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Minimal Amount of Insulin Can Reverse Diabetic Heart Function: Sarcoplasmic Reticulum Ca^{2+} Transport and Phospholamban Protein Expression

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In the present study, the underlying mechanisms for diabetic functional derangement and insulin effect on diabetic cardiomyopathy were investigated with respect to sarcoplasmic reticulum (SR) Ca^{2+}-ATPase and phospholamban at the transcriptional and translational levels. The maximal Ca^{2+} uptake and the affinity of Ca^{2+}-ATPase for Ca^{2+} were decreased in streptozotocin-induced diabetic rat cardiac SR, however, even minimal amount of insulin could reverse both parameters. Levels of both mRNA and protein of phospholamban were significantly increased in diabetic rat hearts, whereas the mRNA and protein levels of SR Ca^{2+}-ATPase were significantly decreased. In case of phospholamban, insulin treatment reverses these parameters to normal levels. Minimal amount of insulin could reverse the protein levels; however, it could not reverse the mRNA level of SR Ca^{2+}-ATPase at all. Thus, the decreased SR Ca^{2+} uptake appear to be largely attributed to the decreased SR Ca^{2+}-ATPase level, which is further impaired due to the inhibition by the increased level of phospholamban. These results indicate that insulin is involved in the control of intracellular Ca^{2+} in the cardiomyocyte through multiple target proteins via multiple mechanisms for the decrease in the mRNA for both SR Ca^{2+}-ATPase and phospholamban which are unknown and needs further study.

Key Words: Phospholamban, SR Ca^{2+}-ATPase, Heart, Diabetes, Mellitus, Insulin

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease. Virtually all forms of a diabetes mellitus are due to a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin (insulin resistance). Insulin elicits a remarkable array of biological responses. The important target tissues for regulation of glucose homeostasis by insulin are liver, muscle, and fat, but insulin exerts potent regulatory effects on other cell types as well. Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. Thus, in the untreated or undertreated diabetic patient, hypertriglyceridemia and hypercholesterolemia often occur. However, the exact mechanism by which these effects are accomplished is not known.

An almost pathognomonic feature of diabetes mellitus is thickening of the capillary basement membrane and other vascular changes that occur during the course of the disease. The cumulative effect is progressive narrowing of the vessel lumina, causing inadequate perfusion of critical regions of certain organs. The matrix is expanded in many vessel walls, in the basement membrane of the retina (Kohner et al, 1982), and in the mesangial cells of the kidney, many large vessels further contributes to luminal narrowing. These pathologic changes contribute to

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some of the major complications of diabetes, including premature atherosclerosis, intercapillary glomerulosclerosis, retinopathy, neuropathy, and ulceration and gangrene of the extremities.

In diabetic patients, cardiovascular complication is a major contributor to mortality and morbidity (Dhalla et al, 1985; Dillmann, 1989). Coronary heart diseases and autonomic neuropathies have been suggested to be the major factors of diabetic dysfunctions of the heart. However, this study is focused on a diabetic cardiomyopathy leading to congestive failure independent of coronary vascular disease (Dillmann, 1989; Shehadeh & Regan, 1995). The abnormality was considered to be most likely due to the decreased myocardial compliance with/without the diminished contractility. A echocardiographic study showed the existence of a distinct diabetic cardiomyopathy (Golderisi et al, 1991), such as the increase in left ventricular mass and wall thickness. Diabetic cardiomyopathy has been suggested to be caused by the intracellular Ca^{2+} overload in the myocardium. Cardiomyopathy in chronic diabetes is associated with the alterations in the sarcolemmal membrane function and in myosin ATPase activity (Garber et al, 1983; Makino et al, 1987) and the defective SR Ca^{2+} uptake and Ca^{2+}-ATPase activity (Afzal et al, 1989). The cause of the decreased SR Ca^{2+}-ATPase activity is not completely clear.

Recently, several studies using the phospholamban-deficient mouse showed that phospholamban is the major regulator of the isoproterenol-mediated cardiac contraction and relaxation (Luo et al, 1994; Hoit et al, 1995; Kiss et al, 1997). During cardiac muscle contraction and relaxation, Ca^{2+} is released from the sarcoplasmic reticulum (SR) for contraction and transported into the SR for relaxation. Ca^{2+} transport across the SR is regulated by the phosphorylation of phospholamban. Dephosphorylated phospholamban is an inhibitor of cardiac SR Ca^{2+}-ATPase, and upon phosphorylation of phospholamban the inhibitory effect is relieved, and this stimulates SR Ca^{2+} transport (Kim et al, 1990; Kim, 1992). The stimulatory effects of the protein kinases on SR function can be reversed by a protein phosphatase which dephosphorylates phospholamban (Kranias & Di Salvo, 1986; Kranias et al, 1988). Therefore, phospholamban appears to be an important regulator of SR function and, thus, a modulator of cardiac muscle function in general, through phosphorylation/dephosphorylation. Previously, we reported that the phosphorylation levels of phospholamban were increased in diabetic cardiac SR, however, phosphatase treatment of phospholamban prior to phosphorylation did not change the level of phosphorylation (Kim et al, submitted). This results suggest that the increased level of phospholamban phosphorylation in diabetic rat may not be due to the autonomic alterations.

Although a defect in the capacity of the cardiac SR isolated from chronically diabetic rats to accumulate Ca^{2+} has been observed, its direct relationship to the phospholamban regulation on the SR Ca^{2+}-ATPase has not been established yet. Therefore, to determine whether the decrease of the cardiac SR function in streptozotocin-induced diabetic rat is associated with changes in levels of both phospholamban and SR Ca^{2+}-ATPase mRNA as well as their protein, RNA dot blot and quantitative immunoblot analyses were studied in diabetic rat heart.

METHODS

Animals

Male Sprague-Dawley rats (8 wk old) weighing about 250 g were randomly divided into two groups. One group was made diabetic with a single injection of streptozotocin (65 mg/kg i.p.) dissolved in citrate buffer (pH 4.5), and the other was injected with citrate buffer as a control group. The development of diabetes mellitus (DM) was confirmed by measuring urine glucose level on the 3rd day of streptozotocin injection. After confirmation of DM, rats were randomly divided into two groups, one group as a diabetic and the other as an insulin-treated diabetic. Insulin-treated diabetic group was injected with minimal amount of insulin (s.c.) throughout the experimental period, which enhances the possibility of their survival, but not enough to treat glycosuria. Rats were maintained on normal animal chow and water *ad libitum* for 12 wks. Blood samples were taken from the rats fasted for 5h and assayed for glycohemoglobin using affinity chromatography (Glyc-Affin GHb, Iso Lab Inc.; Akron, OH), and for glucose using glucose analyzer (Beckman). Routine urine analysis was done using Diastix and Ketostix (Miles Sankyo Co.; Tokyo, Japan). Since the severity of diabetes is a major determinant of myocardial damage in the rat (Thompson et al, 1991), hearts from the diabetic rats of which the glycohemoglobin level was

above 13% (control, 3~5%; DM, 13~17%) were excised and placed in ice-cold saline for 5~10 min prior to use.

Preparation of cardiac sarcoplasmic reticulum vesicles

Membrane fraction enriched with sarcoplasmic reticulum (SR) was isolated according to the method of Feher & LeBolt (1990) with slight modification. Briefly, the ventricular tissue removed from large vessels and the aorta was homogenized in a Virtis homogenizer for 60-sec (15 sec × 4 times with 15 sec intervals) in a buffer containing 10 mM imidazole, pH 7.0, 1 M KCl, and 10 mM sodium metabisulfite. The homogenate was centrifuged at 4,000 × g for 20 min, and the supernatant was centrifuged again at 11,000 × g for 25 min. The supernatant was centrifuged at 40,000 × g for 25 min and this supernatant was centrifuged again at 100,000 × g for 30 min. The resultant pellet was suspended in 10 mM imidazole, pH 7.0, 0.6 M KCl, and 0.3 M sucrose and then centrifuged at 100,000 × g for 45 min. The final pellet was suspended in 10 mM imidazole, pH 7.0, 0.1 M KCl, and 0.3 M sucrose. Protein was measured by the method of Lowry et al (1951) and the Ca^{2+} transport assay was completed within 2 h.

Determination of calcium uptake

Calcium uptake was determined at 37°C by using $^{45}CaCl_2$ and a modification of the Millipore filtration technique described by Martonosi & Feretos (1964). In the Millipore filtration assay, the rate of Ca^{2+} uptake was determined in a medium containing 50 μg of SR per ml, 0.1 M KCl, 5 mM $MgCl_2$, various amounts of $^{45}CaCl_2$, 0.5 mM EGTA, 5 mM ATP, 2.5 mM oxalate, 5 mM NaN_3, and 40 mM histidine-HCl, pH 7.0, using Millipore filters with a pore size of 0.45 μm (type GS). Calcium uptake was initiated by the addition of 5 mM ATP. The initial rates of Ca^{2+} uptake were calculated using least squares linear regression analysis of the 20-, 40-, and 60-sec values of Ca^{2+} uptake.

Western blot analysis

The phospholamban monoclonal antibody (PLB-Ab) was purchased from Upstate Biotechnology Inc. (Lake Placid, USA), and the Ca^{2+}-ATPase monoclonal antibody (SERCA-Ab) was purchased from Affinity Bioreagents Inc. (Golden, USA). The relative ratios of Ca^{2+}- ATPase and phospholamban in cardiac SR from control and diabetic rat hearts were estimated using quantitative immunoblotting. Cardiac SR proteins separated by SDS-polyacrylamide gel electrophoresis (10~18% gradient slab gels) according to the method of Laemmli (1970), were transferred electrophoretically on to polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Bedford, USA). PVDF membranes were reacted with PLB-Ab (1 : 1000 dilution) or SERCA-Ab (1 : 1000 dilution), and incubated with a 1 : 1000 dilution of the anti-mouse IgG, horseradish peroxidase-linked whole antibody (from sheep) from Amersham International (Little Chalfont, UK). Antibody binding was detected using ECL (enhanced chemiluminescence) Western blotting detection reagents were from Amersham International (Little Chalfont, UK). The degree of binding was determined using the densitometer (Bio-Rad, model GS-670) and a computer program (Image-Quant). The density associated with phospholamban was linear in the range of 10~50 μg cardiac SR protein loaded onto the gel lanes, while the corresponding linear range for the SR Ca^{2+}-ATPase was between 5 and 25 μg cardiac SR protein.

RNA isolation and dot blot analysis

Total cellular RNA of hearts was isolated from control and diabetic rats by low temperature guanidine isocyanate method (Chomczynski & Sacchi, 1987). Two-fold serial dilutions of the RNAs, starting with 10 μg, were applied to charged nylon membrane using a dot blot kit (BioRad), prehybridized and hybridized to a specific phospholamban or SR Ca^{2+}-ATPase oligonucleotide, and then 18S rRNA oligonucleotide according to manufacturer's instructions. Prehybridizations were performed in 5 × SSPE (1 × SSPE contains 150 mM sodium chloride, 10 mM sodium phosphate, and 1 mM EDTA, pH 7.4), 50% deionized formamide, 5 × Denhardt's solution, 1% SDS, and 100 μg/ml salmon sperm DNA at 42°C for 3 hrs. Hybridizations were done in the same solution without DNA at 42°C for 24 hrs with oligonucleotides specific to phospholamban or SR Ca^{2+}-ATPase at a concentration of 1×10^6 cpm/ml. The filters were washed twice in a 2 × SSPE at room temperature, once in 0.1 × SSPE and 1% SDS at 50°C for 30 min, and twice in 0.1 × SSPE at room temperature for 15

min, successively. Autoradiography was done with Kodak X-Omat AR film at −70°C for 1 day. To determine the relative RNA levels, the signals in the films were quantitated by transmittance densitometer (BioRad, model GS-670) with comparison of standards which were blotted in another filter and autoradiographed in the same film, and their radioactivities were checked by scintillation counter later. After the membranes were striped of phospholamban or SR Ca^{2+}-ATPase probe in 5 mM Tris-HCl, pH 7.5, and 2 mM EDTA, pH 8.0 at 65°C for 2 hrs, prehybridization and hybridization with a specific probe to 18S rRNA were performed, and autoradiography was done as described above. ^{32}P-labeled probe was added to hybridization solution at a concentration of 4.2×10^6 cpm/ml (1.3 pmol/ml) with cold oligonucleotide at 5 times the concentration of ^{32}P-labeled probe.

For calculation of relative amounts of phospholamban or SR Ca^{2+}-ATPase mRNAs, signals with phospholamban or SR Ca^{2+}-ATPase probes were normalized to those with the 18S rRNA probe in the same membranes. Statistical significance was determined by unpaired Student's t-test.

Oligonucleotides

Three kinds of synthetic 60-mer oligonucleotides were used in the hybridization; one is specific for phospholamban (5' TGA CGC GCT TGC TGG GGC ATT TCA ATA GTC GAG GCT CTC CTG ATA GCC GAG CGA GTA AGG 3'), one for Ca^{2+}-ATPase (5' AGG TGT GTT GCT AAC AAC GCA GAT GCA CGC ACC CGA ACA CCC TTA TAT TTC TGC AAA TGG 3'), and one for 18S rRNA (5' GTA TCT GAT CGT CTT CGA ACC TCC GAC TTT CGT TCT TGA TTA ATG AAA ACA TTC TTG GCA 3'). The oligonucleotides were end-labeled with [γ^{32}P]ATP by using T4 polynucleotide kinase and unincorporated nucleotides were removed by C18 Sep-Pak cartridge (Millipore).

RESULTS

Animals

In contrast to control rats, rats treated with streptozotocin developed the characteristics that confirm diabetes (Table 1). After 12 weeks of streptozotocin-injection, body and heart weights of rats decreased significantly compared to the control group. The ratio of heart to body weight, as an index of cardiac hypertrophy, was increased in diabetic rats (3.26±0.04) compared to that in control rats (2.65±0.02). In this study, rats which exhibited high glycohemoglobin levels (15.67±0.40%) were used as a diabetic group comparing with control glycohemoglobin levels (4.32±0.09%). Minimal amount of insulin could reverse body weight and heart weight to control levels, but not completely. Glycohemoglobin level changes by insulin were minimal (13.55±0.09%).

Calcium transport of cardiac sarcoplasmic reticulum

Rates of Ca^{2+} uptake in cardiac sarcoplasmic reticulum (SR) vesicles were examined in control, diabetic, and insulin-treated diabetic rats at various concentrations of Ca^{2+}. Maximum Ca^{2+} uptake rates

Table 1. Physical characteristics of diabetic rats

Characteristics	Control	Diabetic	Diabetic + Insulin
Body weight, g	467.1±7.6	291.3±11.6*	353.7±5.0*
Heart weight, g	1.24±0.02	0.95±0.06*	1.06±0.04*
Heart/B.W. (mg/g)	2.65±0.02	3.26±0.04*	3.00±0.03*
Glycohemoglobin, %	4.32±0.09	15.67±0.40*	13.55±0.09*

Diabetic animals were made with a single injection of streptozotocin (65 mg/kg i.p.). Minimal amount of insulin (4 units), which is not the sufficient amount of insulin to correct glycosuria, was injected (s.c.) to one group of diabetic rats. Values shown represent means±SE of 18 rats of each group. Blood samples were taken and assayed for glycohemoglobin using affinity chromatography. Statistically significant difference ($p<0.05$) to control group was estimated by unpaired Student's t-test and denoted by asterisk (*). B.W., body weight.

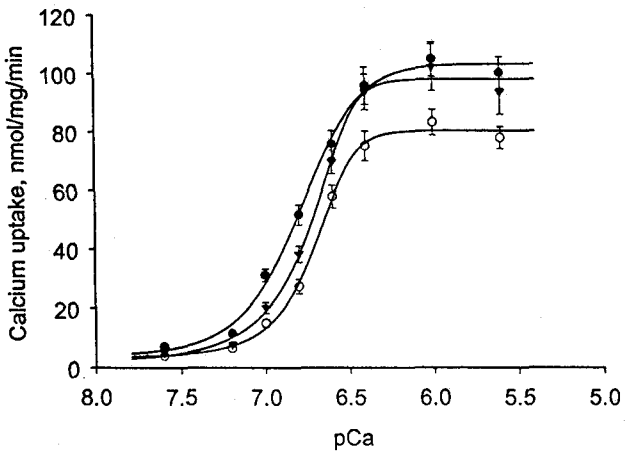

Fig. 1. Ca^{2+} uptake rates in streptozotocin-induced diabetic rat hearts. Initial rates of Ca^{2+} uptake were determined in sarcoplasmic reticulum vesicles (50 μg/ml) from control (●), diabetic (○), and insulin-treated diabetic (▼) rat hearts. Ca^{2+} dependency of Ca^{2+} uptake was determined over a wide range of Ca^{2+} concentrations, as described in the Methods. Each value represents mean±SD of six different preparations, each assayed in triplicate.

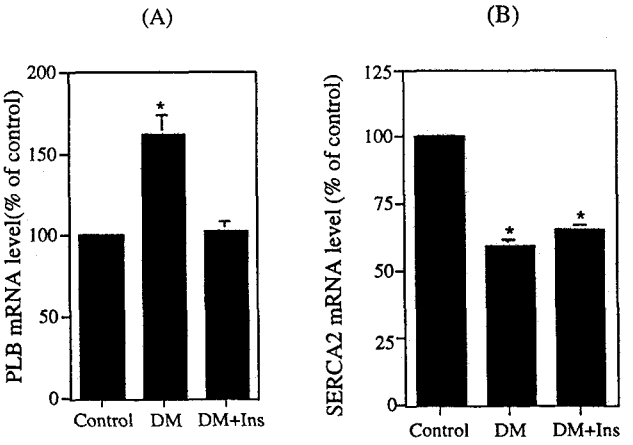

Fig. 2. RNA dot blot analysis of phospholamban (A) and Ca^{2+}-ATPase (B) from control, diabetic, and insulin-treated diabetic hearts. mRNA levels of phospholamban and Ca^{2+}-ATPase corrected by 18S rRNA level in the same membrane, were expressed as percentage of control values. Values are mean±SD of six rat hearts. Statistically significant difference ($P<0.05$) to the control was estimated by unpaired Student's t-test and denoted by asterisk (*).

of SR from diabetic hearts were depressed (80.4±3.8 nmol/min/mg diabetic SR vesicles compared with 103.2±5.4 nmol/min/mg control SR vesicles), which is consistent with a previous report (Afzal et al, 1989). The affinity of Ca^{2+}-ATPase for Ca^{2+} was also decreased in the diabetic cardiac SR in comparison with the control. The EC_{50} values of Ca^{2+} uptake for Ca^{2+} were 0.26 μM in diabetic SR vesicles compared with 0.18 μM in control SR vesicles. Insulin treatment could reverse the above parameters but not completely (Fig. 1).

mRNA levels of phospholamban and SR Ca^{2+}-ATPase in diabetic hearts

To determine whether the observed changes in Vmax and EC_{50} of the SR Ca^{2+} uptake reflected changes at the mRNA levels of the SR Ca^{2+}-ATPase and phospholamban in diabetic rat hearts, the relative mRNA levels of these proteins were studied by RNA dot blot analysis. The SR Ca^{2+}-ATPase mRNA levels were decreased in diabetic hearts, compared with control hearts (Fig. 2). Examination of the phospholamban mRNA levels revealed an opposite trend in the mRNA levels compared with the SR Ca^{2+}-ATPase. There was a significant increase in the phospholamban mRNA levels in the diabetic hearts compared with control hearts (Fig. 2). Insulin treatment reversed the phospholamban mRNA levels, but not SR Ca^{2+}-ATPase mRNA levels.

Protein expression levels of phospholamban and SR Ca^{2+}-ATPase in diabetic hearts

To determine whether the altered mRNA levels of the SR Ca^{2+}-ATPase and phospholamban reflected altered expression of the SR Ca^{2+}-ATPase and phospholamban in diabetic rat hearts, the relative levels of these proteins were examined by quantitative immunoblotting. The labeling of phospholamban by the phospholamban antibody (PLB-Ab) and of Ca^{2+}-ATPase by the SR Ca^{2+}-ATPase antibody (SERCA-Ab) was proportional to the amount of cardiac SR protein electrophoresed in the range of 10 to 50 μg and 5 to 25 μg protein, respectively. In diabetic hearts, changes in the protein expression of both SR Ca^{2+}-ATPase and phospholamban were closely correlated with the changes of mRNA levels of these proteins (Table 2). Table 2 summarizes the data obtained with six different hearts of each group. The relative ratios of the binding sites for PLB-Ab and SERCA-Ab, which reflect the relative levels of these

Table 2. Percent changes in relative protein amounts of phospholamban and sarcoplasmic reticulum Ca^{2+}-ATPase in rat hearts

	Control	Diabetic	Diabetic + Insulin
Phospholamban	100	122.9±6.7*	102.0±3.4
Ca^{2+}-ATPase	100	77.6±4.7*	91.7±2.7*
PLB/Ca-A	1	1.58*	1.11

Sarcoplasmic reticulum Ca^{2+}-ATPase and PLB protein levels were determined by quantitative immunoblotting as described in the Methods. The labeling of PLB by the PLB-Ab or of Ca^{2+}-ATPase by the Ca-A-Ab was assayed by using three protein concentrations (12.5, 25, 37.5 μg) in the linear range, assayed in triplicate. After the densitometric scan, the values found in control rats were taken as 100%, and the changes in diabetic and insulin-treated diabetic hearts were expressed as percentage of control values. Values are mean±SD of six rat hearts. Statistically significant differences ($P<0.05$) to control group were estimated by unpaired Student's t-test and denoted by asterisk (*). PLB, phospholamban; Ca-A, Ca^{2+}-ATPase.

proteins in the same hearts, were estimated from the quantitative immunoblots and are presented as the ratio of phospholamban to Ca^{2+}-ATPase (Table 2). This relative ratio of phospholamban to SR Ca^{2+}-ATPase was observed to increase in diabetic hearts (phospholamban/Ca^{2+}-ATPase: 1.58), and these changes appeared to be significant compared to control hearts. Minimal amount of insulin could reverse the protein levels of phospholamban and SR Ca^{2+}-ATPase.

DISCUSSION

Conflicting results have been presented regarding the relative roles of microvascular changes, interstitial fibrosis, and metabolic derangements in the pathogenesis of diabetic cardiomyopathy. Although the cardiac dysfunction is frequently associated with the enhanced coronary atherosclerosis in diabetic patients, evidence has been accumulated for the existence of a specific diabetic cardiomyopathy (Zarich & Nesto, 1989). There are several hypotheses regarding the pathogenesis of diabetic cardiomyopathy. One of them is the diabetic autonomic neuropathy. Defective β-adrenergic responsiveness has been suggested as the characteristic feature of both insulin-dependent (Heyliger et al, 1982; Atkins et al, 1985; Nishio et al, 1988) and non-insulin-dependent (Schaffer et al, 1991) diabetes. Previously, we have shown that the phosphorylation of phospholamban with the catalytic subunit of cAMP-dependent protein kinase resulted in improved SR Ca^{2+} pump activity in both control and diabetic hearts (Kim et al, 1993). However, the stimulation of Ca^{2+} transport was higher in SR prepared from diabetic hearts than that in SR from control hearts (Kim et al, 1993).

We have found that the phosphorylation level of phospholamban was higher in diabetic hearts compared to control hearts (Kim et al, 1993; Kim et al, submitted). Kahn et al (1986) reported that plasma catecholamine levels were significantly reduced in diabetic subjects. Furthermore, myocardial catecholamine depletion frequently occurs in patients with heart failure. Thus, it was possible that the increase in phospholamban phosphorylation by exogenous catalytic subunit of cAMP-dependent protein kinase (Kim et al, submitted) is due to the lower basal phosphorylation level of phospholamban by the decreased endogenous norepinephrine levels in diabetic heart. However, there were no changes in phospholamban phosphorylation levels in both control and diabetic hearts with or without phosphatase pretreatment (Kim et al, submitted). Thus, increased levels of phospholamban phosphorylation are not due to the autonomic dysfunction.

Using quantitative immunoblotting, we demonstrated that the functional changes in the diabetic rat hearts were associated with a decrease in the levels of SR Ca^{2+}-ATPase protein. Furthermore, the alterations of mRNA levels of SR Ca^{2+}-ATPase in diabetic hearts (Fig. 2) are in close correlation with the changes in these protein levels (Table 2). However, examination of the phospholamban protein levels in the same hearts revealed an opposite trend of changes compared with the SR Ca^{2+}-ATPase. The phospholamban levels increased in diabetic hearts, and these findings at the protein level are in agreement with the observation at mRNA levels (Table 2, Fig. 2). This was not altogether an unexpected finding as others have reported that SERCA2 and phospholamban genes can be independently regulated (Nagai et al, 1989; Kiss et al, 1994). For example, Kiss et al (1994) found that thyroid hormone can simultaneously upregulate the expression of SERCA2 and downregulate the expression of phospholamban in the rat heart.

The inverse changes in the expression of the two SR proteins resulted in a significant increase in the relative ratio of phospholamban to SR Ca^{2+}-ATPase in diabetic rat hearts compared with control rat hearts. These changes in the relative levels of phospholamban and the SR Ca^{2+}-ATPase in diabetic rats were indicative of the alterations in SR Ca^{2+} uptake properties in these hearts. The decreases in SR Ca^{2+}-ATPase protein and mRNA levels were reflected by decreases in the Vmax of the SR Ca^{2+} pump. Furthermore, the changes in the relative ratio of phospholamban to Ca^{2+}-ATPase correlated with changes in the EC_{50} of the SR Ca^{2+} pump for Ca^{2+} measured in the same hearts. This suggests that the amount of phospholamban in relation to the SR Ca^{2+}-ATPase is an important determinant of the affinity of this enzyme for Ca^{2+}. The functional significance of inverse changes in phospholamban and the SR Ca^{2+}-ATPase levels in diabetic rat hearts was reflected in the relaxation parameters. In diabetic rat hearts, the SR Ca^{2+} uptake which is an index of the myocardial relaxation rate, was depressed as a consequence of down-regulation of Ca^{2+}-ATPase gene expression and overexpression of its inhibitor, phospholamban.

Insulin elicits a remarkable array of biological responses. The heart is neither one of the important target tissues for regulation of glucose homeostasis by insulin, such as liver, muscle, and fat, nor one of the major complication sites, such as retina, kidney, and nerve. Even if heart is not the major complication site, heart failure is the leading cause of death in diabetic patients. Thus, the exact mechanism by which effects of insulin on diabetic cardiomyopathy itself are accomplished needs to be determined.

In the present study, the maximal Ca^{2+} uptake and the affinity of Ca^{2+}-ATPase for Ca^{2+} were decreased in streptozotocin-induced diabetic rat SR, however, even minimal amount of insulin could reverse both parameters. In case of phospholamban, insulin treatment reverses levels of both mRNA and protein to normal. Minimal amount of insulin could reverse the protein levels of SR Ca^{2+}-ATPase, however, it could not reverse the mRNA level of SR Ca^{2+}-ATPase at all. These results may suggest two possibilities. One possibility is that the translational steps of the above two proteins are under control by insulin. The other one is that insulin may regulate both transcriptional and translational levels of phospholamban, but in case of SR, the translational step may be regulated by insulin. A recent analysis of the time course of mRNA and protein expression during chronic stimulation of fast-twitch skeletal muscle indicated a close correspondence between the appearance of phospholamban mRNA and phospholamban protein, whereas induction of the SERCA2a protein lagged behind expression of its mRNA (Hu et al, 1995). A follow-up study indicated that the transcription rates of the SERCA2 and phospholamban genes in chronically stimulated fast-twitch skeletal muscle are discoordinately regulated (Hu et al, 1998).

In summary, our data indicate that the phospholamban and the SR Ca^{2+}-ATPase protein and mRNA levels are inversely regulated in the diabetic heart. These expressional changes in the phospholamban and the SR Ca^{2+}-ATPase would explain, at least in part, the alterations in SR Ca^{2+} uptake rates, and the relaxation defect in diabetic hearts. The exact role of insulin in phospholamban regulation of diabetic cardiac SR function and myocardial contractility needs to be determined in future studies.

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