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Decrease in Ca^{2+} Storage in the Cardiac Sarcoplasmic Reticulum of Diabetic Rat

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In order to elucidate the molecular mechanism of the intracellular Ca^{2+} overload frequently reported from diabetic heart, diabetic rats were induced by the administration of streptozotocin, the membrane vesicles of junctional SR (heavy SR, HSR) were isolated from the ventricular myocytes, and SR Ca^{2+} uptake and SR Ca^{2+} release were measured. The activity of SR Ca^{2+}-ATPase was 562 ± 14 nmol/min/mg protein in control heart. The activity was decreased to 413 ± 30 nmol/min/mg protein in diabetic heart and it was partially recovered to 485 ± 18 nmol/min/mg protein in insulin-treated diabetic heart. A similar pattern was observed in SR $^{45}Ca^{2+}$ uptakes; the specific uptake was the highest in control heart and it was the lowest in diabetic heart. In SR $^{45}Ca^{2+}$ release experiment, the highest release, 45% of SR $^{45}Ca^{2+}$, was observed in control heart. The release of diabetic heart was 20% and it was 30% in insulin-treated diabetic heart. Our results showed that the activities of both SR Ca^{2+}-ATPase and SR Ca^{2+} release channel were decreased in diabetic heart. In order to evaluate how these two factors contribute to SR Ca^{2+} storage, the activity of SR Ca^{2+}-ATPase was measured in the uncoupled leaky vesicles. The uncoupling effect which is able to increase the activity of SR Ca^{2+}-ATPase was observed in control heart; however, no significant increments of SR Ca^{2+}-ATPase activities were measured in both diabetic and insulin-treated diabetic rats. These results represent that the Ca^{2+} storage in SR is significantly depressed and, therefore, Ca^{2+}-sequestering activity of SR may be also depressed in diabetic heart.

Key Words: Diabetic heart, Sarcoplasmic reticulum, Streptozotocin, Ca^{2+} release, Ca^{2+} uptake, Ca^{2+}-ATPase

INTRODUCTION

Sarcoplasmic reticulum (SR) in cardiac muscle is an intracellular Ca^{2+} store and plays major roles in contraction and relaxation of myocytes by controlling cytosolic Ca^{2+} concentration (Fleischer, 1985). During the excitation of cardiac muscle, membrane action potential activates L-type Ca^{2+} channels on the membranes of transverse tubules and sarcolemma and the influx of extracellular Ca^{2+} triggers the release of SR Ca^{2+} through the activation of SR Ca^{2+} release channel (ryanodine receptor), known as Ca^{2+}-induced Ca^{2+} release. The process of Ca^{2+}-induced Ca^{2+} release is necessary for the contraction of cardiac muscle. In order to elucidate the physiological functions of SR on myocardial contraction, the roles of SR Ca^{2+}-ATPase and SR Ca^{2+} release channel on the SR Ca^{2+} uptake, storage, and release have been intensively studied (Coronado et al, 1994; Kurihara, 1994).

Cardiac dysfunctions accompanied with cytosolic Ca^{2+} accumulation have been reported in chronic diabetic mellitus (Levy et al, 1994) and it is believed to play a role in diabetes-induced congestive heart failure (Dhalla et al, 1985; Pierce & Russell, 1997: Mahgoub & Abd-Elfattah, 1998). The depressions of cardiac function were investigated from streptozo-

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tocin-induced diabetic rats and both a depressed shortening velocity and a prolonged relaxation were observed (Fein et al, 1980). These abnormal functions of diabetic heart in contractility may be due to the defects of contractile proteins and cytoplasmic Ca^{2+} regulations. Although the mechanism of diabetic heart failure is not known, the alterations in Ca^{2+} metabolism have been suggested to be involved in the diabetic intracellular Ca^{2+} overload and cardiomyopathy. Depressions in the activities of Ca^{2+}-ATPase, Na^{+}-Ca^{2+} exchanger in sarcolemma (Heyliger et al, 1987; Makino et al, 1987) and that of Ca^{2+}-ATPase in sarcoplasmic reticulum (Rupp et al, 1989) have been demonstrated in diabetic heart. Specially, a slowed relaxation of diabetic myocardium has been known to correlate with the decreases in SR Ca^{2+} uptake and SR Ca^{2+}-ATPase activity; however, the molecular mechanisms for these changes are poorly understood.

In order to investigate the abnormal functions of SR in diabetic ventricular myocytes, we have prepared cardiac SR vesicles and studied the characteristics of SR Ca^{2+} uptake and SR Ca^{2+} release. The activity of SR Ca^{2+}-ATPase was decreased and both SR Ca^{2+} uptake and SR Ca^{2+} release were also depressed in diabetic HSR vesicles. We have evaluated the physiological significance of the functional alterations of diabetic SR and found the decrease in the capacity of SR Ca^{2+} storage in diabetic heart.

METHODS

Materials

Sucrose was purchased from Fluka BioChemical Co. (Switzerland). Triton X-100 and $MgCl_2$ were supplied by Wako Pure Chemical LTD (Japan). $^{45}CaCl_2$ was purchased from DuPont-NEN Research Products (Boston, MA, U.S.A.). All other chemicals and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Diabetic model

Male Sprague-Dawley rats weighing ~200 g were randomly divided into three groups, control, diabetic, and insulin-treated diabetic groups. The diabetic and the insulin-treated diabetic groups were received an intraperitoneal injection of streptozotocin at a dosage of 65 mg/kg body weight (Heyliger et al, 1987). Streptozotocin was dissolved in a solution containing 0.1 M citric acid and 0.1 M sodium phosphate (pH 4.5) at a concentration of 50 mg/ml. Rats in control group were received the citrate buffer without streptozotocin. Hyperglycemia was developed within 3 days after the injection of streptozotocin. The animals were maintained for 12 weeks and chronic hyperglycemia was tested qualitatively by using Glukotest® (Boehringer Mannheim, Germany) during the duration. After the induction of diabetes, the rats of insulin-treated diabetic group were received insulin once a day at a dosage of 4 U.

Preparation of cardiac HSR vesicles

Animals were sacrificed by decapitation and hearts were immediately removed. Junctional vesicles of sarcoplasmic reticulum (HSR) were prepared from ventricular muscles as described by Valdivia et al (1992). Briefly, ventricular muscles freed from connective tissues and large vessels were cut into small pieces and homogenized with a glass-Teflon homogenizer. HSR vesicles were precipitated by centrifugation at 10,000×g. The pellet was resuspended and incubated in a buffer containing 0.6 M KCl, 5 mM Tris-Mes, pH 6.8. The suspension was centrifuged for 1 hour in a type SW-28 rotor (Beckman Instruments, INC., Palo Alto, CA, U.S.A.) at 25,000 rpm (95,000×g). The pellet containing vesicles was resuspended and subjected to a discontinuous sucrose gradient centrifugation in a SW-28 rotor for 5 hours at 25,000 rpm. Fractions were recovered from the interfaces of 10, 20, 30, 35, 40% of sucrose layers and HSR vesicles were obtained from the interface between 30% and 35% sucrose layers. Each fractions were centrifuged and 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 (Lowry et al, 1951).

Measurement of SR Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of HSR vesicles was measured by the method of Niggli et al (1979). Briefly, the activity was monitored in a solution containing 120 mM KCl, 30 mM Hepes (pH 7.4), 1 mM $MgCl_2$, 0.5 mM ATP, 50 μM $CaCl_2$, 0.4 mM NADH, 2 mM

phosphoenolpyruvate, 1 IU/ml pyruvate kinase, and 1 IU/ml lactate dehydrogenase. The formation of ADP by Ca^{2+}-ATPase activity is quantitatively coupled to the oxidation of NADH and the oxidation of NADH in the reaction solution decreases the absorbance at 340 nm. The absorbance at 340 nm was continuously monitored and the activity was calculated from the slope of the decrease in absorbance. Although mitochondrial contamination was minimized by differential centrifugation, ATPases of contaminated mitochondria were inhibited by potassium cyanide treatment during the analysis.

The Ca^{2+}-dependence of SR Ca^{2+}-ATPases was measured in solutions containing various concentrations of free Ca^{2+}. The concentration of free Ca^{2+} was adjusted by the addition of various amounts of EGTA and calculated by using a computer program that used the stability constants of Fabiato (1988). To determine the activity of Ca^{2+}-ATPases in leaky HSR vesicles, Triton X-100 (0.001%) was added to permeabilize the vesicular membrane and the Ca^{2+}-ATPase activity was measured. Uncoupling of HSR vesicles by Triton X-100 was checked by the leakage of SR $^{45}Ca^{2+}$ while SR Ca^{2+} release channels were blocked by 10 mM Mg^{2+}.

SR $^{45}Ca^{2+}$ uptake and SR $^{45}Ca^{2+}$ release

SR $^{45}Ca^{2+}$ uptake and $^{45}Ca^{2+}$ release were measured by a modified method of Ghosh et al (1988). Briefly, $^{45}Ca^{2+}$ uptake was performed in an uptake medium containing 1.8 μM $^{45}CaCl_2$ (with 2.16 Ci/mmol $^{45}Ca^{2+}$), 50 μM $CaCl_2$, 120 mM KCl, 30 mM Hepes (pH 7.4), 1 mM $MgCl_2$, and 5 mM KCN. The uptake was initiated by the addition of 1 mM ATP. After the completion of uptake, HSR vesicles were transferred onto a filter paper (Whatman GF/B) and washed three times with a washing solution containing 120 mM KCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, and 30 mM Hepes (pH 7.4). The radioactivity remained in the HSR vesicles was determined by a liquid scintillation spectrometer. SR $^{45}Ca^{2+}$ release was commenced by adding release-inducing reagents after 10 min of $^{45}Ca^{2+}$ uptake.

RESULTS

Experimental animals

Experimental rats were divided into three groups, control, diabetic, and insulin-treated diabetic rats. Streptozotocin (65 mg/kg, i.p.) was injected into the rats of the diabetic and the insulin-treated diabetic groups. Hyperglycemia was developed within 3 days after streptozotocin injection. The insulin-treated diabetic rats were received insulin once a day. General characteristics of three groups presented in Table 1 were measured at 12 weeks after the induction of diabetes by streptozotocin. The diabetic rats failed to gain weight as rapidly as the controls and 25% of diabetic rats died during 12 week-period. At the time of sacrifice, the body weights of diabetic and insulin-treated diabetic rats were lower than those of controls. Average weights of ventricles among these three groups showed highest in control and lowest in diabetic rats. However, the ratios of ventricular weight versus body weight were not significantly different in three groups. The ratio of control group was 3.4±0.3 (mg/g, n=8) and that of diabetic group was 3.9±1.3 (mg/g, n=11). The ratio of insulin-treated diabetic rats was 4.2±0.6 (mg/g, n=8), slightly higher than that of diabetic rats.

Table 1. General characteristics of experimental rats

	Control (n=8)	DM (n=11)	DM+Insulin (n=8)
Body wt, g	478±13	306±30	341±17
Ventricular wt, g	1.6±0.2	1.2±0.4	1.4±0.2
(Ventricular/body) ratio, mg/g	3.4±0.3	3.9±1.3	4.2±0.6

Values are means±SD; n is the number of rats.

SR Ca^{2+}-ATPase activity

HSR vesicles derived from the terminal cisternae of SR were obtained by a discontinuous sucrose gradient centrifugation. The activity of SR Ca^{2+}-ATPase in HSR vesicles was measured by an enzyme-coupled assay. The rate of decrease in absorbance of NADH at 340 nm represents the activity of SR Ca^{2+}-ATPase (Fig. 1A). Average activity of SR Ca^{2+}-ATPase was calculated from the slope of the decrease in absorbance and it was 562±14 nmol/min/mg protein in control rats (Fig. 1B). The activity in diabetic rats was significantly decreased to

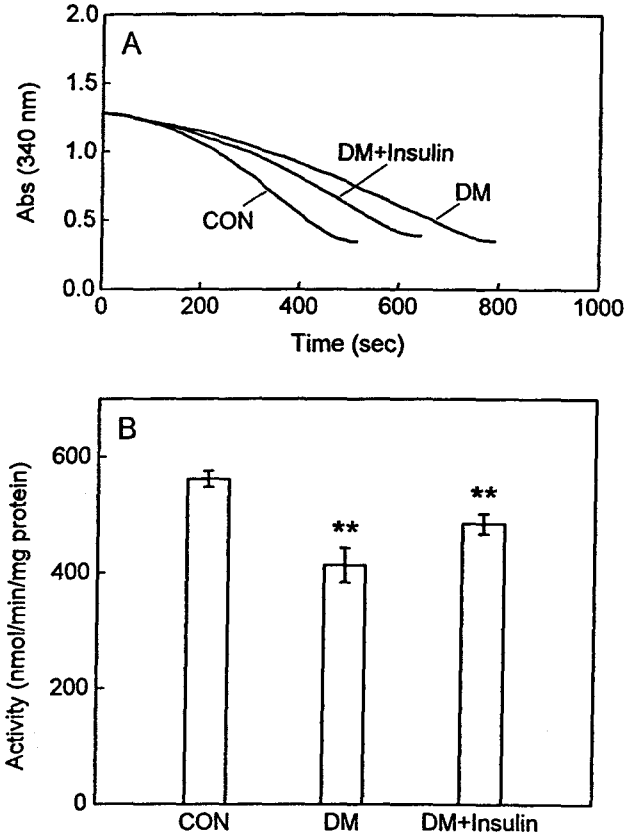

Fig. 1. Influence of streptozotocin-induced diabetes on SR Ca^{2+}-ATPase activity. (A) The activities of SR Ca^{2+}-ATPases obtained from control (CON), diabetic (DM), and insulin-treated diabetic (DM+Insulin) rats. SR Ca^{2+}-ATPase activity was monitored by the decrease in absorbance at 340 nm using an enzyme-coupled system. Each experiment was done with 50 μg/ml SR proteins. (B) The average activities of SR Ca^{2+}-ATPases. The activities were calculated from the slopes of the decreases in absorbance. The activities in diabetic and insulin-treated diabetic rats were significantly decreased (**$p < 0.01$ vs. control value by Anova test). Values are means ±SD (n=5~7).

413±30 nmol/min/mg protein while the activity was slightly recovered to 485±18 nmol/min/mg protein by insulin treatment in insulin-treated diabetic rats. These data indicated that SR Ca^{2+}-ATPase activity was depressed in diabetic SR preparations in comparison with controls.

Ca^{2+}-dependence of SR Ca^{2+}-ATPase activity

The activity of SR Ca^{2+}-ATPase was measured in the solutions containing various concentrations of free

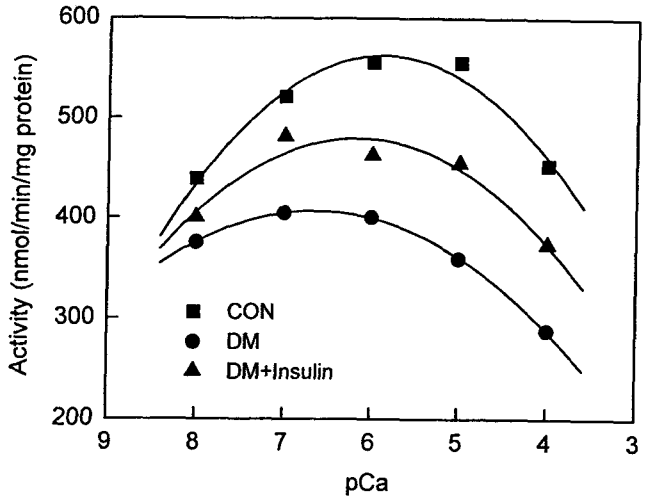

Fig. 2. Ca^{2+}-dependence of SR Ca^{2+}-ATPase. HSR vesicles were obtained from the hearts of control (CON), diabetic (DM), and insulin-treated diabetic (DM+Insulin) rats and average activities of SR Ca^{2+}-ATPase were measured at various concentrations of free Ca^{2+}. The concentration of free Ca^{2+} was adjusted by the addition of EGTA and calculated by a computer program that used the stability constants. Fitted lines were obtained by polynomial regressions. Negative value of logarithmic concentration of free Ca^{2+} was expressed as pCa. Each activity represents an average value of 2 experiments.

Ca^{2+}. The Ca^{2+}-dependence of SR Ca^{2+}-ATPase was appeared to be very similar among three groups (Fig. 2). When the concentration of free Ca^{2+} in the reaction solution was changed from 10 nM to 500 μM, the activity of SR Ca^{2+}-ATPase was appeared biphasic in all three groups. In control, the activities were 439, 521, 556, 556, and 452 nmol/min/mg protein at free Ca^{2+} concentrations of 10 nM, 100 nM, 1 μM, 10 μM and 100 μM, respectively. A maximal activity of ~560 nmol/min/mg protein was obtained in the medium containing 1~10 μM free Ca^{2+} and at below or above this concentration the activity was gradually decreased. The biphasic Ca^{2+}-dependences of SR Ca^{2+}-ATPase activity were also observed in the HSR vesicles of diabetic and insulin-treated diabetic rats. The activities of SR Ca^{2+}-ATPase in insulin-treated diabetic rats were the values between those of control and diabetic rats at the whole range of free Ca^{2+} concentration. These data showed no significant changes in Ca^{2+}-dependence of SR Ca^{2+}-ATPase activity among three groups although the activity was decreased in diabetic

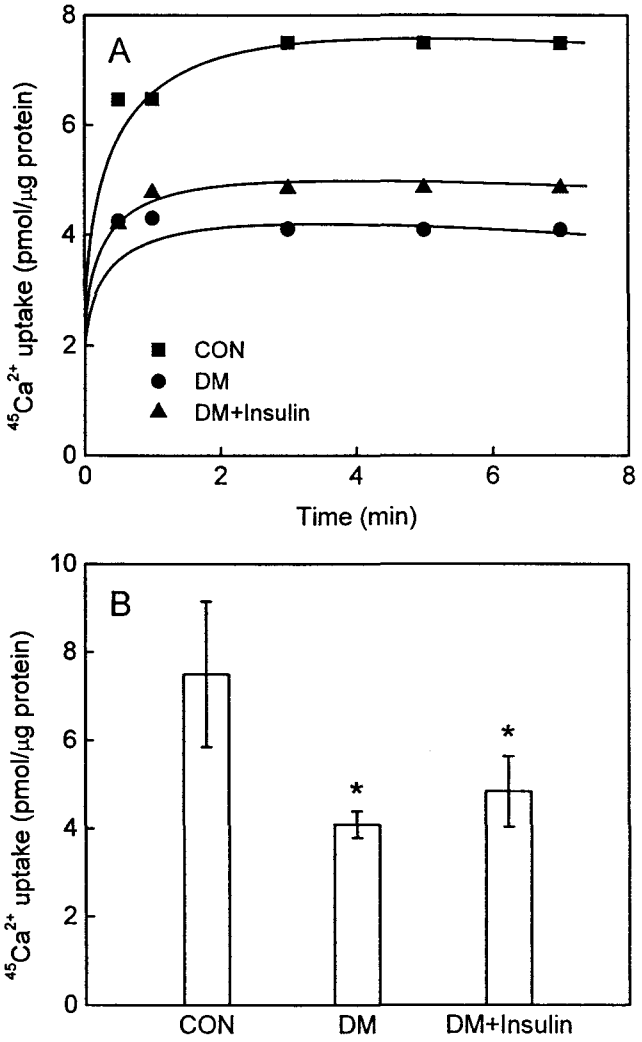

Fig. 3. SR $^{45}Ca^{2+}$ uptakes by HSR vesicles. (A) Time course of SR $^{45}Ca^{2+}$ uptake. The uptake was initiated by the addition of ATP in an uptake medium containing 50 μM Ca^{2+} and 1.83 μM $^{45}Ca^{2+}$. HSR vesicles were washed by filtering through a Whatman filter (GF/B). The washing solution contained 10 mM Ca^{2+} and 10 mM Mg^{2+} to block $^{45}Ca^{2+}$ releases from HSR vesicles during washing procedure. (B) Specific $^{45}Ca^{2+}$ uptakes by cardiac HSR vesicles. The specific uptake was calculated after active uptake for 7 min. The specific SR $^{45}Ca^{2+}$ uptakes of diabetic and insulin-treated diabetic rats were significantly lower than that of control (*$p<0.05$ vs. control value by Anova test).

rats.

SR $^{45}Ca^{2+}$ uptakes

The time courses of active $^{45}Ca^{2+}$ uptakes are shown in Fig. 3A. SR $^{45}Ca^{2+}$ uptake was measured by a filtration method and the amount of $^{45}Ca^{2+}$ taken up by HSR vesicles was calculated after incubations of 0.5, 1, 3, 5, 7 min. Rapid uptakes reached more than 90% saturation were achieved within 1 min in all three groups. Specific uptakes were calculated after 7 min of active uptake, shown in Fig. 3B. The highest uptake was obtained from the HSR vesicles of control rats and was 7.5 pmol/μg protein. However, the uptake of HSR vesicles in diabetic rats was only ~55% of the control uptake. The specific SR Ca^{2+} uptakes of diabetic and insulin-treated diabetic rats were 4.1 and 4.8 pmol/μg protein, respectively.

SR $^{45}Ca^{2+}$ releases

Releases of SR $^{45}Ca^{2+}$ were measured after 10 min of active uptake and were initiated by either decreasing free Ca^{2+} concentration or adding caffeine in the medium (Fig. 4). When the free Ca^{2+} concentration was decreased from 51.8 μM to 1 μM, $^{45}Ca^{2+}$ release was 43.5±6.5% in the SR vesicles of control rats. However, the SR $^{45}Ca^{2+}$ releases of diabetic and insulin-treated diabetic rats were lower than the control release and were 19.6±7.8% and 29.6±7.0%, respectively (Fig. 4A). The caffeine-induced releases were of similar pattern in the medium containing 51.8 μM of free Ca^{2+} concentration shown in Fig. 4B. Caffeine (10 mM) released 32±9% of stored $^{45}Ca^{2+}$ in the control SR vesicles. Caffeine-induced releases were 11.5±4.5% and 17.5±3.5% in the SR vesicles of diabetic and insulin-treated diabetic rats, respectively.

Uncoupling effect on SR Ca^{2+}-ATPase activity

The physiological roles of SR Ca^{2+}-ATPase and SR Ca^{2+} channel are opposite on the regulation of intracellular Ca^{2+} concentration. In order to evaluate the net contributions of these two factors to intracellular Ca^{2+}, the effect of uncoupling was investigated in HSR vesicles (Fig. 5). When HSR vesicles were made leaky by treating the vesicles with 0.001% Triton X-100, the activity of SR Ca^{2+}-ATPase was increased only in control by ~20% from 570±52 nmol/min/mg protein to 720±19 nmol/min/mg protein. The increase in SR Ca^{2+}-ATPase activity was due to the dissipation of accumulated SR Ca^{2+} and the consequent decrement of Ca^{2+} concentration gradient across the SR membrane (Kim et al, 1996). These uncoupling effects

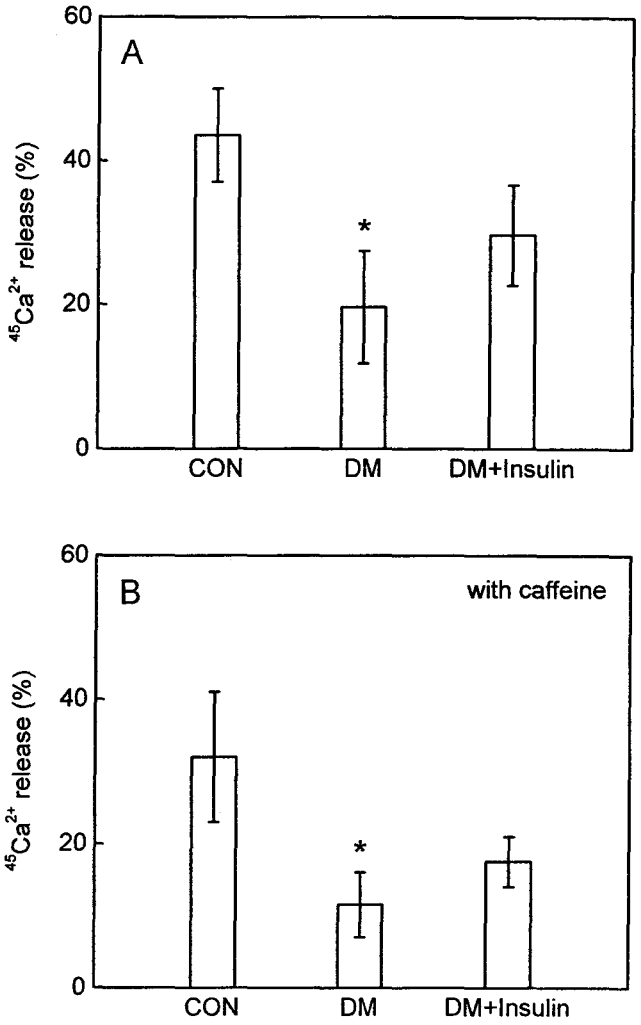

Fig. 4. $^{45}Ca^{2+}$ releases by HSR vesicles prepared from control, diabetic, and insulin-treated diabetic hearts. (A) SR $^{45}Ca^{2+}$ releases induced by the addition of EGTA. After the active uptake of 3 min, free Ca^{2+} concentration of release medium was adjusted to 1 μM and the amount of $^{45}Ca^{2+}$ release was calculated after 1 min (n=5~7). SR $^{45}Ca^{2+}$ release in diabetic heart was lower than that of control (*$p<0.05$ vs. control value by Anova test). (B) Caffeine-induced $^{45}Ca^{2+}$ release. Caffeine (10 mM) was added to the release medium and the amount of $^{45}Ca^{2+}$ release was calculated after 1 min (n=5~7). The caffeine-induced $^{45}Ca^{2+}$ release was lowest in the HSR vesicles of diabetic rats (*$p<0.05$ vs. control value by Anova test).

were not observed in the activities of SR Ca^{2+}-ATPase in both diabetic and insulin-treated diabetic rats.

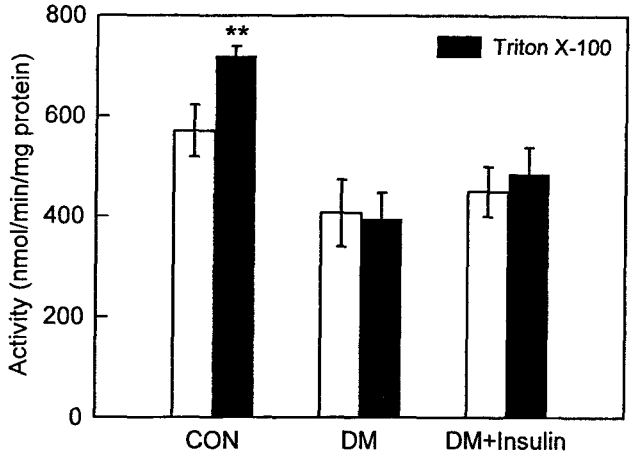

Fig. 5. Uncoupling effect on SR Ca^{2+}-ATPase activity. Leaky SR vesicles were made by the addition of Triton X-100 (0.001%). Average activity of cardiac SR Ca^{2+}-ATPase was calculated from the rate of absorbance decrease. The effect of uncoupling on SR Ca^{2+}-ATPase activity was only appeared in the HSR vesicles of control heart (**$p<0.01$ vs. the control activity in the absence of Triton X-100 by Anova test). Values are means±SD (n=5).

DISCUSSION

In insulin-dependent diabetes as well as chronic diabetes due to streptozotocin administration, the dysfunctions of cardiac myocytes have been frequently reported. These abnormal functions may be due to intracellular Ca^{2+} overload as a defect in Ca^{2+} homeostasis (Levy et al, 1994; Yu et al, 1994a & 1994b). Although the molecular mechanisms of altered Ca^{2+} handling are still poorly understood, significant impairments of Ca^{2+}-ATPase activity have been found in the membranes of both sarcolemma and SR. Sarcolemmal Ca^{2+} transports mediated by both Na^{+}-Ca^{2+} exchanger and Ca^{2+}-ATPase were shown to be depressed in diabetic heart and these changes were reversible upon insulin treatment (Heyliger et al, 1987; Makino et al, 1987; Takeda et al, 1996). Defects in SR Ca^{2+} transports were also demonstrated in diabetic cardiomyopathy and the decrease in SR Ca^{2+}-ATPase activity was observed in the diabetic ventricular myocytes (Ganguly et al, 1983; Bouchard & Bose, 1991).

In the regard of abnormal Ca^{2+} regulation in diabetic heart, it is still controversial on what factors really associate with alterations in Ca^{2+} homeostasis. It has been suggested that Ca^{2+} transport in SR is

significantly affected in diabetes (Lagadic-Gossmann et al, 1996); however, the effect of diabetes on Ca^{2+} influx or efflux across the plasma membrane may not be significant. Stimulus-evoked cytosolic Ca^{2+} transients were identical between normal and diabetic myocytes in the presence of thapsigargin, a specific antagonist of SR Ca^{2+}-ATPase (Kirby et al, 1992). In addition, because SR Ca^{2+} accounts for more than 90% of the myocardial Ca^{2+} transient (Bers, 1985), the alteration of SR functions, such as uptake, storage, and release of SR Ca^{2+}, may be the major defect in diabetic cardiomyopathy (Yu et al, 1994b).

In this study, our results demonstrated that the Ca^{2+}-transporting functions of SR were altered in diabetic heart and, specially, both SR Ca^{2+}-ATPase activity and SR Ca^{2+} uptake were depressed. In diabetic heart, the activity of SR Ca^{2+}-ATPase was decreased by 27% and SR Ca^{2+} uptake was depressed to 55% (Fig. 1 and Fig. 3). These reductions were partially recovered by insulin treatment. Our results indicate that the sequestration of cytosolic Ca^{2+} by SR Ca^{2+}-ATPases can be reduced in diabetic myocytes because of the decrease in SR Ca^{2+}-ATPase activity.

In order to elucidate the cytosolic Ca^{2+} overload in diabetic heart, the functional roles of SR Ca^{2+} release channel should be also characterized since SR Ca^{2+} release channel could be another major factor in the regulation of cytosolic Ca^{2+} concentration. Releases of SR $^{45}Ca^{2+}$ were measured in the condition of either decreasing extravesicular Ca^{2+} concentration or adding caffeine in the medium (Fig. 4). When the extravesicular Ca^{2+} concentration was decreased to 1 μM by the addition of EGTA, the amount of SR $^{45}Ca^{2+}$ release was $43.5 \pm 6.5\%$ in the control HSR vesicles. Meanwhile, in the presence of 10 mM caffeine, SR $^{45}Ca^{2+}$ release was $32 \pm 9\%$. These data demonstrated the accumulation and spontaneous release of SR Ca^{2+}, indicating that the major parts of HSR vesicle preparation exist as tight-sealed vesicles. However, the SR $^{45}Ca^{2+}$ releases in diabetic hearts were reduced by $55 \sim 70\%$ as shown in Fig. 4. and these reductions were partially recovered by insulin treatment in insulin-treated diabetic hearts. Our observations are in agreement with a previous study (Yu et al, 1994b). In ryanodine binding experiments, the number of high-affinity binding sites was decreased in diabetic heart and thus a decrease in SR Ca^{2+} release had been suggested (Yu et al, 1994b).

The activities of both Ca^{2+}-ATPase and Ca^{2+} release channel were decreased in the SR vesicles of diabetic rats. The effects of these two factors are opposite on the regulation of intracellular Ca^{2+} concentration. Defect in SR Ca^{2+}-ATPase activity would increase cytosolic Ca^{2+} concentration and, in contrast, the decrease in SR $^{45}Ca^{2+}$ release could result in the opposite effect. Therefore, in order to evaluate how SR contributes to the changes in cytosolic Ca^{2+} concentration in control and diabetic myocytes, the characteristics of SR Ca^{2+} storage were measured by investigating the uncoupling effect. Recent work from this laboratory showed that the dissipation of Ca^{2+} concentration gradient across SR membrane (uncoupling) by activating SR Ca^{2+} release channels increased the activity of SR Ca^{2+}-ATPase (Kim et al, 1996).

Our study showed that the accumulation of Ca^{2+} occurred in all three HSR preparations; however, a significant effect of uncoupling was observed in the SR vesicles of control and the activity of SR Ca^{2+}-ATPase was increased by 25% (Fig. 5). These results represent the formation of significant concentration gradient of free Ca^{2+} only in the HSR vesicles of control heart. HSR vesicles of diabetic and insulin-treated diabetic hearts could accumulate $^{45}Ca^{2+}$ through the $^{45}Ca^{2+}$-binding to luminal calsequesterins (Raichman et al, 1995); however, the gradient formation of free Ca^{2+} may be not sufficient enough to increase the SR Ca^{2+}-ATPase activity upon uncoupling since the increases in SR Ca^{2+}-ATPase activities were negligible or no uncoupling effects were observed in these vesicles.

In conclusion, this study provides evidence for a specific subcellular abnormality that can account for the reduced capacity of diabetic SR in Ca^{2+} storage. Thus, the observed depressions in both SR Ca^{2+}-ATPase activity and SR $^{45}Ca^{2+}$ uptake in diabetic heart can be conceived to result in the genesis of intracellular Ca^{2+} overload. Lagadic-Gossmann et al (1996) measured intracellular Ca^{2+} transient of diabetic myocytes and they hypothesized a similar idea that the reduced caffeine-induced SR Ca^{2+} release in diabetic heart was due to the decrease in SR Ca^{2+} content.

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