Myosin Light-Chain Phosphorylation in Diabetic Cardiomyopathy in Rats

Xueliang Liu, Nobuakira Takeda, and Naranjan S. Dhalla

The regulatory myosin light chain (MLC) is phosphorylated in cardiac muscle by Ca^{2+}/calmodulin-dependent MLC kinase (MLCK) and is considered to play a modulatory role in the activation of myofibrillar adenosine triphosphatase (ATPase) and the process of force generation. Since the depression in cardiac contractile function in chronic diabetes is associated with a decrease in myofibrillar ATPase activity, we investigated changes in MLC phosphorylation in diabetic heart. Rats were made diabetic by injecting streptozotocin (65 mg/kg intravenously), and the hearts were removed 8 weeks later; some 6-week diabetic animals were injected with insulin (3 U/d) for 2 weeks. Changes in the relative MLC and MLCK protein contents were measured by electrophoresis and immunoblot assay, whereas phosphorylated and unphosphorylated MLCs were separated on 10% acrylamide/urea gel and identified by Western blot. MLC and MLCK contents were decreased markedly (40% to 45%) and MLC phosphorylation was decreased significantly (30% to 45%) in the diabetic rat heart homogenate in comparison to control values. The changes in MLC and MLCK content in diabetic heart were partially reversible, whereas changes in MLC phosphorylation were normalized upon treatment with insulin. These results suggest that decreased protein contents of MLC and MLCK and phosphorylation of MLC may contribute to the depression of cardiac myofibrillar ATPase activity and heart dysfunction in diabetic cardiomyopathy.

CHRONIC DIABETES in animals and humans has been reported to produce heart dysfunction.[1-5] Previous studies have shown that cardiac contractile proteins in the diabetic heart are altered with respect to myofibrillar adenosine triphosphatase (ATPase), actomyosin ATPase, and myosin ATPase activities, as well as myosin isozyme composition.[1,6-9] The changes in regulatory proteins, such as troponin I and T subunits, have also been reported in the diabetic heart.[10,11] Although these changes in myosin isozyme composition and regulatory proteins can explain the depressed myofibrillar ATPase activity and subsequent contractile dysfunction in diabetic heart, the status of myosin light chain (MLC), MLC phosphorylation, and MLC kinase (MLCK) are not known during the development of diabetic cardiomyopathy. It should be pointed out that the phosphorylation of MLC by MLCK has been shown to play a modulatory role in the generation of contractile force in vertebrate striated muscle.[12] Several investigations have provided information concerning the function of MLC phosphorylation in cardiac muscle. For example, a positive correlation was found between MLC phosphorylation and the rate of left ventricular pressure development in rats subjected to exercise.[13] Perfusion of rat hearts with some negative and positive inotropic agents for a prolonged period was observed to result in an excellent relationship between active tension and the extent of MLC phosphorylation.[14] The rate of isometric force development was also shown to be associated with an increase of MLC phosphorylation in a permeable rabbit psoas fiber preparation.[15,16] In fact, increased isometric force was reported to be induced by phosphorylation of MLC in permeable rabbit psoas fibers,[17] and a similar effect of phosphorylation on the submaximal force development was subsequently shown in skinned cardiac trabecula.[18]

A detailed analysis of the literature showed that MLC phosphorylation in both skinned skeletal and cardiac muscle fibers showed a leftward shift in the force-pCa relationship, as well as decreased cooperativity.[19] However, little information regarding the status of MLC phosphorylation in myocardium under different pathophysiological conditions is available in the literature. In particular, virtually nothing is known concerning alterations in MLCK that may occur during the development of diabetic cardiomyopathy. The present study was therefore undertaken to investigate changes in cardiac contractile activity, myofibrillar ATPase activity, protein concentrations of MLC and MLCK, and the level of MLC phosphorylation in diabetic rat heart. In addition, this study examined whether these cardiac alterations are reversible upon treatment of diabetic rats with insulin.

MATERIALS AND METHODS

Experimental Model

Male Sprague-Dawley rats weighing approximately 200 g were randomly divided into control and experimental groups. Experimental animals received an intravenous injection of 0.1-mol/L citrate-buffered streptozotocin (pH 4.5) at a dosage of 65 mg/kg body weight, whereas control animals received a similar injection of vehicle alone. In some experiments, randomly selected diabetic animals at 6 weeks after streptozotocin injection were administered subcutaneous injections of protamine zinc insulin 3 U/d for 2 weeks and labeled as the insulin-treated group. Blood samples were analyzed for glucose and insulin levels using the Worthington (Biochemical Co, Freehold, NJ) Statzyme Reagent Kit and standard radioimmunoassay techniques (Amersham, Oakville, Canada), respectively. Some of the animals were used for measurement of cardiac contractile activity in terms of the rate of contraction (+dP/dt) and rate of relaxation (−dP/dt) according to the method described previously.[20] The experimental model used in this study was similar to that used previously for establishing the presence of diabetic cardiomyopathy as indicated by alterations in cardiac function, metabolism, and ultrastructure.[1,2,4,6,21,22]

From the Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, Winnipeg; the Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada; and the Department of Internal Medicine, Aoto Hospital, Jikei University, Tokyo, Japan.

Submitted April 5, 1996; accepted July 3, 1996.

Supported by grants from the Canadian Diabetes Association, Toronto, and the Racing Automobile Memorial Foundation, Tokyo.

Address reprint requests to Naranjan S. Dhalla, PhD, MD, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, 351 Tache Ave, Winnipeg, Manitoba, Canada R2H 2A6.

0026-0495/97/4601-0014$03.00/0

Measurements of Myofibrillar and Myosin ATPase Activities

Myofibrils were isolated according to the procedure used previously.[6,9] Marker enzyme analysis and the action of various inhibitors of ATPase activity[4,20,21] showed that the myofibrillar preparations from control and diabetic hearts were completely devoid of contamination by mitochondria, sarcolemma, and sarcoplasmic reticulum. Myofibrillar Mg^{2+}-ATPase and Ca^{2+}-ATPase activities were determined according to the methods used previously.[6,9] Myosin was purified as described previously,[23] and myosin Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were measured according to procedures described elsewhere.[7,8,24]

MLC Phosphorylation

The muscle (20 to 30 mg) was homogenized with a Brinkmann (Mississauga, Canada) homogenizer with Kinematica 87/Polytron PTA 7K1 for 4 to 6 seconds in the buffer (100 μL buffer/2 mg muscle) containing 60 mmol/L KCl, 1 mmol/L cysteine, 20 mmol/L imidazole (pH 6.9), 1 mmol/L $MgCl_2$, 1 mmol/L ouabain, 10 mmol/L NaN_3, 1 mmol/L $CaCl_2$, 0.01% leupeptin, 250 μmol/L phenylmethylsulfonyl fluoride, and 1 mmol/L dithiothreitol (DTT). The level of MLC phosphorylation in the homogenate was estimated by monitoring the ratio of phosphorylated and total (phosphorylated plus unphosphorylated) MLC.[25-27] Tissue homogenate (10 mg per assay tube) was equilibrated for 10 minutes at 30°C before initiating the assay procedures. For the MLC phosphorylation reaction, all samples were separated into two sets of assay tubes: one set contained 1 mmol/L Mg^{2+}-ATP plus 1 mmol/L $CaCl_2$ for maximal activity, and the other set had no Mg^{2+}-ATP and $CaCl_2$. The samples were further incubated at 30°C for 10 minutes and the reaction was stopped by adding ice-cold 10% (wt/vol) trichloroacetic acid (TCA). The TCA-precipitated homogenate was centrifuged at 15,000 × *g* for 30 minutes, and then the pellet was suspended in acetone containing 10 mmol/L DTT and placed on a rotator for 1 hour at room temperature. These samples were centrifuged, and the pellets were solubilized in 400 μL of the buffer (6.4 mmol/L urea, 17 mmol/L Tris hydrochloride, 19.5 mmol/L glycine [pH 8.6], 10 mmol/L DTT, 10 mmol/L EGTA, 1 mmol/L EDTA, 5 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, and 0.04% bromophenol blue in final concentration) by suspending and placing the tubes on a rotator for at least 2 hours. These samples were centrifuged (15,000 × *g*) for 30 minutes and then immediately loaded onto native polyacrylamide gels. Phosphorylated and unphosphorylated MLCs were separated by 10% polyacrylamide electrophoresis.[26] All gels were subjected to preelectrophoresis for 1.5 hours at 400 V; the running-tank buffer contained 20 mmol/L Tris hydrochloride, 22 mmol/L glycine, 1 mmol/L sodium thioglycate, and 1 mmol/L DTT. Samples (60 μL) were applied to each well and subjected to electrophoresis at 350 V for 19 to 21 hours at 4°C (assays were stopped within 1 to 2 hours after the bromophenol blue tracking dye exited the gel). Separated proteins were then electroblotted onto nitrocellulose sheets using a 25-mmol/L Na_2HPO_4 transfer buffer at a current of 1.5 A and a temperature of 15°C for 1 hour. After transfer, bands of phosphorylated and unphosphorylated MLCs on nitrocellulose sheets were detected by enhanced chemiluminescence (ECL) Western blot analysis with mouse anti-MLC antibody (1:1,000; Sigma Immuno Chemicals, St Louis, MO). The imaging densitometer was used to scan the developed films and to quantify MLC phosphorylation.[27]

Analysis of MLC and MLCK

The relative protein content in control, diabetic, and insulin-treated cardiac muscle homogenates was obtained by 12% mini–sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure described previously.[27] The proteins in homogenate separated by SDS-PAGE were electroblotted onto Immobilon-P transfer membrane (Millipore, Bedford, MA) in a transfer buffer that contained 25 mmol/L Tris hydrochloride, 120 mmol/L glycine, and 20% methanol (vol/vol) for the determination of relative contents with immunoblotting analysis.[28] The transferred membranes were incubated for 1 or 2 hours at room temperature with monoclonal mouse anti-MLC antibody (1:1,000; Sigma Immuno Chemicals) or mouse anti-MLCK antibody (1:1,000; Sigma Immuno Chemicals). The transferred membranes were subsequently incubated with biotinylated anti–mouse IgG (1:1,000; Amersham) for 40 minutes, and then finally with streptavidin-conjugated horseradish peroxidase (1:5,000; Amersham) for 40 minutes. For ECL detection, the membrane sheets were developed on Hyperfilm-ECL (Amersham) to visualize proteins. The relative protein content was determined by a model GS-670 imaging densitometer (Bio-Rad, Mississuaga, Canada) with Image Analysis Software Version 1.0.[27]

Data Analysis

The data are expressed as the mean ± SE. An unpaired Student's *t* test was used to examine the difference between control and experimental samples. A *P* value less than .05 was taken to represent a significant difference.

RESULTS

General Characteristics, Cardiac Performance, and Myofibrillar/Myosin ATPase Activities of Diabetic and Insulin-Treated Animals

In comparison to control animals, body weight and ventricular growth were significantly decreased and the ventricular weight to body weight ratio was increased in rats 8 weeks after streptozotocin injection (Table 1). In diabetic animals, plasma glucose was increased markedly and plasma insulin was depressed compared with control values. Daily injection of insulin in diabetic animals for 2 weeks normalized plasma glucose and insulin concentrations and ventricular weight and the ratio of ventricular weight to body weight; however, insulin-treated diabetic rats still had a lower body weight. Diabetic rats exhibited a depression of +dP/dt and −dP/dt compared with control animals; depressed +dP/dt and −dP/dt values in diabetic hearts were reversed by insulin treatment (Table 1). These characteristics are similar to those reported for diabetic animals from this laboratory and others.[2,4,6,8,9]

Table 2 shows that myofibrillar Mg^{2+}-ATPase and Ca^{2+}-ATPase activities were depressed in diabetic animals, and that these effects were reversed by treatment of diabetic rats with insulin. Myosin isolated from the diabetic heart exhibited significantly lower Ca^{2+}-ATPase and Mg^{2+}-ATPase activities than control preparations, but insulin administration to diabetic rats resulted in normalization of these activities. Control values for myofibrillar and myosin ATPase activities and the changes in diabetic hearts are similar to those reported previously.[9,29]

Table 1. General Characteristics of the Control and Experimental Animals

Characteristic	Control	Diabetic	Diabetic + Insulin
Body weight (g)	556 ± 23	309 ± 18*	362 ± 26*
Ventricular weight (mg)	1,079 ± 197	848 ± 38*	1,020 ± 28
Ventricular weight/body weight (mg/g)	2.27 ± 0.04	2.75 ± 0.060*	2.40 ± 0.064
Plasma glucose (mg/dL)	159 ± 9.3	498 ± 5.2*	192 ± 7.1
Plasma insulin (μU/mL)	29 ± 2.9	12 ± 0.9*	34 ± 1.2
+dP/dt (mm Hg/s)	5,624 ± 253	3,578 ± 212*	4,790 ± 256
−dP/dt (mm Hg/s)	5,498 ± 123	3,258 ± 221*	4,580 ± 134

NOTE. Values are the mean ± SE of 6 experiments.
*$P < .05$, significantly different from control.

Table 2. Myofibrillar and Myosin ATPase Activities in Control and Experimental Animals

Activity	Control	Diabetic	Diabetic + Insulin
Myofibrillar (nmol P_i/mg/5 min)			
Mg^{2+}-ATPase	211 ± 12	148 ± 7*	184 ± 10
Ca^{2+}-ATPase	888 ± 28	562 ± 33*	847 ± 31
Myosin (μmol P_i/mg/min)			
Ca^{2+}-ATPase	1.21 ± 0.03	0.53 ± 0.04*	1.01 ± 0.03
Mg^{2+}-ATPase	0.229 ± 0.007	0.149 ± 0.002*	0.212 ± 0.004

NOTE. Values are the mean ± SE of 6 experiments.
*$P < .05$, significantly different from control.

Phosphorylation of MLC

Figure 1 shows the representative immunoblots of unphosphorylated (upper bands) and phosphorylated (lower bands) MLC in homogenates without (Fig 1A) and with (Fig 1B) Mg^{2+}-ATP plus $CaCl_2$ from control, diabetic, and insulin-treated diabetic hearts. Figure 2 shows the data regarding the ratios of phosphorylated and total (phosphorylated plus unphosphorylated) MLC. There was a significant increase in the phosphorylation level of MLC (30% to 45%) in homogenates with Mg^{2+}-ATP plus $CaCl_2$ compared with the respective homogenates without Mg^{2+}-ATP plus $CaCl_2$ in control, diabetic, and insulin-treated diabetic animals. However, in homogenates either without (Fig 2A) or with (Fig 2B) Mg^{2+}-ATP plus $CaCl_2$, the phosphorylation level in the homogenate of diabetic

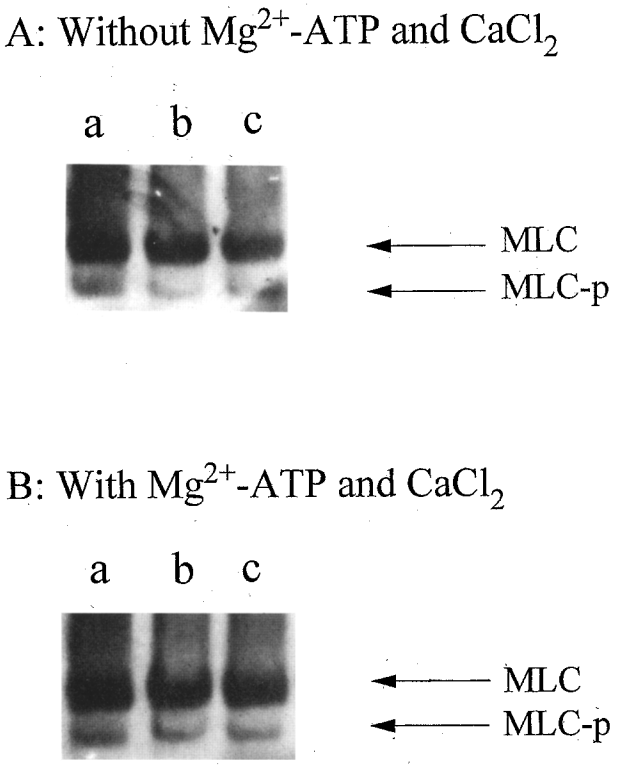

Fig 1. Western blot analysis of unphosphorylated and phosphorylated MLC in cardiac muscle homogenates. MLC phosphorylation in control (a), diabetic (b), and insulin-treated diabetic (c) cardiac muscle homogenates without (A) and with (B) Mg^{2+}-ATP plus $CaCl_2$. MLC, unphosphorylated MLC; MLC-p, phosphorylated MLC.

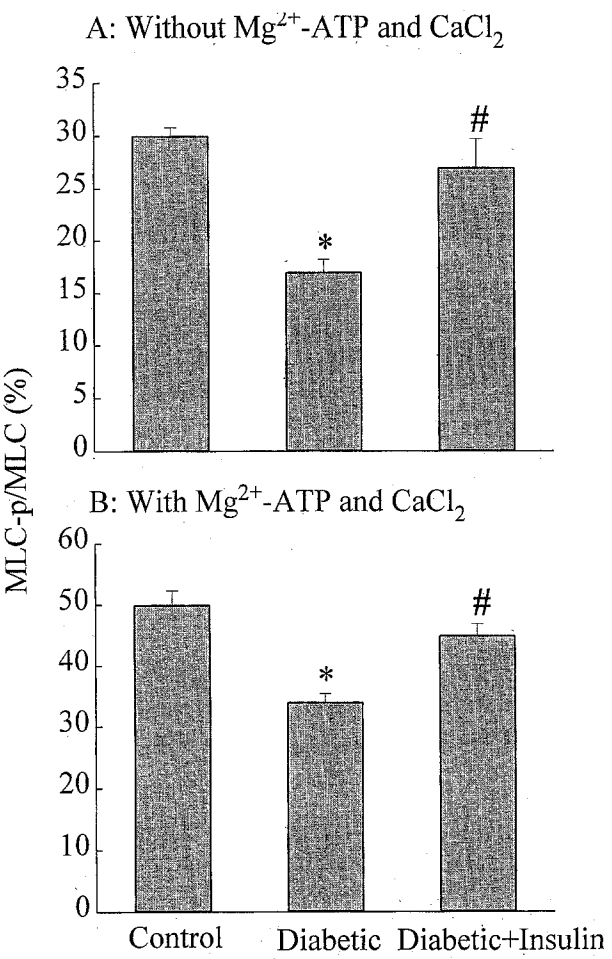

Fig 2. MLC phosphorylation in the homogenate of control, diabetic, and insulin-treated diabetic rats. Phosphorylation of MLC in cardiac muscle homogenates without (A) and with (B) Mg^{2+}-ATP plus $CaCl_2$. *$P < .05$ *v* control; #$P < .05$ *v* diabetic. Each value is the mean ± SE of 6 separate experiments.

cardiac muscles was decreased by 30% to 45% in comparison to the control values. This decrease in phosphorylation of MLC in diabetic animals was normalized by insulin administration to the diabetic rats.

Relative MLC and MLCK Protein Contents

MLC and MLCK in the cardiac muscle from control, diabetic, and insulin-treated diabetic rats were identified by ECL Western blot. The representative bands for MLC and MLCK with a molecular weight of approximately 18.5 and 70 kD are shown in Figs 3 and 4, respectively. Laser densitometric analysis of MLC bands showed a significant decrease in MLC protein content (~40%) in the homogenate of diabetic heart in comparison to the controls (Fig 3). Figure 4 shows that the MLCK protein content in the homogenate of diabetic heart was decreased by approximately 50% compared with control values. Insulin administration to diabetic rats resulted in partial normalization of the decreased relative protein contents of MLC and MLCK in diabetic heart homogenate (Figs 3 and 4).

DISCUSSION

The presence of cardiac dysfunction including a depression in contractile force generation, cardiac relaxation, and cardiac

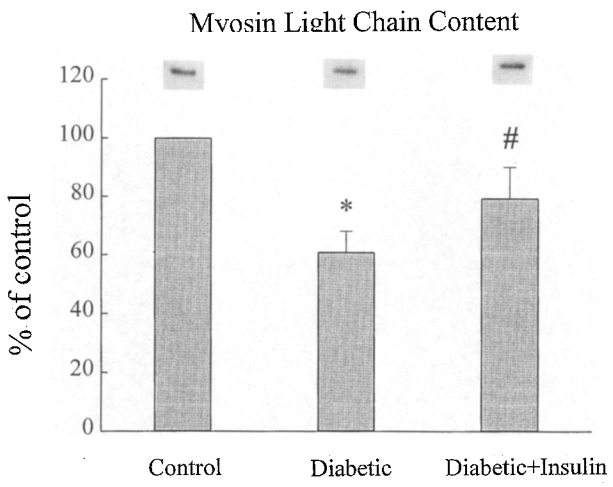

Fig 3. Semiquantification of MLC protein in cardiac muscle homogenate with immunoblot analysis. A typical immunoblot and the analysis of results for relative protein content of MLC in control, diabetic, and insulin-treated diabetic hearts are shown. Each value is the mean ± SE of 6 separate experiments. *$P < .05$ *v* control; #$P < .05$ *v* diabetic.

pumping has been reported in clinical and experimental chronic diabetes.[1,5,30,31] This dysfunction in diabetic cardiomyopathy is associated with a decrease in the activity of myofibrillar and myosin ATPase.[1,7-9,29] In the present study, we confirmed the occurrence of a significant decrease in myocardial function, as reflected by the depressed +dP/dt and −dP/dt and a significant depression in myofibrillar and myosin Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in the diabetic animals. Previous investigations have shown that the depressed myofibrillar and myosin ATPase activities in diabetic animal hearts may be related to changes in the myosin isozyme composition[7,8] and in the troponin I and T subunits.[10,11] However, in this study we observed decreased MLC phosphorylation in the homogenate of diabetic heart. Since MLC phosphorylation is considered to play a modulatory role in the interaction between actin and myosin, changes in MLC phosphorylation activity can be seen to alter the myofibrillar or myosin ATPase activities. In this regard, biochemical studies with purified myosin and actin have shown an increase in actomyosin ATPase activity by MLC phosphorylation, and this change was shown to be due to an increase in the affinity of myosin for actin.[32-34] Some investigators have shown no changes in myosin ATPase activity,[35] but others have reported a depression in myofibrillar ATPase activity upon MLC phosphorylation.[36,37] These variable effects of MLC phosphorylation were considered to be due to aging of the myosin preparations, as well as the assay conditions,[34,38] and thus are difficult to interpret in terms of the myosin and actin interaction.

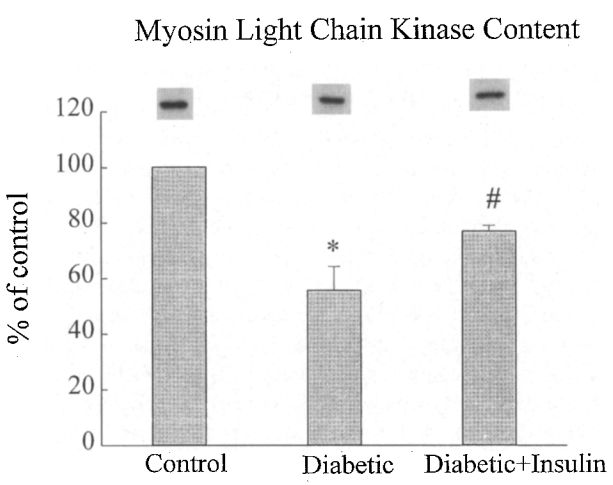

Fig 4. Semiquantification of MLCK protein in cardiac muscle homogenate. A typical immunoblot and the analysis of results for relative protein content of MLCK in homogenate of control, diabetic, and insulin-treated diabetic hearts are shown. Each value is the mean ± SE of 6 separate experiments. *$P < .05$ *v* control; #$P < .05$ *v* diabetic.

The depressed MLC phosphorylation observed in the homogenate of diabetic heart may partly explain the depression in myofibrillar and myosin ATPase activities and the impaired contractile function in chronic diabetes. It should be pointed out that some investigators have reported that the rates of MLC phosphorylation and dephosphorylation are low because of the small amounts of both MLCK and MLC phosphatase present in cardiac muscle.[39,40] Furthermore, the degree of MLC phosphorylation is dependent on the concentrations of MLC and MLCK, as well as the dynamics of changes in Ca^{2+}/calmodulin concentrations and MLC phosphatase activity in the tissue. Since the data in this study have revealed a decrease in the protein contents of MLC and MLCK in the diabetic heart, it appears that the observed decrease in the contents of MLC and MLCK in homogenates of diabetic rat heart may explain the decreased level of MLC phosphorylation. On the other hand, changes in Ca^{2+}/calmodulin concentrations may not be responsible for the observed alterations, because the decrease in MLC phosphorylation in the diabetic heart homogenate with and without Mg^{2+}-ATP plus $CaCl_2$ was similar (30% to 45%) in the diabetic animals. Since the level of MLC phosphorylation attained reflects the end result of the relative activities of MLCK and MLC phosphatase, experiments are needed to identify whether any change in MLC phosphatase content or activity occurs in cardiac muscle of diabetic animals.

Treatment of diabetic rats with insulin, which has been reported to reverse the depressed myofibrillar and myosin ATPase activities and heart dysfunction,[6-9] was observed to reverse the decreased level of MLC phosphorylation and the decreased concentrations of MLC and MLCK. This can be seen to provide evidence that depression of MLC phosphorylation may represent one of the mechanisms associated with decreased myofibrillar and myosin ATPase activities in the diabetic heart. Our experiments concerning changes in plasma glucose and insulin levels in diabetic animals with or without insulin treatment indicate that the observed decrease in MLC phosphorylation may be a consequence of metabolic stress; however, the exact metabolic signal leading to changes in regulatory proteins affecting MLC phosphorylation remains unclear at present. Although the approach used here may provide some indication for the modification of MLC phosphorylation to occur in vivo, it should be noted that the analysis of MLC phosphorylation in this study is of indirect nature and may not reflect the in vivo situation. Thus, some caution should be exercised in interpreting the data, considering the technical difficulties of directly quantifying the extent of MLC phosphorylation in vivo.

REFERENCES

1. Fein FS, Strobeck JE, Malhotra A, et al: Reversibility of diabetic cardiomyopathy with insulin in rats. Circ Res 49:1251-1261, 1981
2. Penpargkul S, Schaible T, Yipintsoi T, et al: The effect of diabetes on performance and metabolism of rat hearts. Circ Res 47:911-921, 1980
3. Regan TJ, Wu CF, Yeh CK, et al: Myocardial composition and function in diabetes. Circ Res 49:1268-1277, 1981
4. Ganguly PK, Pierce GN, Dhalla KS, et al: Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am J Physiol 244:E528-E535, 1983
5. Dhalla NS, Pierce GN, Innes IR, et al: Pathogenesis of cardiac dysfunction in diabetes mellitus. Can J Cardiol 1:263-281, 1985
6. Pierce GN, Dhalla NS: Mechanisms of the defect in cardiac myofibrillar function during diabetes. Am J Physiol 248:E170-E175, 1985
7. Dillmann WH: Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes 29:579-582, 1980
8. Malhotra A, Penpargkul S, Fein H, et al: The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. Circ Res 49:1243-1250, 1981
9. Pierce GN, Dhalla NS: Cardiac myofibrillar ATPase activity in diabetic rats. J Mol Cell Cardiol 13:1063-1069, 1981
10. Liu X, Takeda N, Dhalla NS: Troponin I phosphorylation in heart homogenate from diabetic rat. Biochim Biophys Acta 1316:78-84, 1996
11. Akella ÁB, Ding XL, Cheng R, et al: Diminished Ca^{2+} sensitivity of skinned cardiac muscle contractility coincident with troponin T-band shifts in the diabetic rat. Circ Res 76:600-606, 1995
12. Sweeney HL, Bowman BF, Stull JT: Myosin light chain phosphorylation in vertebrate striated muscle: Regulation and function. Am J Physiol 264:C1085-C1095, 1993
13. Fitzsimons DP, Bodell PW, Baldwin KM: Phosphorylation of rodent cardiac myosin light chain 2: Effects of exercise. J Appl Physiol 67:2447-2453, 1989
14. Kopp SJ, Bárány K: Phosphorylation of the 19,000-dalton light chain of myosin in perfused rat heart under the influence of negative and positive inotropic agents. J Biol Chem 254:1207-1212, 1979
15. Metzger JM, Greaser ML, Moss RL: Variations in cross-bridge attachment rate and tension with phosphorylation potentiation in intact muscle. J Gen Physiol 93:855-883, 1989
16. Sweeney HL, Stull JT: Alteration of cross-bridge kinetics by myosin light chain phosphorylation in rabbit skeletal muscle: Implications for regulation of actin-myosin interaction. Proc Natl Acad Sci USA 87:414-418, 1990
17. Persechini A, Stull JT, Cooke R: The effect of myosin phosphorylation on the contractile properties of skinned rabbit skeletal muscle fibers. J Biol Chem 260:7951-7954, 1985
18. Morano I, Rüegg JC: Calcium sensitivity of myofilaments in cardiac muscle—Effect of myosin phosphorylation. Basic Res Cardiol 81:17-23, 1986
19. Sweeney HL, Stull JT: Phosphorylation of myosin in permeabilized mammalian cardiac and skeletal muscle cells. Am J Physiol 250:C657-C660, 1986
20. Dixon IMC, Hata T, Dhalla NS: Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ Res 66:782-788, 1990
21. Pierce GN, Dhalla NS: Sarcolemmal Na^+-K^+-ATPase activity in diabetic rat heart. Am J Physiol 245:C241-C247, 1983
22. Pierce GN, Kutryk MJB, Dhalla NS: Alterations in Ca^{2+} binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc Natl Acad Sci USA 80:5412-5416, 1983
23. Bhan AK, Malhotra A: Trypsin digestion of canine cardiac myosin. Low molecular weight fragment of myosin with high adenosine triphosphatase activity. Arch Biochem Biophys 174:27-35, 1976
24. Zak B, Epstein E, Baginski ES: Determination of liver microsomal glucose-6-phosphatase. Ann Clin Lab Sci 7:160-177, 1977
25. Sobieszek A, Jetschin P: Urea-glycerol-acrylamide gel electrophoresis of acidic low molecular weight muscle proteins: Rapid determination of myosin light chain phosphorylation in myosin, actomyosin and whole muscle samples. Electrophoresis 7:417-425, 1986
26. Hathaway DR, Haeberle JR: A radioimmunoblotting method of measuring myosin light chain phosphorylation levels in smooth muscle. Am J Physiol 249:C345-C351, 1985
27. Liu X, Shao Q, Dhalla NS: Myosin light chain phosphorylation in cardiac hypertrophy and failure due to myocardial infarction. J Mol Cell Cardiol 27:2613-2631, 1995
28. Burnette WN: 'Western blotting': Electrophoretic transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 112:195-203, 1981
29. Rubinstein M, Schaible TF, Malhotra A: Effects of graded insulin therapy on cardiac function in diabetic rats. Am J Physiol 246:H453-H458, 1984
30. Makino N, Dhalla KS, Elimban V, et al: Sarcolemmal Ca^{2+} transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am J Physiol 253:E202-E207, 1987
31. Regan TJ, Lyons MM, Ahmed SS, et al: Evidence for cardiomyopathy in familial diabetic mellitus. J Clin Invest 60:885-899, 1977
32. Michnicka M, Kasman K, Kakol I: The binding of actin to phosphorylated and dephosphorylated myosin. Biochim Biophys Acta 704:470-475, 1982
33. Pemrick SM: The phosphorylated L_2 light chain of skeletal myosin is a modifier of the actomyosin ATPase. J Biol Chem 255:8836-8841, 1980
34. Persechini A, Stull JT: Phosphorylation kinetics of skeletal muscle myosin and the effect of phosphorylation on actomyosin adenosine triphosphatase activity. Biochemistry 23:4144-4150, 1984
35. Morgan M, Perry SV, Ottaway J: Myosin light-chain phosphatase. Biochem J 157:687-697, 1976
36. Coole R, Franks K, Stull JT: Myosin phosphorylation regulates the ATPase activity of permeable skeletal muscle fibers. FEBS Lett 144:33-37, 1982
37. Kakol I, Kasman K, Michnicka M: The phosphorylation-dephosphorylation process as a myosin-linked regulation of superprecipitation of fast skeletal muscle actomyosin. Biochim Biophys Acta 704:437-443, 1982
38. Stepkowski D, Szczesna D, Wrotek M, et al: Factors influencing interaction of phosphorylated and dephosphorylated myosin with actin. Biochim Biophys Acta 831:321-329, 1985
39. Herring BP, England PJ: The turnover of phosphate bound to myosin light chain-2 in perfused rat heart. Biochem J 240:205-214, 1986
40. Stull JT, Sweeney HL, Kamm KE: Myosin light chain phosphorylation in striated and smooth muscles, in Taylor AW, et al (eds): Biochemistry of Exercise, vol 21 (ed 7). Champaign, IL, Human Kinetics, 1990, pp 195-208