy chains were immunologically identical with ventricular myosin heavy chains, in contrast, there was 8.0% relative cross-reactivity of atrial myosin light chains with left ventricular myosin light chains by radioimmunoassay. According to charge separation on two-dimensional polyacrylamide urea gels, atrial myosin light chains were different from those of ventricular myosins. Variances in ATPase activities between atrial and ventricular myosins were strongly demonstrated. There was a lower K^+ activated ATPase activity in atrial myosin, however the Ca^{2+} activated ATPase activity, at ATP saturation levels, was higher in atrial myosin as compared to ventricular myosins.

Introduction

Both a gradient (as described by O'Farrell) (1)), and a high percent of polyacrylamide (made possible by the use of a slab gel (1)), facilitates greater resolution of low molecular weight proteins. With the use of a gradient of polyacrylamide (5-20%) on a slab gel (dodecylsulfate), atrial myosin light chains can be distinguished from those of the ventricles. Resolution of this type showed that atrial myosin light chains were different from light chains of other myosins (2-5). Immunological and kinetic differences were also noted between atrial and ventricular myosins.

Procedures

Myosin Purification. Myosin was purified according to procedures defined earlier (6). Atria of dogs were dissected free from ventricles and blood vessels. Six left and right atria were pooled for atrial myosin purification. Single left or right ventricles dissected free of atria, were used for ventricular myosin. After the tissue was minced, it was sheared, washed three times in a low salt buffer (0.05 M KH_2PO_4, pH 6.8, 0.001 M EDTA, 0.01 M Na PPi, 0.001 M DTT), and centrifuged (9,000 x g, 10 min), after each wash. Myosin was then extracted from the pellet in a buffer with a high salt concentration (0.1 M KH_2PO_4, 0.3 M KCl, 0.01 M NaPPi, 0.001 M DTT, 0.001 M EDTA, and 0.05 M K_2HPO_4, pH 7.5). After stirring the homogenate for 15 min (7) the solution was filtered and centrifuged (9,000 x g, 15 min). Myosin was crystalized with a 9-fold dilution of water containing 0.002 M EDTA. Myosin, after pelleting at 9,000 x g for 10 min, was homogenized in 0.05 M NaPPi, pH 7.5, 0.001 M DTT, 0.001 M EDTA, and 0.002 M ATP, stirred for 10 min and centrifuged (40,000 x g for 20 min). Myosin was collected from the supernatant by a salt fractionation using the 35% to 42% $(NH_4)_2SO_4$ fraction, dialyzed, and centrifuged as described earlier (7).

ISSN 0006-291X

Myosin ATPase Activity. Potassium-EDTA-activated ATPase activity of myosin was measured in a mixture containing 0.1 M Tris.HCl, pH 7.5, 5mM EDTA, 0.65 M KCl and various concentrations of ATP. Calcium-activated ATPase activity was measured in 0.2 M Tris. maleate, pH 6.5, 0.01 M $CaCl_2$ and various concentrations of ATP. Incubation with enzyme was carried out at 37° for 5 min. The reaction was stopped by addition of 1 ml of 20% trichloroacetic acid; from the 2 ml reaction mixture, 1 ml aliquots were assayed for inorganic phosphate by the method of Fiske and SubbaRow (8). All glassware was acid washed. Myosin solutions were dialyzed against the specific assay buffer used for enzymatic analyses. Protein concentrations were determined according to Lowry (9). All other for analyses of myosin ATPase activity were described earlier (10).

Immunological Analyses of Myosin. Preparation of antisera was the same as described earlier (6,7), as well as development of double diffusion immunoassays (6,7). A radioimmunoassay was developed for myosin light chains. Goat antimyosin was diluted 1:85 in 0.05 M potassium phosphate, pH 7.5, 0.001 M EDTA, and 0.1% serum albumin. The reaction mixture contained 300 µl of potassium phosphate buffer, 100 µl of diluted antiserum, and 50 µl of ^{125}I myosin light chains; components were added in that order. (Iodination of myosin light chains was carried out as described earlier (11). Samples were incubated overnight at 4° C. After incubation 2 ml of charcoal suspension was added (1.25 g charcoal, 0.125 g Dextran 250, 0.75 g NaCl in 200 ml of potassium phosphate buffer without albumin). The slurry was centrifuged, the supernatant decanted to analyze the ^{125}I antigen-antibody complex, and the pelleted charcoal assayed for the free ^{125}I-myosin light chains.

Affinity Chromatography. For coupling either myosin light chains or myosin heavy chains to Sepharose (Pharmacia) the procedures described by Pharmacia were followed. Five grams CNBr-activated Sepharose 4B were washed in 0.001 M HCl and filtered. The swollen gel was transferred to a 60 ml plastic bottle containing either 2 mg light chains or 20 mg heavy chains in 0.2 M sodium phosphate buffer, pH 8.0, 0.5 M KCl and shaken 20 hours at 4°. To remove uncoupled antigen the mixture was filtered and washed with 50 ml of 0.2 M sodium phosphate buffer, pH 8.0, 0.5 M KCl. Two additional washing cycles were then used to remove noncovalently bound contaminants. Each cycle consisted of a pH 4.0 wash (250 ml of 0.1 M acetic acid, and 0.5 M KCl) and a pH 8.0 wash (250 ml of 0.1 M boric acid and 0.5 M KCl). The Sepharose coupled myosin chains were then washed in 500 ml of 0.3 M KCl, and 0.05 M Tris, pH 7.5.

The filtered complex was suspended in 25 ml of 0.05 M Tris, pH 7.5, and 0.3 M KCl, containing 10 ml of goat antimyosin chains from dog hearts, partially purified by $(NH_4)_2SO_4$ precipitation and treated as described earlier (11). The myosin-Sepharose immunoadsorbant was shaken at room temperature for 2.5 hours. Five hundred ml of 0.05 M Tris, pH 7.5 and 0.3 M KCl were added to the Sepharose coupled antigen-antibody complex. The slurry was filtered, resuspended in 0.05 M Tris, pH 7.5, 0.3 M KCl, transferred to a Pharmacia K9/30 Column and washed until absorbancy at 280 mµ was below 0.03 absorbance units. The antibody specific for each of the defined myosin chains was eluted from the myosin-Sepharose immunoadsorbant prepared for either myosin heavy chains or myosin light chains with 0.2 M acetic acid, pH 2.8 and 0.3 M KCl.

One-Dimensional Slab Gel Electrophoresis. The gradient of polyacrylamide was a modification of that of O'Farrell (1). A water-cooled Bio Rad slab gel apparatus (of which the glass plates were 10 cm square with 1.5 mm spacers) was used in the following sodium dodecylsulfate gel gradients of 5-20% acrylamide. The 5% acrylamide well contained: 3.75 ml 1.5 M Tris.HCl, pH 8.8, 0.15 ml 10% sodium dodecylsulfate, 0.75 ml glycerol, 2.5 ml (30% acrylamide + 0.8% N,N' methylene-bisacrylamide), 7.77 ml H_2O, 0.05 ml 10% TEMED, 0.03 ml 10% ammonium persulfate. The 20% acrylamide well contained: 1.80 ml 3 M Tris.HCl, pH 8.8, 0.15 ml 10% sodium dodecylsulfate, 3.0 ml glycerol, 10.0 ml (30% acrylamide + 0.8% N, N' methylene-bisacrylamide), 0.2 ml 10% TEMED, and 0.03 ml 10% ammonium persulfate. The 3% stacking gel contained: 2.5 ml 0.5 M Tris.HCl, pH 6.8, 0.1 ml 10% sodium doedecylsulfate, 1.0 ml (30% acrylamide + 0.8% N,N' methylene-bisacryl-

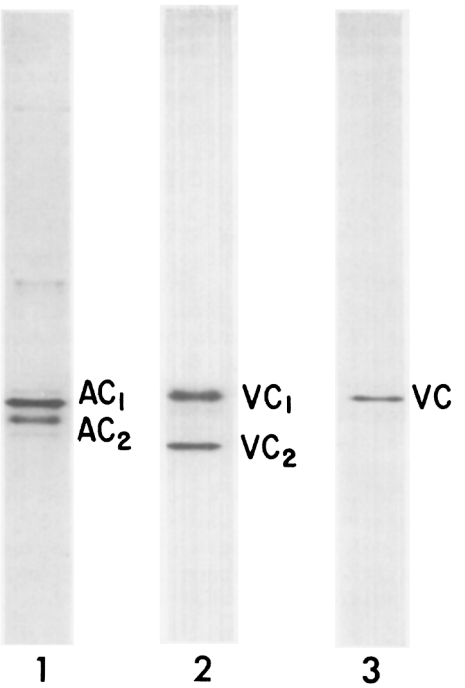
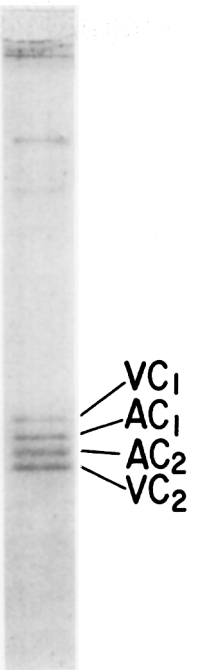


Figure 1

Light chains were purified from whole myosin with 8 M urea as described earlier (6). One-dimensional slab gel electrophoresis in dodecylsulfate as described in Methods is shown for atrial myosin light chains (AC_1 and AC_2) (1); (2) ventricular myosin light chains (VC_1 and VC_2), as well as (3) reduced and carboxymethylated light chain C_1 are shown. (Light chain C_1 tends to form a doublet on dodecylsulfate gels unless reduced and carboxymethylated). Atrial light chains (AC_1 and AC_2) and ventricular myosin light chains (VC_1 and VC_2) give four bands when combined (4). Atrial myosin light chains (5), ventricular myosin light chains (6), and atrial + ventricular myosin light chains (7) were subjected to isoelectrofocusing as described by O'Farrell (1), except the 2% ampholines were comprised of 1.6% of pH range 4 to 6 and 0.4% pH range 3 to 10, and then electrophoresed in the second dimension through a gradient of polyacrylamide (SDS). A stained isoelectrofocusing gel of atrial myosin (5), ventricular myosin (6), and atrial + ventricular myosin (7), are placed above each respective slab gel.

amide), 6.2 ml H_2O, 0.01 ml TEMED, 0.2 ml 10% ammonium persulfate. In preparation of protein sample for electrophoresis, 0.10 ml of myosin (0.10 ml of myosin (0.1-0.5 mg) was mixed with 0.10 ml of sample buffer. (The sample buffer contained: 2.5 ml 0.5 M Tris·HCl, pH 6.8, 2.0 ml glycerol, 4.0 ml 20% sodium dodecylsulfate, 0.5 ml 0.1% bromphenol blue, 0.5 ml β-mercaptoethanol, 0.25 ml H_2O). Aliquots (5-20 µl) were added to separate wells and electrophoresed at 20 ma (70-225 v) for 4 hours. The electrophoresis buffer contained 6 gm Tris, 28.3 gm glycine, and 10 ml 10% sodium dodecylsulfate in 1 liter (pH 8.3).

Two-Dimensional Slab Gel Electrophoresis. Conditions for two-dimensional gel electrophoresis of myosin in urea were as those described by Traugh and Porter (12) except gel dialysis was omitted (13). The first dimensional urea gel was subjected to electrophoresis at pH 8.7; the running buffer was pH 8.2. The second dimensional urea gel was subjected to electrophoresis at pH 4.6.



Atrial LC + Ventricular LC

4

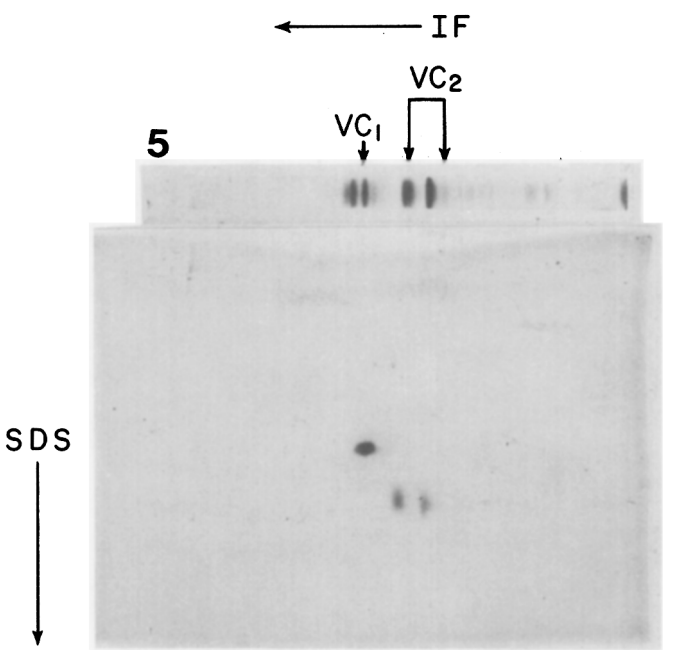


Figure 1 (continued)

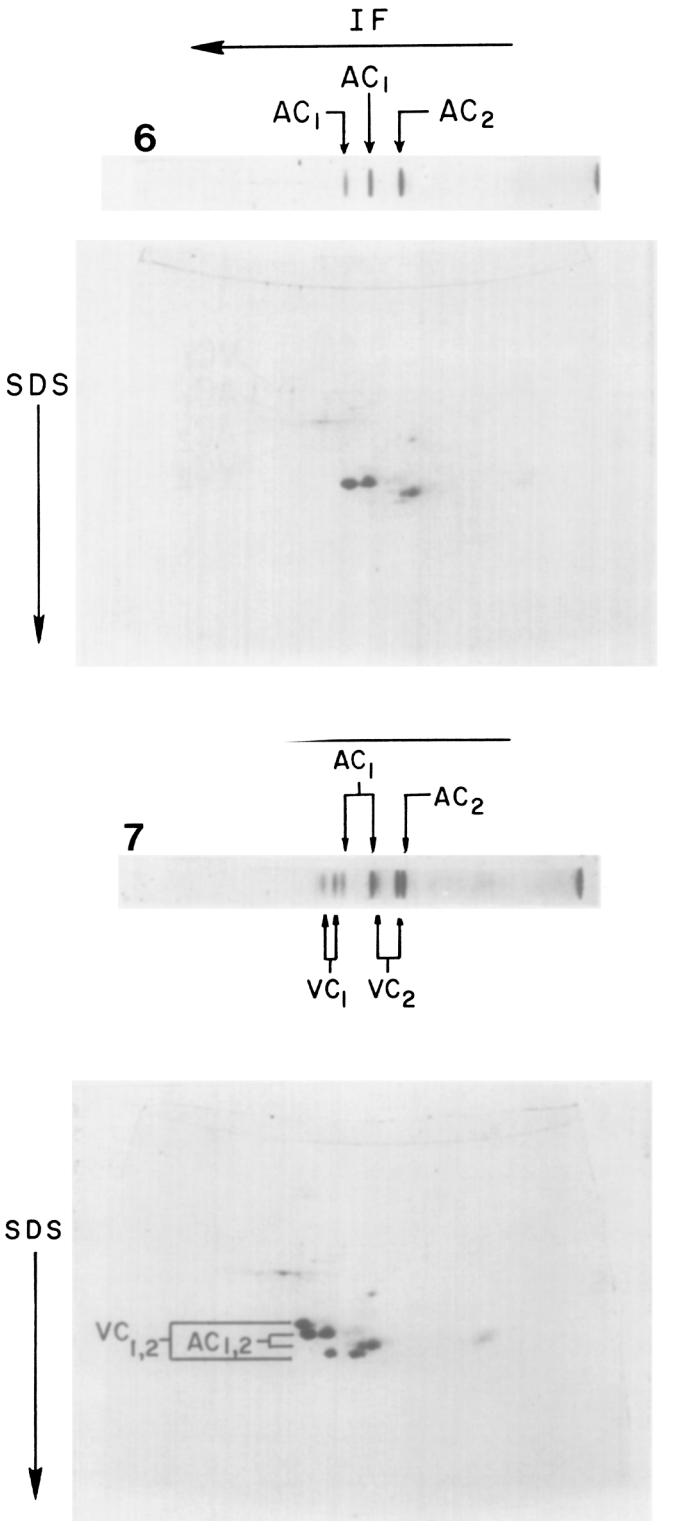


Figure 1 (continued)

Two-dimensional electrophoresis of myosin, where the first dimension was isoelectrofocusing and the second dimension was carried out in sodium dodecylsulfate, were as described by O'Farrell (1).

Results and Discussion

Canine atrial myosin light chains have molecular weights of 26,000 and 21,200 on sodium dodecylsulfate (SDS) gels (Figure 1 (1)) as compared to the 28,000 and 18,500 molecular weights for ventricular myosin light chains on this type of gel (Figure 1 (2 and 3)). The combined atrial and ventricular myosin light chains gave four bands on SDS gels (Figure 1 (4)). When atrial and ventricular myosin light chains were subjected to isoelectrofocusing in the first dimension followed by electrophoresis in sodium dodecylsulfate in the second dimension, patterns shown in Figure 1 (5-7) were obtained. With isoelectrofocusing, each of the light chains gave more than one band (14). The heterogeneity of myosin light chain C_2 is possib a consequence of phosphorylation (14, 16-18) or spontaneous modification as describ by Frearson and Perry (18). Ventricular myosin light chain C_1 gave two or more closely aligned bands on a first dimensional isoelectrofocusing gel, however, these components merged as one spot when electrophoresed in the second dimension. It is not known at this time why differently charged species are obtained for myosin l chain C_1 on urea gels. Reducing and carboxymethylating the light chains does not reduce the number of bands present after isoelectrofocusing. Using isoelectrofocus various species of actin were shown to be present in cultured muscle cells (15). Each of the atrial myosin light chains are slightly different from those of ventricular myosin. The atria constitutes a small part of the heart so that in the past, if one used the whole heart in preparation of myosin, the myosin light chains specific to the atria could have been overlooked. Both atrial and ventricular myosin light chains were obtained from cultured cardiac cells when the whole heart was used.

According to charge separation on 2-dimensional polyacrylamide urea gels after electrophoresis, atrial myosin light chains were distinct, and different from those of ventricular myosin (Figure 2). Ventricular myosin light chain C_1 gave two components, C_{1c} and C_{1d}, on this type of gel system (13), while light chain C_2 remained one component. Different from that of ventricular myosin, atrial myosin light chain C_1 remained one component (Figure 2).

Light chains from the atria were immunologically distinct from ventricular myosin light chains, giving an average of only 8% cross-reactivity by radioimmunoassay (Figure 3).

Differences in ATPase activities between atrial and ventricular myosins were strongly demonstrated. There was low K^+ ATPase activity in atrial myosin, using

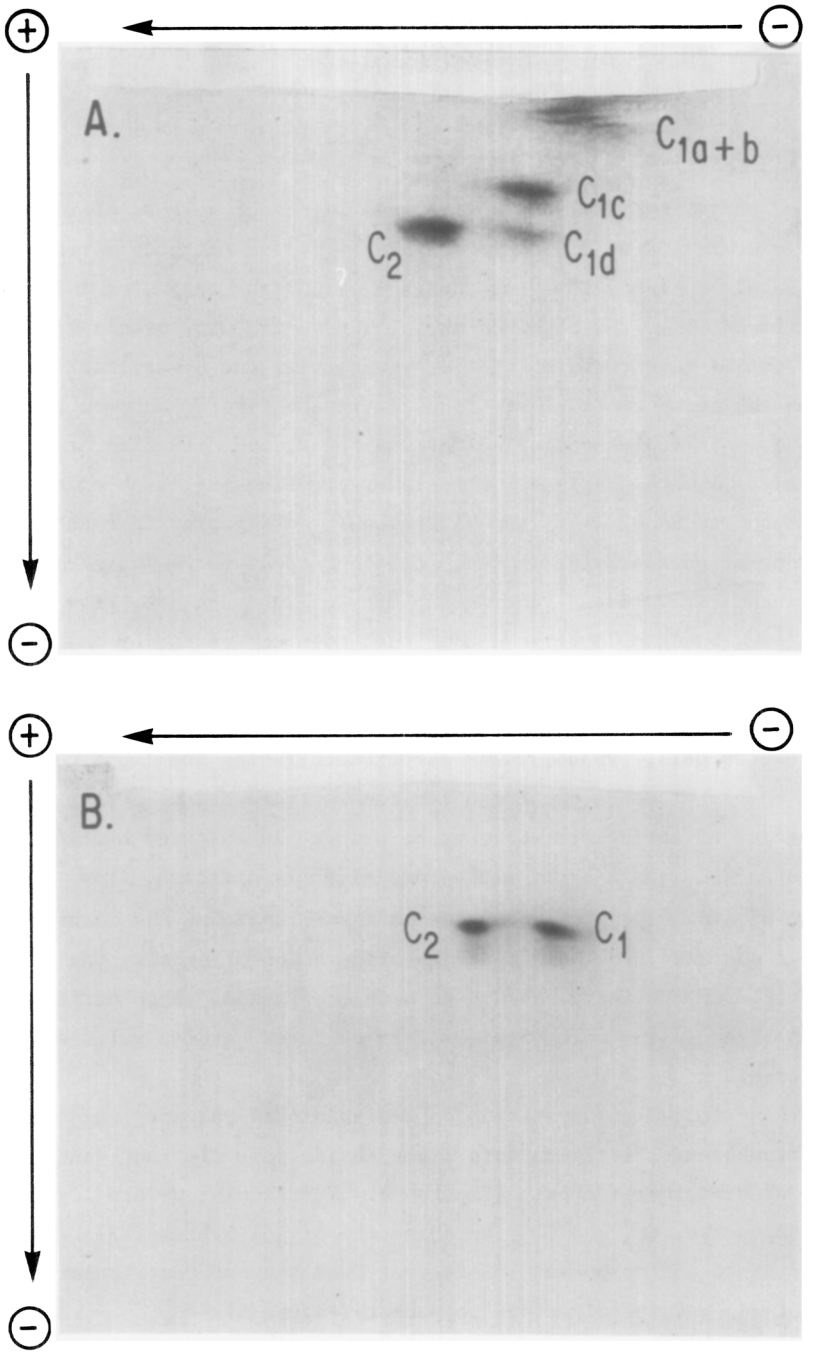


Figure 2

Two dimensional polyacrylamide slab gel electrophoresis in urea as described in Methods (A) ventricular myosin, (B) atrial myosin (C_{1ab} are non specific complexing of C_{1c}). For (A) electrophoresis in the first dimension was 6 hours (5ma/tube) whereas for (B) electrophoresis was 9 hours (5ma/tube). (If atrial myosin light chains were electrophoresed for the same period of time as ventricular myosin light chain, the 2 atrial myosin light chains superimposed on ventricular myosin light chain C_{1c})

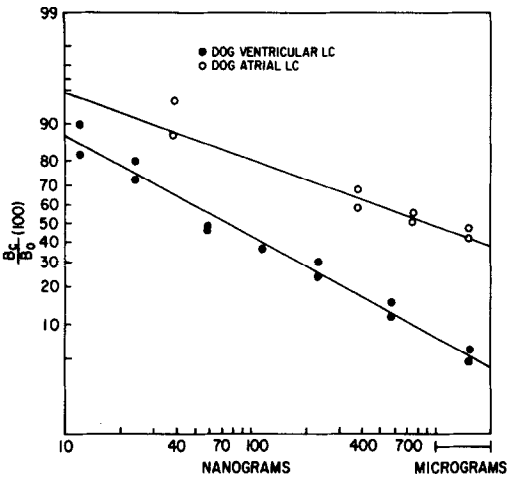


Figure 3

Radioimmunoassay against antimyosin (dog) using iodinated dog left ventricle light chains (LV-LC) competing with dog right ventricle light chains (RV-LC) and dog atrial light chains (A-LC). (Antimyosin, or antimyosin light chains gave similar results). Conditions are described in Methods. Percent cross-reactivity, calculated according to Grota (22) gave 100% relative cross-reactivity for right and left ventricular myosin light chains but only an average of 8% (three analyses) for atrial myosin light chains. (Left and right ventricular myosin light chains were immunologically identical.)

TABLE I

MYOSIN ATPase ACTIVITY
(μMoles Pi/mg myosin.min^{-1})

K^+ Activated Atrial Myosin	S.D. 1.05 ± 0.29	Left Ventricular Myosin	S.D. 1.56 ± 0.17
	($p<.001$)		
Ca^{2+} Activated Atrial Myosin	1.34 ± 0.16	Left Ventricular Myosin	0.91 ± 0.14
	($p<.01$)		

Optimal conditions for K^+ and Ca^{2+} activated myosin ATPase, where each cation is used alone in the reaction mixture was described earlier (10); assays were carried out at ATP saturation levels of 3 mM. Other conditions used here are described in Methods. Standard deviations (S.D.) are for 3 to 5 animals.

conditions optimal for these enzymatic properties in the ventricle (Table I) (16). Ca^{2+} ATPase activity, on the other hand, was higher in atrial myosin as compared to ventricular myosins. Data for left ventricular myosin are shown. Right ven-

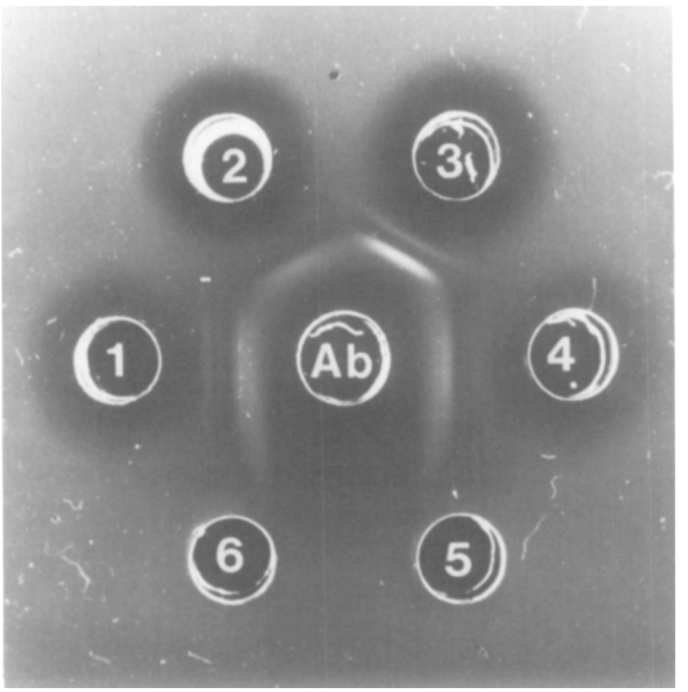


Figure 4
Ouchterlony pattern of dog left ventricular heavy chains (1); dog atrial heavy chains (2); sheep atrial heavy chains (3); and sheep left ventricle heavy chains (4). (0.1 mg of protein was added to each well.) The center well contained about 0.1 mg of goat γG antimyosin heavy chains purified by affinity chromatography.

tricular myosin had 25% lower K^+ and Ca^{2+} ATPase activities as compared to left ventricular myosin (17). Similar differences between atrial and ventricular myosins were noted in other species. A physiological explanation for this high Ca^{2+} ATPase activity in atrial myosin relative to ventricular myosins is not possible at this time, however, these comparative studies may give further insight into the properties of myosin in the various chambers of the heart, and the important role myosin light chains play in regulating the enzymatic activity of myosin.

If there are differences in the heavy chains between atrial and ventricular myosins it is not perceivable by immunodiffusion techniques (Figure 4). Atrial myosin precipitin bands fused with those of ventricular myosin for both dog and sheep myosin when incubated against goat antimyosin (heavy chains) of dog in an Ouchterlony reaction. Goat antimyosin heavy chains was purified by affinity chromatography.

The importance of the intensity and time course of contractile activity has

been described by Mommaerts (19); contractility appears to be, at least in part, related to the rate myosin hydrolyzes ATP (20, 21). From these results it appears that the velocity of contraction is greater in the atria as compared to the ventric

References

1. O'Farrell, P.H. (1975) J. Biol. Chem., 250, 4007-4021
2. Adelstein, R.S., and Conti, M.A. (1972) Symposia on Quantitative Biology 37, 599-605
3. Sarkar, S., Sreter, F.A., and Gergely, J. (1971) Proc. Nat. Acad. Sci., 68, 946-950
4. Perrie, W.T., Smillie, L.B., and Perry, S.V. (1973) Cold Spring Harbor Symposia on Quantitative Biology, 37, 17-18
5. Ash, J.F. (1975) J. Biol. Chem., 250, 3560-3566
6. Wikman-Coffelt, J., Zelis, R., Fenner, C., and Mason, D.T. (1973) Biochem. Biophys. Res. Commun., 51, 1097-1104
7. Wikman-Coffelt, J., Zelis, R., Fenner, C., and Mason, D.T. (1973) Biochem. Biophys. Res. Commun., 51, 1097-1104
8. Fiske, C.H., and SubbaRow, Y. (1925) J. Biol. Chem., 66, 375-379
9. Lowry, O.H., Rosebraugh, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem., 193, 265-275
10. Fenner, C., Mason, D.T., Zelis, R., and Wikman-Coffelt, J. (1973) Proc. Nat. Acad. Sci., 70, 3205-3209
11. Wikman-Coffelt, J. (1972) Anal. Biochem., 48, 339-345
12. Traugh, J.A., and Porter, G.G. (1976) Biochem., 15, 610-616
13. McPherson, J., Traut, R.R., Mason, D.T., Zelis, R., and Wikman-Coffelt, J. (1974) J. Biol. Chem., 249, 994-996
14. McPherson, J., Fenner, C., Smith, A., Mason, D.T., and Wikman-Coffelt, J. (1974 FEBS Letters, 47, 149-154
15. Whalen, R.G., Butler-Browne, G.S., and Gros, F. (1976) Proc. Nat. Acad. Sci., 73, 2018-2022
16. Wikman-Coffelt, J., Fenner, C., Zelis, R., and Mason, D.T. (1975) Effect of variations in pH on kinetics of myosin, In: Recent Advances in Studies on Cardiac Structure and Metabolism (eds. Paul-Emile Roy and Peter Harris) Vol.8 University Park Press, Baltimore
17. Wikman-Coffelt, J., Fenner, C., Smith, A., and Mason, D.T. (1975) J. Biol. Chem 250, 1257-1262
18. Frearson, N., and Perry, S.V. (1975) Biochem. J., 151, 99-107
19. Mommaerts, W.F.H.M. (1971) Annals of the New York Academy of Sciences, 185, 425-432
20. Wikman-Coffelt, J., Walsh, R., Fenner, C., Kamiyama, T., Salel, A., and Mason, D.T. (1976) J. Mol. Cell. Cardiol., 8, 263-270
21. Barany, M. (1967) J. Gen. Physiol., 50, pt-197
22. Grota, L.J., and Brown, G.M. (1976) Endocrinology, 98, 615-622