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Unchanged Protein Level of Ryanodine Receptor but Reduced [^{3}H] Ryanodine Binding of Cardiac Sarcoplasmic Reticulum from Diabetic Cardiomyopathy Rats

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The ryanodine receptor, a Ca^{2+} release channel of the sarcoplasmic reticulum (SR), is responsible for the rapid release of Ca^{2+} that activates cardiac muscle contraction. In the excitation-contraction coupling cascade, activation of SR Ca^{2+} release channel is initiated by the activity of sarcolemmal Ca^{2+} channels, the dihydropyridine receptors. Previous study showed that the relaxation defect of diabetic heart was due to the changes of the expressional levels of SR Ca^{2+}ATPase and phospholamban. In the diabetic heart contractile abnormalities were also observed, and one of the mechanisms for these changes could include alterations in the expression and/or activity levels of various Ca^{2+} regulatory proteins involving cardiac contraction. In the present study, underlying mechanisms for the functional derangement of the diabetic cardiomyopathy were investigated with respect to ryanodine receptor, and dihydropyridine receptor at the transcriptional and translational levels. Quantitative changes of ryanodine receptors and the dihydropyridine receptors, and the functional consequences of those changes in diabetic heart were investigated. The levels of protein and mRNA of the ryanodine receptor in diabetic rats were comparable to these of the control. However, the binding capacity of ryanodine was significantly decreased in diabetic rat hearts. Furthermore, the reduction in the binding capacity of ryanodine receptor was completely restored by insulin. This result suggests that there were no transcriptional and translational changes but functional changes, such as conformational changes of the Ca^{2+} release channel, which might be regulated by insulin. The protein level of the dihydropyridine receptor and the binding capacity of nitrendipine in the sarcolemmal membranes of diabetic rats were not different as compared to these of the control. In conclusion, in diabetic hearts, Ca^{2+} release processes are impaired, which are likely to lead to functional derangement of contraction of heart. This dysregulation of intracellular Ca^{2+} concentration could explain for clinical findings of diabetic cardiomyopathy and provide the scientific basis for more effective treatments of diabetic patients. In view of these results, insulin may be involved in the control of intracellular Ca^{2+} in the cardiomyocyte via unknown mechanism, which needs further study.

Key Words: Ryanodine receptor, Sarcoplasmic reticulum, Heart, Diabetes mellitus

INTRODUCTION

For cardiac muscle relaxation, SR Ca^{2+}-ATPase mediates the uptake of Ca^{2+} from the cytosol, which is controlled by phospholamban, the inhibitor of the SR Ca^{2+}-ATPase. Previously we have shown that the maximal Ca^{2+} uptake and the affinity of Ca^{2+}-ATPase for Ca^{2+} decreased in the cardiac SR from diabetic rats (Kim et al, 1993). Furthermore, the experimental cardiomyopathy produced by streptozotocin administration resulted in a decreased rate of Ca^{2+} uptake, which was associated with coordinate decreases in the expression of Ca^{2+}-ATPase mRNA and protein level and increases in the levels of both phospholamban mRNA and protein (Kim et al,

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1999a). Our previous study showed that the protein levels in diabetic hearts increased to 133.3% for phospholamban and decreased to 70.8% for Ca^{2+}-ATPase, both of which were expressed as percentage of control values (100%). Consequently, the relative phospholamban/Ca^{2+}-ATPase ratio increased to 1.88 in diabetic rat hearts, which was in correlation with changes in the rate of SR Ca^{2+} uptake for Ca^{2+} (Kim et al, in press). Since phospholamban is an inhibitor of SR Ca^{2+}-ATPase, the above data could explain the mechanism for the relaxation defect of diabetic cardiac muscle, while the cause of the contractile defect besides the altered myosin ATPase activity (Garber et al, 1983) is not clear.

SR is also an important determinant in the regulation of intracellular Ca^{2+} fluxes, contributing to myocardial contractility (Luo et al, 1994). During the excitation of cardiac muscle, extracellular Ca^{2+} entry is believed to be primarily mediated by the dihydropyridine receptor (DHPR), which functions as a voltage-dependent L-type Ca^{2+} channel localized in the T-tubule membranes and is the sensor of their depolarization (Marty et al, 1995). The influx of extracellular Ca^{2+} triggers the release of SR Ca^{2+} through the activation of SR Ca^{2+} release channel (ryanodine receptor), which is called as Ca^{2+}-induced Ca^{2+} release. The process of Ca^{2+}-induced Ca^{2+} release is necessary for the contraction of cardiac muscle.

Diabetic cardiomyopathy is a disease of heart muscle associated with diabetes (Bell, 1995). Cardiac dysfunction accompanied with cytosolic Ca^{2+} accumulation has been reported in chronic diabetes mellitus (Levy et al, 1994) and it seems to play a role in diabetes-induced heart failure (Pierce & Russell, 1997; Mahgoub & Abd-Elfattah, 1998). Although it has been reported that the Ca^{2+} storage in SR is significantly depressed and, therefore, Ca^{2+} release activity of SR may be also depressed in diabetic heart (Kim et al, 1998), the exact defect of SR Ca^{2+} release channel in diabetic heart has not been determined yet.

The objective of the present study was to examine the mechanisms altering SR function in diabetic rat heart, with the following specific aims: 1) to determine the quantitative changes in the expressional levels of mRNA encoding SR Ca^{2+} release channel and DHPR that are responsible for calcium release in rat hearts, 2) to determine whether the functional states of SR Ca^{2+} release channel and DHPR were altered in diabetic rat heart, and 3) to determine the correlation between quantitative changes in SR Ca^{2+} release channel and DHPR levels and the functional consequences of these changes in diabetic rat heart.

METHODS

Animal models

Male Sprague-Dawley (SD) rats weighing 250 g (8 weeks after birth) were randomly divided into two groups (control vs diabetic). One animal 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 group of control animals was injected with citrate buffer. The development of diabetes mellitus (DM) was confirmed by urine analysis (Diastix and Ketostix, Miles Sankyo Co., Tokyo, Japan) on the 3 rd day of streptozotocin injection. After the confirmation of DM, diabetic rats were divided into two groups (the diabetic vs the insulin-treated diabetic). Small amount of insulin (3.5 u/d, s.c.) was injected throughout the experimental period, which enhances the possibility of survival, but not enough to correct glycosuria. All rats were maintained on normal animal chow and water *ad libitum* for 12 w. Blood samples were taken from the rats fasted for 5 h and assayed for glycohemoglobin using affinity chromatography (Glyc-Affin GHb, Iso Lab Inc., Akron, Ohio, USA) and for glucose using glucose analyzer (Beckman). 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% were excised and placed in ice-cold saline for 5～10 min prior to use.

cDNA expression and isolation

E. coli DH5 α cells (Pharmacia Biotec.) trasfected with the pCRII (vector inserted 389bp ryanodine receptor cDNA, Pharmacia Biotec.) constructs or the pGEX (vector inserted 378 bp dihydropyridine receptor cDNA, Pharmacia Biotec.) constructs were grown in 5～10 ml of Luria Bertani (LB) medium supplemented with 100 μg of ampicillin/ml, to an A_{600}=1.0～1.5 at 37°C, then treated with 1 mM isopropyl-1-thio-β-D-galactopyranoside and incubated 12～16 h at 20°C with constant shaking. The cells were harvested by centrifugation (10,000×g, 5 min) and the pellet

was used as the DNA purification source. The cardiac-specific ryanodine receptor cDNA and dihydropyridine receptor cDNA were a generous gift from Dr. Do Han Kim in Gwang-Ju Institute for Science and Technology. DNA was isolated using DNA purification system (Promega) according to the procedure described by the manufacturer.

RNA isolation and dot blot analysis

Total cellular RNA of hearts was isolated by guanidine isothiocyanate method (Chomczynski & Sacchi, 1987). Dot blot analysis for the ryanodine receptor and dihydropyridine receptor mRNA were done as we described previously (Kim et al, 1999b). RNAs were prehybridized and hybridized to the 389 bp cardiac-specific ryanodine receptor cDNA probe or the 378 bp dihydropyridine receptor cDNA probe, and then 18 S rRNA oligonucleotide. The specificity of the probe was tested in preliminary studies by Northern blot analysis of mRNA separations (Data not shown). Autoradiography was done with Kodak X-Omat AR film at $-70^{\circ}C$ 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 radioactivity's were checked by scintillation counter later. For the calculation of relative amounts of ryanodine receptor or dihydropyridine receptor mRNAs, signals with ryanodine receptor or dihydropyridine receptor probe were normalized to those with the 18 S rRNA probe in the same membranes.

A synthetic 60-mer oligonucleotides were used in the hybridization, which is specific for 18 S 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).

Quantitative immunoblots

The cardiac-specific ryanodine receptor and dihydropyridine receptor monoclonal antibodies (RyR-Ab and DHPR-Ab) were purchased from Affinity Bioreagents Inc. (Golden, USA). The relative ratios of ryanodine receptor and dihydropyridine receptor in cardiac SR from control, diabetic, insulin-supplemented diabetic hearts were estimated by quantitative immunoblotting. Cardiac SR proteins separated by SDS-polyacrylamide gel electrophoresis (4% for ryanodine receptor and 6% for dihydropyridine receptor) according to the method of Laemmli (1970) were transferred electrophoretically onto polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Bedford, USA). The transferred proteins were incubated with blocking solution containing 5% dried milk, and 0.1% Tween 20 in phosphate buffered saline for 1 h at room temperature. After blocking, the transblots were reacted with a monoclonal antibody (1 : 1000 dilution) specific for ryanodine receptor or dihydropyridine receptor, and incubated with a secondary antibody (goat anti-mouse-HRP conjugate) from Amersham International (Little Chalfont, UK) at 1 : 2000 for 2 h. Antibody binding was detected using ECL (enhanced chemiluminescence) horseradish peroxidase developing agents from Amersham International (Little Chalfont, UK), and membranes were exposed to a sheet of autoradiography film. The developed bands were quantitated by the scanned image using ImageQuant software, with data reported as integrated density units. The density associated with ryanodine receptor and dihydropyridine receptor was linear in the range of $10 \sim 50\,\mu g$ cardiac SR protein loaded onto the gel lanes.

Preparation of sarcolemmal membranes and heavy sarcoplasmic reticulum vesicles

Crude plasma membrane fractions were prepared by the previously described method (Ogawa et al, 1995) with some modifications. Briefly, rats were killed and the gastrocnemius muscle was quickly removed, minced, and homogenized in 6 vol. of cold 20 mM Tris-HCl, pH 7.0, 0.1 M KCl, 5 mM EDTA, 0.3 mM phenylmethylsulfonyl fluoride (PMSF), 50 U/ml aprotinin, and $10\,\mu M$ benzetonium chloride. Homogenates were centrifuged at $500 \times g$ for 10 minutes, and supernatants were centrifuged at $12,000 \times g$ for 30 min. The pellets were suspended and homogenized in 10 mM histidine, pH 7.0, 0.6 M KCl to extract contractile protein. The suspension was centrifuged at $40,000 \times g$ for 15 minutes. The pellets were then resuspended and centrifuged at $120,000 \times g$ for 60 minutes. The resultant precipitates were washed with 20 mM Tris-HCl, pH 7.0, 0.1 M KCl, 0.1 mM PMSF and were used as crude skeletal muscle mem-

brane fractions.

For the preparation of heavy sarcoplasmic reticulum (HSR) vesicles (Valdivia et al, 1992), the last pellet was resuspended in 10% sucrose solution and subjected to a discontinuous sucrose gradient centrifugation for 5 h at 100,000×g. Microsomal fractions were recovered from the interfaces of 20, 30, 35, 40% of sucrose layers and HSR vesicles were obtained from the interface between 30% and 35% sucrose layers. The subfraction was diluted with 10% sucrose solution and centrifuged for 90 min at 100,000×g. The pellet was resuspended in a solution containing 0.3 M sucrose, 0.1 M KCl, 5 mM Na-Pipes, pH 6.8, frozen in liquid nitrogen, and stored at −80°C before use. The concentration of protein was determined by the Lowry method (1951).

[^{3}H]ryanodine binding assay

[^{3}H]ryanodine binding was carried out for 90 min at 37°C in 0.1 ml of 0.2 M KCl, 1 mM Na_2EGTA, 0.995 mM $CaCl_2$, 10 mM Na-PIPES, pH 7.2. Free Ca^{2+} concentration was 10 μM, which was calculated with a computer program using affinity constants of Fabiato (1988). [^{3}H]ryanodine (60 mCi/mmol) was purchased from Du Pont-New England Nuclear and was diluted directly in the incubation medium to achieve concentrations in the saturable range of 1~30 nM. During incubation, cardiac SR (0.3~0.5 mg/ml) were the last reagent to be added to the medium. Aliquots of the samples were placed on Whatman GF/B glass fiber filters, using a Brandel (Gaithersburg, Maryland, USA) cell harvester. Filters were washed three times with 5-ml of ice-cold 0.1 M KCl, 1 mM K-PIPES, pH 7.0. Nonspecific [^{3}H]ryanodine binding was determined in the presence of 10 μM unlabeled ryanodine and was subtracted from each sample. In order to establish whether [^{3}H]ryanodine binding was affected by proteolytic degradation that may have occurred during the binding assay, control incubations were carried out in the presence or absence of the protease inhibitors pepstatin A (1 μM), iodoacetamide (1 mM), phenylmethylsulfonyl fluoride (0.1 mM), leupeptin (1 μM), and benzamidine (1 mM). Addition of the protease inhibitor mixture did not result in a significant change in the site density or in the affinity of [^{3}H]ryanodine binding. Radioactivity was measured by scintillation counting.

[^{3}H]nitrendipine binding assay

Binding of [^{3}H]nitrendipine to membrane fractions was monitored according to a method reported earlier (Finkel et al, 1987). Membrane preparations (0.08~0.1 mg protein/tube) were incubated with 0.035~5 nM [^{3}H]nitrendipine, in the absence or presence of 2.5 μM unlabeled nitrendipine, a concentration sufficient to inhibit more than 95% of the specific [^{3}H]nitrendipine binding. Assays were terminated after 1 h at room temperature by filtration (GF/C filters, Whatman, Clifton, New Jersey, USA). Filters were washed twice with 5 ml cold Tris-HCl buffer. The radioactivity of the filters was counted in a scintillation counter at an efficiency of 39~41%. The nonspecific [^{3}H]nitrendipine binding (in the presence of nitrendipine) was subtracted from the total binding (in the absence of nitrendipine) to obtain the specific binding of [^{3}H]nitrendipine.

Statistical analysis

Values in each experiment were expressed as the mean±SE of six different hearts, unless otherwise specified. Statistical significance between two groups was assessed by unpaired Student's t-test. P values less than 0.05 were defined as statistically significant.

RESULTS

Expression of SR Ca^{2+} release channel and dihydropyridine receptor

Since systolic contraction which is in part controlled by the activities of SR Ca^{2+} release channel and dihydropyridine receptor was also impaired in diabetic hearts (Kim et al, 1998), we examined the expression of these genes and proteins of hearts in control, diabetic, and insulin-treated diabetic rats. We analyzed the expression level of mRNA for cardiac ryanodine receptor using an RNA dot blot method. The mRNA level of cardiac SR Ca^{2+} release channel was not significantly changed in diabetic rats, and insulin had no influence on mRNA level of this SR Ca^{2+} release channel (Fig. 1). Similar analysis on protein expression levels of SR Ca^{2+} release channel showed that cardiac SR Ca^{2+} release channel protein was also unchanged in diabetic rats (Fig. 2).

The expression levels of the mRNA and the protein

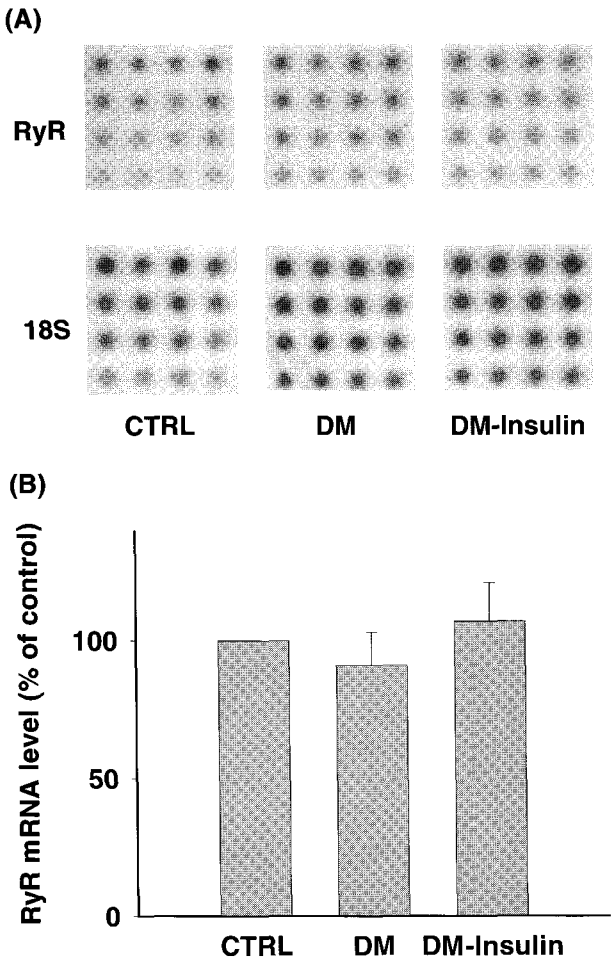

Fig. 1. RNA dot blot analysis of ryanodine receptor from control, diabetic, and insulin-treated diabetic rat hearts. (A) Representative RNA dot blots of ryanodine receptor. (B) Quantitative representation of percent changes in SR ryanodine receptor RNA levels. Levels of ryanodine receptor mRNA corrected by 18S rRNA level in the same membrane were normalized to the mean of control group. 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 ± S.E. of six different hearts, each assayed in triplicate. RyR: ryanodine receptor, CTRL: control, DM: diabetes mellitus.

for dihydropyridine receptor localized in the T-tubule membranes were also examined in these heart preparations. In diabetic heart, the mRNA and protein levels of dihydropyridine receptor were not changed (Fig. 3, 4), which is similar to the findings of ryanodine receptor (Fig. 1, 2).

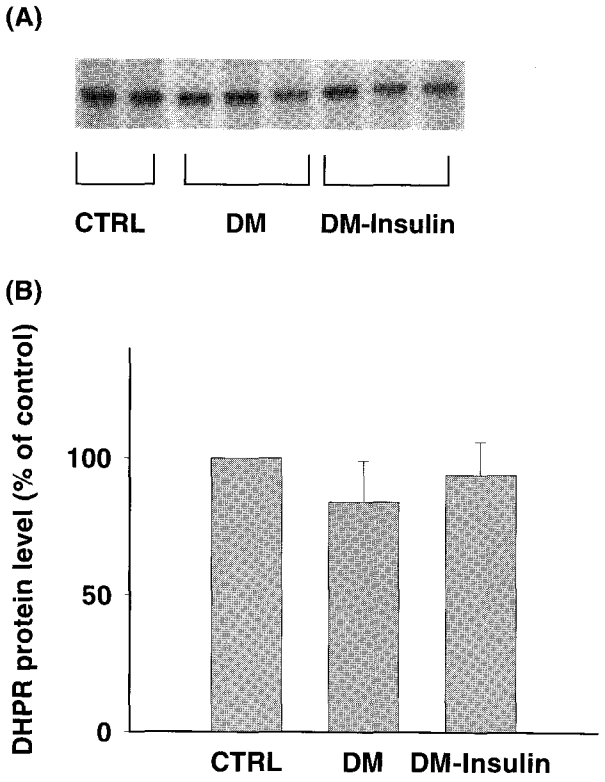

Fig. 2. Western blot analysis of ryanodine receptor in control, diabetic, and insulin-treated diabetic rat hearts. (A) Representative Western blots of ryanodine receptor. Immunoblots were reacted with 1 : 1000 dilution of ryanodine receptor monoclonal antibody. (B) Quantitative representation of percent changes in SR ryanodine receptor protein. 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 ± S.E. of six different hearts, each assayed in triplicate. RyR: ryanodine receptor, CTRL: control, DM: diabetes mellitus.

[^{3}H]ryanodine binding

Even if there were no significant changes in the expression levels of SR Ca^{2+} release channel and DHPR mRNA, it was of our special interest to determine whether diabetic heart has an impaired SR Ca^{2+} release that is a parameter of cardiac contraction. [^{3}H]ryanodine binding can be used to monitor changes in the functional state of the Ca^{2+} release channel (Hawkes et al, 1992; Meissner & El-Hashem, 1992). We studied specific [^{3}H]ryanodine binding to preparations of cardiac heavy SR vesicles from control, diabetic, and insulin-treated diabetic rats to determine whether changes in the functional state of

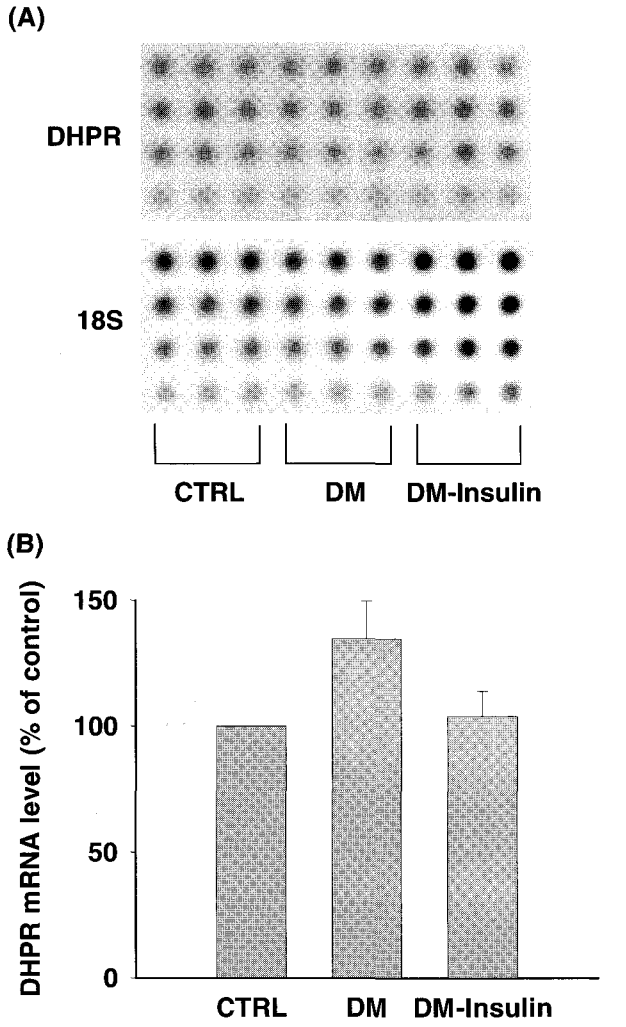

Fig. 3. RNA dot blot analysis of dihydropyridine receptor from control, diabetic, and insulin-treated diabetic rat hearts. (A) Representative RNA dot blot of dihydropyridine receptor. (B) Quantitative representation of percent changes in SR dihydropyridine receptor RNA levels. Levels of dihydropyridine receptor mRNA corrected by 18 S rRNA level in the same membrane were normalized to the mean of the control. 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±S.E. of six different hearts, each assayed in triplicate. DHPR: dihydropyridine receptor, CTRL: control, DM: diabetes mellitus.

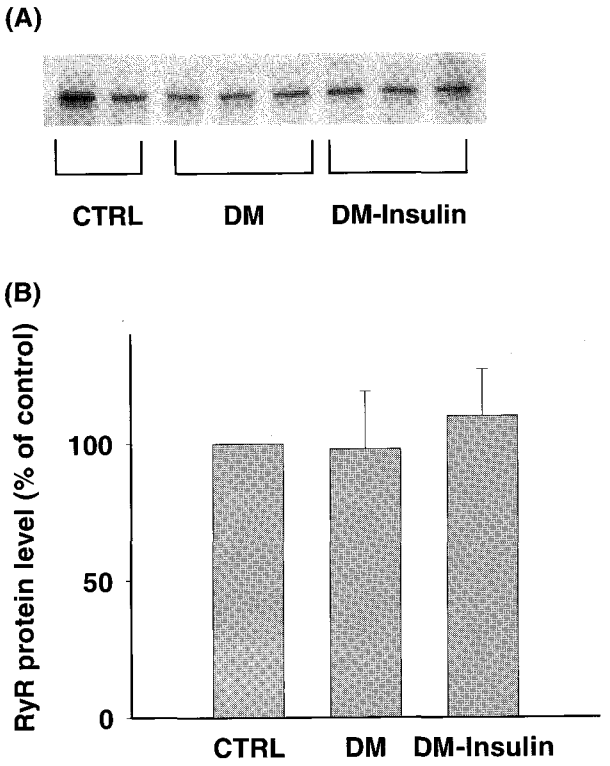

Fig. 4. Western blot analysis of dihydropyridine receptor in control, diabetic, and insulin-treated diabetic rat hearts. (A) Representative Western blots of dihydropyridine receptor. Immunoblots were reacted with 1 : 1000 dilution of dihydropyridine receptor monoclonal antibody. (B) Quantitative representation of percent changes in SR dihydropyridine receptor protein. 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±S.E. of six different hearts, each assayed in triplicate. DHPR: dihydropyridine receptor, CTRL: control, DM: diabetes mellitus.

SR Ca^{2+} release channel occurs in these conditions. [^{3}H]ryanodine binding decreased in diabetic rats, which was restored in insulin-treated diabetic rats (Fig. 5). This result shows that SR Ca^{2+} release channel was also affected by diabetic conditions. The above findings indicate that there were no transcriptional and/or translational changes but only the functional and/or structural modification of Ca^{2+} release channel occurred in this diabetic condition.

[^{3}H]nitrendipine binding

In order to determine whether diabetic condition possibly affects sarcolemmal Ca^{2+} handling, we also examined sarcolemmal Ca^{2+} channels, i.e. dihydropyridine receptor. Studies of [^{3}H]nitrendipine binding to cardiac sarcolemmal membrane revealed no change in diabetic hearts when compared with the control

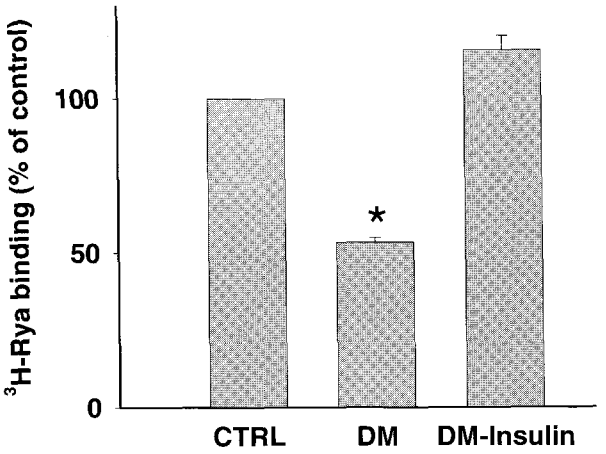

Fig. 5. [^{3}H]ryanodine binding of cardiac heavy sarcoplasmic reticulum vesicles from control, diabetic, and insulin-treated diabetic rats. [^{3}H]ryanodine binding was carried out for 90 min at 37°C in 0.1 ml of 0.2 mM Na_2EGTA, 0.995 mM $CaCl_2$, 10 mM Na-PIPES, pH 7.2. The calculated free Ca^{2+} was 10 μM. [^{3}H]ryanodine (60 mCi/mmol) (Du Pont-New England Nuclear) was diluted directly in the incubation medium to achieve concentrations in the saturable range of 1~30 nM. During incubation, cardiac (0.3~0.5 mg/ml) microsomes were the last reagent to be added to the medium. Values are mean ±S.E. of six different hearts, each assayed in triplicate. Statistical significant differences ($P<0.05$) to the control were estimated by unpaired Student's t-test and denoted by asterisk (*). Rya: ryanodine, CTRL: control, DM: diabetes mellitus.

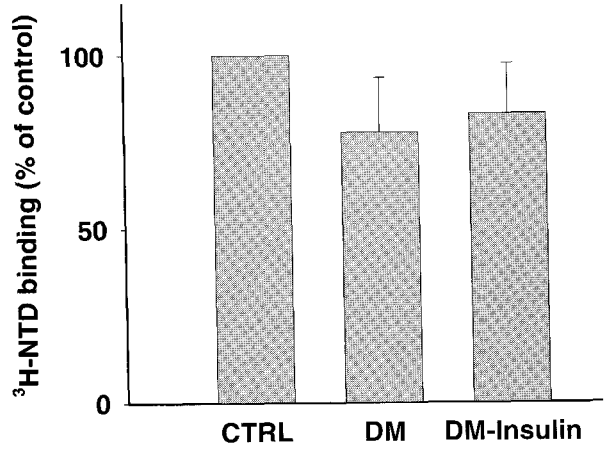

Fig. 6. [^{3}H]nitrendipine binding of cardiac heavy sarcoplasmic reticulum vesicles from control, diabetic, and insulin-treated diabetic rats. Values are mean±S.E. of six different hearts, each assayed in triplicate. NTD: nitrendipine, CTRL: control, DM: diabetes mellitus.

(Fig. 6). This result was similar to the expression level of dihydropyridine receptor mRNA and protein (Fig 3, 4).

DISCUSSION

The present study shows that (1) both protein and mRNA levels of the ryanodine receptor as well as the dihydropyridine receptor are not significantly altered in the diabetic rat hearts; (2) [^{3}H]nitrendipine binding to the dihydropyridine receptor is not changed in the diabetic rat hearts; (3) however, [^{3}H]ryanodine binding to the ryanodine receptor is reduced significantly in the diabetic rat hearts and this reduced binding is reversed to normal by insulin treatment.

Previously we have demonstrated that the expression of the mRNA and protein levels of SR Ca^{2+}-ATPase was downregulated in the diabetic rat hearts (Kim et al, 1999a; Kim et al, in press), which was responsible for the cardiac relaxation defect. The sarcoplasmic reticulum (SR) Ca^{2+} release channel (ryanodine receptor) plays an important role in excitation-contraction coupling. Therefore, an altered expression of Ca^{2+} release channel might be responsible for the contractile dysfunction in the diabetic rat hearts. In the present study, however, the expression level of the mRNA coding for the cardiac SR Ca^{2+} release channel was not changed in the diabetic rat hearts (Fig. 1), and there was also no change observed in the protein level for SR Ca^{2+} release channel in diabetic rat hearts (Fig. 2). However, Teshima et al (2000) showed a diminished ryanodine receptor mRNA expression in diabetic rats. There is no definite explanation for this discrepancy and further studies on ryanodine receptor mRNA expression and degradation may be required to clarify these contradicts.

A previous study reported a decrease in ^{3}H-labeled ryanodine binding sites in diabetic myocardium, suggesting a decreased density of ryanodine receptor (Yu et al, 1994). Lagadic-Gossmann et al (1996) reported that a rise in $[Ca^{2+}]_i$ to rapid application of caffeine was suppressed in diabetic myocytes. We studied functional state of the Ca^{2+} release channel as monitored by [^{3}H]ryanodine binding to the ryanodine receptor and [^{3}H]nitrendipine binding to dihydropyridine receptor in control, diabetic, and insulin-treated diabetic hearts. [^{3}H]ryanodine binding was decreased in diabetic hearts, and was restored to the control level in insulin-treated diabetic hearts (Fig. 5).

This result suggests that, in the diabetic rats, the specific affinity of [^{3}H]ryanodine to the SR Ca^{2+} release channel was significantly decreased in cardiac SR with no change in the SR Ca^{2+} release channel density. [^{3}H]ryanodine binding to the SR Ca^{2+} release channel might be defective because of structural alterations of the ryanodine receptors, abnormal interactions with modulators, and defects in other proteins that may interact with the receptor.

Dysfunction of L-type Ca^{2+} channel i.e. a decrease in L-type Ca^{2+} current in diabetic hearts has been also reported (Wang et al, 1995). However, in our study there was no significant difference in mRNA as well as protein expression levels of dihydropyridine receptor in diabetic hearts (Fig. 3, 4), which is consistent with a recent report (2000). [^{3}H]nitrendipine binding to L-type Ca^{2+} channel, dihydropyridine receptor, in cardiac sarcolemmal membranes showed no change in the diabetic rats (Fig. 6).

In summary, in diabetic hearts as compared with controls, there was no significant change occurred in the expression levels of the mRNA and SR Ca^{2+} release channel and dihydropyridine receptor proteins, whereas functional changes in the SR Ca^{2+} release and [^{3}H]ryanodine binding to SR Ca^{2+} release channel were apparent. Directly characterizing the channel properties will be helpful in future studies.

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