Rapid Publications

Diabetes Mellitus Induces Changes in Cardiac Myosin of the Rat

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SUMMARY

Decreased contractility has been reported in the diabetic heart. Because a close correlation exists between contractility and the activity of Ca^{2+} ATPase of purified actinomyosin and myosin, Ca^{2+} ATPase activity was determined in control and diabetic rats. In control rats, actinomyosin ATPase was 0.59 ± 0.05 μmol Pi/mg protein/min and had decreased by 35% to 0.38 ± 0.04 in diabetic rats ($P < 0.025$). Myosin ATPase activity was 1.25 ± 0.09 μmol Pi/mg protein/min in control rats and had decreased by 45% to 0.67 ± 0.05 in diabetic animals ($P < 0.01$). To investigate the decrease in myosin ATPase activity further, ventricular myosin was separated by pyrophosphate polyacrylamide electrophoresis into its three authentic components, V_1, V_2, and V_3 myosin. V_1 has the highest mobility and Ca^{2+} ATPase activity (1.27 ± 0.3 arbitrary units) and represents 72% of control myosin, whereas V_3 has the lowest mobility and Ca^{2+} ATPase activity (0.16 ± 0.08 units) and constitutes 13% of myosin. A marked change in the predominance of V_1 and V_3 myosin components occurs in diabetic rats where V_3 myosin predominates, representing 68% of total myosin with V_1 myosin constituting only 15%. Ca^{2+} ATPase activities of V_1, V_2, and V_3 myosin in control and diabetic hearts are similar; however, the predominance of V_3 myosin in diabetic rats can account for the decreased Ca^{2+} ATPase activity of diabetic myosin. The diabetes-induced changes in myosin ATPase activity and myosin isoenzyme distribution can be reverted to control levels by insulin administration. DIABETES 29: 579–582, July 1980.

The frequent occurrence of cardiac failure in diabetic patients due to coronary vascular disease is well established.[1] More recent reports describe abnormalities in cardiac performance in the diabetic heart in the absence of significant large vessel disease.[2,3] This process has been termed "diabetic cardiomyopathy." Although this clinical entity is still somewhat controversial, several reports seem to confirm its existence.[2–4] Decreased cardiac contractility in diabetic animals seems quite well established.[5,6] The pathophysiologic mechanisms that underlie the decreased contractility are currently unclear. In particular, it is unknown if changes in the state of contractile proteins contribute to the decreased contractility of the diabetic heart. The activity of Ca^{2+}-activated ATPase of actinomyosin and myosin has not been determined in diabetic hearts. A close correlation between the activity of Ca^{2+}-activated ATPase of actinomyosin or myosin and cardiac contractility has been well established.[7] Furthermore, recent reports have described the occurrence of cardiac myosin isoenzymes.[8–10] These myosin isoenzymes can be separated by electrophoretic techniques into three components that differ in Ca^{2+} ATPase activity.[8,9] The following studies were undertaken to assess the activity of Ca^{2+} ATPase of actinomyosin and myosin and to determine if changes in the distribution of myosin isoenzymes occur in diabetic rat hearts.

MATERIALS AND METHODS

Male Sprague-Dawley rats between 6 and 8 wk of age were used for all experiments. Control and diabetic animals were age- and litter-matched. Rats were made diabetic by i.v. administration of 65 mg streptozotocin/kg body wt and maintained in a diabetic state for 4 wk. After being diabetic for 4 wk, rats were injected s.c. with 2 U protamine zinc insulin (PSI)/day for 4 wk.[11] To assure that animals were diabetic, blood sugars were determined 4 days and 4 wk after streptozotocin administration. Blood sugars in control animals were 120 ± 15 mg/dl, in diabetic animals 420 ± 30 mg/dl, and in insulin-treated diabetic rats 175 ± 18 mg/dl. Actinomyosin and myosin were prepared from rat ventricles by previously described methods.[12,13] In some experiments, 0.1 mM phenylmethanesulfonylfluoride (PMSF) was included during the myosin isolation procedure and the ATPase assay.[14] The purified myosin was free of contaminating pro-

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teins as judged by SDS polyacrylamide electrophoresis.[15] The Ca^{2+}-activated ATPase activity of actinomyosin and myosin was determined as previously described.[12,13] The Ca^{2+}-activated ATPase of actinomyosin was measured under the following conditions: 0.18 M KCl, 0.05 M Tris-maleate (pH 7.6), 0.01 M $CaCl_2$, and 0.002 M ATP. The assay was performed using 250 μg purified actinomyosin in a final volume of 1 ml for 15 min at 30°C. The reaction was stopped with the addition of 0.5 ml of ice-cold 10% trichloroacetic acid. The assay conditions for Ca^{2+}-activated ATPase of purified myosin were: 0.6 M KCl, 0.05 M Tris (pH 7.6), 0.01 M $CaCl_2$, 0.005 M dithiothreitol, and 0.005 M ATP. Per assay, 100 μg purified myosin was incubated in the final volume of 1 ml for 10 min at 30°C. Under the indicated "low ionic strength" assay conditions (0.18 M KCl) the actin-activated ATPase of myosin is measured in determinations using actinomyosin as starting material.[16] The assays using purified myosin measure the Ca^{2+}-activated ATPase of myosin.[16] Inorganic phosphate was determined by the method of Fiske and Subbarow.[17] Protein concentrations were determined by the Biuret method.[18] To check the specificity of actinomyosin ATPase, its activity was determined in the absence of calcium and the presence of 10^{-4} M EDTA. Under these conditions, ATPase activity was completely abolished.

A modification of the pyrophosphate polyacrylamide electrophoresis technique described by Hoh[8] was used to separate ventricular myosin into its three components, V_1, V_2, and V_3. The modification consisted of the inclusion of 0.25% agarose in the gel to prevent melting of the gels during the run, and the addition of 1 mM EDTA and 0.01% mercaptoethanol to the electrophoresis buffer as described by d'Albis et al.[9] Ca^{2+}-activated ATPase was determined in the three forms of myosin separated on polyacrylamide gels as described by Hoh.[8] The ratio of the peak height of the $Ca_3(PO_4)_2$ peak to the peak height of the protein peak was used to determine the specific activity of Ca^{2+} ATPase in arbitrary units (U) in V_1, V_2, and V_3 after 24 h of electrophoresis. The mean Ca^{2+} ATPase activity after 3 h of electrophoresis was determined from the ratio of the area under the calcium phosphate peak to the area under the protein peak. The areas under the peaks were planimetered by hand with a Lasico planimeter. The percent of total myosin represented by V_1, V_2, and V_3 myosin in control and diabetic rats was determined from the area under the protein peaks.

RESULTS

To gain further insight into the biochemical basis of the decreased contractility occurring in diabetic hearts, the activity of Ca^{2+}-activated ATPase of actinomyosin and myosin was determined. The Ca^{2+} ATPase activities of actinomyosin and myosin in control and diabetic animals are shown in Table 1. In control rats, actinomyosin Ca^{2+} ATPase was 0.59 ± 0.05 μmol Pi/mg protein/min and showed a 35% decrease to 0.38 ± 0.04 μmol Pi/mg protein/min in diabetic animals. A 45% decrease occurred in the activity of Ca^{2+} ATPase of purified myosin. In control animals, myosin ATPase activity was 1.25 ± 0.09 μmol Pi/mg protein/min and decreased to 0.67 ± 0.05 μmol Pi/mg protein/min in diabetic hearts. The lower ATPase activity of diabetic myosin is most likely not due to proteolytic damage. Myosin isolated from control and diabetic animals showed an identical migration pattern of one myosin heavy chain and two light chains on SDS electrophoresis. Occasionally, several faint bands were visible directly below the heavy chain in control and diabetic myosin;[19] however, this did not result in a change of ATPase activity. In addition, the inclusion of the protease inhibitor PMSF during the isolation procedure and ATPase assay did not alter the results.

TABLE 1
Activity of Ca^{2+}-activated ATPase of actinomyosin (AM) and myosin (M) in control and diabetic rat hearts

	Ca^{2+} ATPase AM (μmol Pi/mg protein/min)	Ca^{2+} ATPase M (μmol Pi/mg protein/min)
Control	0.59 ± 0.05 (6)	1.25 ± 0.09 (8)
Diabetes	0.38 ± 0.04 (6)*	0.67 ± 0.05 (8)*

To determine whether changes in the distribution of myosin components occur in diabetic rat hearts, ventricular myosin was separated into its three components by nondenaturing pyrophosphate polyacrylamide gel electrophoresis.[8] In agreement with the findings of others, we observed in a highly reproducible manner three components, V_1, V_2, and V_3 in rat ventricles after 24 h of electrophoresis (Figure 1). V_1 myosin was found to have the highest mobility and V_3 the lowest. In control animals, V_1 myosin predominates and represents 72% of total myosin. A redistribution of myosin isoenzymes occurs in diabetic animals. After 4 wk of diabetes, V_3 was the predominant form and represented 68% of all myosin (Figure 1, Table 2). The ATPase activity was then quantitated in electrophoretically separated myosin components to see if the predominance of V_3 myosin in the diabetic heart may account for the decreased Ca^{2+} ATPase activity observed in chemically purified diabetic myosin. Determination of ATPase activity of V_1 myosin in control rats gave an activity of 1.27 ± 0.3 arbitrary units. The accurate determination of the Ca^{2+} ATPase activity in the minor components, V_2 and V_3, was difficult because the calcium phosphate precipitates could not be adequately resolved as individual peaks separated by distinct valleys from the adjacent components. Resolution of coomassie blue-stained protein bands of V_1, V_2, and V_3 presented no problem, as shown in Figure 1. Using the peak height of absorbance for each component, the specific activity of ATPase was 0.6 ± 0.14 U for V_2 and 0.16 ± 0.08 U for V_3 myosin. These values are in good agreement with those found by other investigators for the myosin components of control animals.[8] In diabetic animals, ATPase activity of the predominant V_3 component was 0.17 ± 0.03 U. It is similar to the value obtained for V_3 in control animals. The Ca^{2+} ATPase value for V_1 myosin was 1.35 ± 0.3 U and 0.7 ± 0.15 U for V_2 myosin in diabetic hearts. The ATPase activities of V_1, V_2, and V_3 myosin were determined in five control and diabetic animals and were significantly different from each other ($P < 0.001$). However, V_1 ATPase activity in control and diabetic animals was not significantly different. The same was true for V_2 and V_3 ATPase activity.

It is possible to calculate the mean ATPase activity for electrophoretically purified myosin using the specific ATPase activity of the components and their predominance (Table 2). The calculated mean ATPase activity for control rats is 1.02 U versus 0.43 U for diabetic animals.

Because it was difficult to accurately determine the spe-

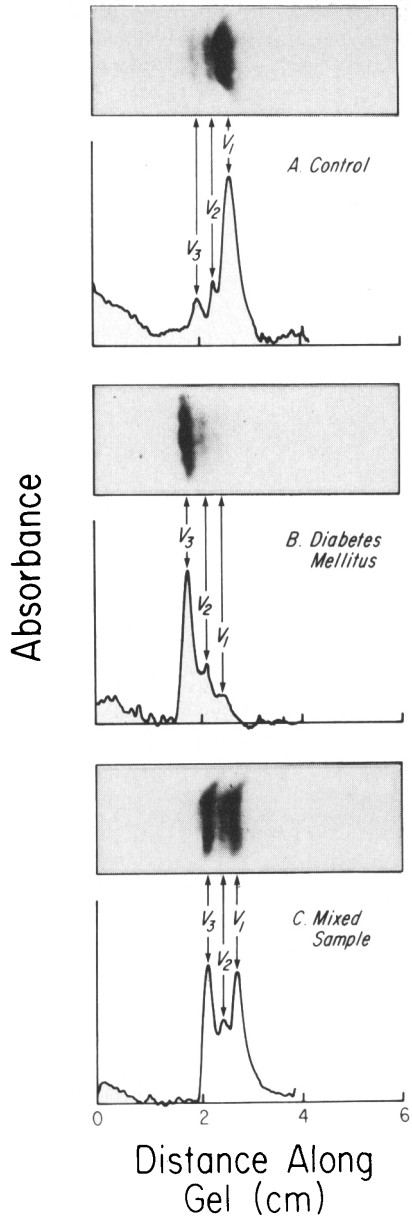

FIGURE 1. Typical electrophoretic pattern and densitometric profile of the three components of myosin from ventricles of control and diabetic hearts. Contractile proteins extracted with 100 mM pyrophosphate served as starting material. Protein bands were stained with coomassie blue and densitometrically scanned. In control hearts (8 wk old), V_1 myosin predominates (panel A), whereas in litter-matched rats that were diabetic for 4 wk, V_3 myosin is the predominant form (panel B). Mixing of control and diabetic cardiac proteins led to the myosin distribution pattern shown in panel C. Similar patterns were observed when purified myosin was used as starting material.

TABLE 2
Distribution of myosin components in control and diabetic hearts

Myosin component	Control Percent of total myosin	Diabetes Percent of total myosin
V_1	72 ± 9	15 ± 2
V_2	15 ± 5	17 ± 4
V_3	13 ± 3	68 ± 8

cific ATPase activities in the less predominant components of myosin, we took the following approach to quantitate the Ca^{2+} ATPase of electrophoretically purified myosin. Electrophoretic purification of equal amounts of control and diabetic myosin was performed for only 3 h. During the relatively brief period of electrophoresis, the individual myosin components were not resolved so that a mean ATPase activity of the myosin component was obtained. The Ca^{2+} ATPase of electrophoretically purified myosin was 1.13 ± 0.14 U in control ventricles and 0.4 ± 0.05 U in diabetic ventricles. These values are in good agreement with the calculated values.

That the three myosin components do not represent artifacts of the myosin isolation procedure or the gel system has been shown by Hoh[8] and d'Albis.[9] We could confirm their results. The electrophoretic pattern is highly reproducible and V_1 myosin of control and diabetic animals shows an identical migration behavior. The same is true for V_2 and V_3 myosin components. In addition, in gels of varying polyacrylamide gel concentration (3.5–6%), a similar migration pattern occurred. Rerunning of the main isoenzyme, V_1 from control rats or V_3 from diabetic rats on a second gel, led to a single protein band with identical migration distance. Identical myosin components were observed when total ventricular protein or purified myosin was used as starting material. Mixing of equal amounts of control and diabetic ventricular myosin led to the migration pattern shown in Figure 1. The V_1 and V_3 myosin of the mixed samples showed identical migration behavior and Ca^{2+} ATPase activity as V_1 and V_3 myosin of control or diabetic hearts.

To assure that changes in the myosin ATPase activity and myosin isoenzyme distribution are not due to toxic effects of streptozotocin, the following experiments were performed. Rats were made diabetic by streptozotocin administration and maintained in a diabetic state for 4 wk. At that time Ca^{2+}-activated myosin ATPase activity and myosin isoenzyme distributions were determined in one group of diabetic animals (five rats). Ca^{2+}-activated ATPase of myosin was 0.64 ± 0.07 μmol Pi/mg protein/min and the distribution of ventricular myosin was V_1, 13 ± 4%; V_2, 17 ± 6%; and V_3, 70 ± 10%. The second group of diabetic animals received 2 U PZI/day for an additional 4 wk. In the insulin-treated animals Ca^{2+}-activated myosin ATPase was 1.15 ± 0.11 μmol Pi/mg protein/min and myosin isoenzyme distribution was V_1, 67% ± 8%; V_2, 18% ± 5%; and V_3, 15% ± 5%. Insulin treatment normalized myosin ATPase activity and myosin isoenzyme distribution.

DISCUSSION

The results of our studies show that diabetes mellitus induces marked changes in the state of cardiac myosin. The Ca^{2+}-activated ATPase of purified actinomyosin and myosin is decreased in diabetic hearts by 35% and 45%, respectively, in comparison with enzyme activities in control rats. Although it appears likely that, in the intact myofibril, myosin ATPase is activated in the presence of actin and magnesium,[20] a close correlation between the activity of Ca^{2+}-activated ATPase of actinomyosin and myosin and cardiac contractility is well established.[7] It is thus likely that the decrease in enzyme activity observed in diabetic animals mediates in part the diminished contractility of the diabetic heart. Several other mechanisms may contribute to the de-

creased contractile performance of the diabetic heart. Decreases in glucose utilization[21] and increased wall stiffness of the ventricle[5] due to glycoprotein deposition in the interstitium have been reported.

To explore the basis for the decrease in Ca^{2+} ATPase activity of myosin in further detail, myosin from control and diabetic hearts was separated by pyrophosphate polyacrylamide electrophoresis[8] into three components, V_1, V_2, and V_3. A marked change in distribution of myosin components occurs in diabetic animals. V_3 myosin is the predominant component in diabetic hearts, representing 68% of total myosin, whereas in control hearts it represents only 13%. The predominance of V_3 myosin, which has a 10-fold lower ATPase activity than V_1 myosin, may account for the decreased myosin ATPase activity of diabetic myosin. The calculated mean ATPase activity of electrophoretically purified myosin and the mean ATPase activity of myosin determined after a short electrophoretic run is 60% lower in diabetic animals in comparison with control rats. This decrease is in line with the 45% decrease in ATPase activity found in chemically purified diabetic myosin.

The biochemical basis for the separation of myosin into three components on the pyrophosphate gel system has not been completely clarified. The reports by Hoh[8] and d'Albis,[9] as well as our own results, establish that the electrophoretically separated myosin components represent authentic forms of myosin and do not result from artifacts induced by the gel system or the protein isolation procedure. Furthermore, Hoh[22] could show that V_1 and V_3 myosin contain different species of myosin heavy chain and thus represent genetic isozymic forms of myosin. It is currently unclear if, in addition, different species of light chains compose V_1 and V_3 myosin. In addition, posttranslational changes (e.g., phosphorylation) may contribute to the different migration distance of V_1, V_2, and V_3 myosin during the electrophoresis.

The identical migration pattern and Ca^{2+} ATPase activity of V_1, V_2, and V_3 myosin in diabetic and control hearts makes it likely that the only alteration that diabetes induces is a change in the predominance of the V_1 and V_3 myosin components. If the decrease in myosin ATPase in diabetic animals was caused by de novo changes in myosin, like glycosylation, a different myosin component, which is not present in control animals, should have resulted.

It is currently unclear which of the pathophysiologic alterations that occur in diabetic rats mediate the changes in ATPase activity and lead to the predominance of different myosin components. These changes could result from insulin lack itself or may be due to ketosis, hyperglycemia, weight loss, or other factors. It is unlikely that these changes are caused by a toxic extrapancreatic effect of streptozotocin because they are reversible by insulin administration.

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