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Effects of Insulin on Altered Mechanical and Electrical Papillary Muscle Activities of Diabetic Rats

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Abstract Since insulin compounds can restore some metabolic parameters and lipid profile alterations of the diabetic rat heart, we investigated whether these beneficial effects extend to diabetic rat cardiac dysfunctions. Twenty-four male Wistar albino rats, 6 months of age with an average body weight of 250–320 g, were divided randomly into three groups, each consisting of eight rats: control-group (C) rats were fed with standard rat nutrient and water; diabetic-group (D) rats were treated with a single intramuscular injection of streptozotocin (STZ, 45 mg/kg), dissolved in 0.01 M sodium citrate, pH adjusted to 4.5; and insulin-treated diabetic group (D + INS) rats were treated with subcutaneous injections of 1 IU/l insulin (INS) twice a day after a single intramuscular injection of STZ (45 mg/kg). Treatment of D rats with INS caused a time-dependent decrease in blood glucose. We found that the lipid profile and HbA_{1c} levels in the D + INS group reached the values of control rats at the end of the treatment period. Contraction force in group D was compared with values from groups C and D + INS ($p < 0.05$). Values were obtained at a muscle contraction and relaxation time of milliseconds, with contraction time in D compared to C and D compared to D + INS and C ($p < 0.05$). Rate-dependent changes in action potential configuration in left ventricular papillary muscle obtained from 8-week control, STZ-treated D and D + INS rats showed significant membrane potential changes between C and STZ-treated D animals. Action potential amplitude showed significant changes between matched D + INS and STZ-treated D animals. Depolarization time showed significant changes between C and STZ-treated D animals and between the D + INS and D groups. Half-repolarization time showed significant changes between D + INS and STZ-treated D animals and compared to the D and C groups. Our data suggest that the beneficial effects of insulin treatment on the mechanical and electrical activities of the diabetic rat heart appear to be due to restoration of the diminished K^+ currents, partially related to the restoration of hyperglycemia.

Keywords Diabetes mellitus · Insulin · Papillary muscle · Contraction force · Electrophysiology

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Introduction

Hyperglycemia can be an important contributor to the development of vascular complications (Sowers 2004). In diabetes mellitus (DM), prevalence and mortality from cardiovascular diseases is severalfold higher in patients in the general population. One of the most important cardiovascular complications of insulin-dependent DM is a diabetic cardiomyopathy characterized by early diastolic and later systolic dysfunctions (Maghoub and Abd-Elfattah 1998).

In experimental animal studies, diabetes is most commonly induced by application of streptozotocin (STZ). In STZ-treated rats diminished cardiac contractility was found (Penpargkul et al. 1980; Brown et al. 2001), and this was related to significant alterations of myocardial calcium metabolism; reduction of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (Ganguly et al. 1983), sarcolemmal Ca^{2+}-ATPase (Heyliger et al. 1987), the Na^+–Ca^{2+} exchanger (Makino et al. 1987) and L-type calcium current (Chattou et al. 1999); and decreased cross-bridge cycle rate (Ishikawa et al. 1999). In cardiac myocytes, insulin profoundly

affects calcium handling; e.g., it stimulates L-type calcium current (Aulbach et al. 1999), the Na^+–Ca^{2+} exchanger (Algenstaedt et al. 1997) and interaction between insulin receptor substrate proteins and SR Ca^{2+}-ATPase (Algenstaedt et al. 1997). The effects of insulin on overall cardiac contraction force vary among species. A positive inotropic effect of insulin was described in guinea pigs (Von Arnim and Bolte 1980) and rabbits (Snow 1976). In piglets, the inotropic effect of insulin was biphasic, the initial negative action being followed by an increase in the contraction force (Lee and Downing 1976).

The most prominent electrical alteration is prolongation of the QT interval in diabetic patients, probably owing to attenuation of K^+ currents in diseased human myocardium (Lo et al. 1993). This prolongation of action potential duration (APD) may aggravate the reduction of diastolic filling and stroke volume at high heart rates. It may also decrease the normal endocardial–epicardial APD gradient, distorting in this way the normal temporal pattern of ventricular repolarization and resulting in flattening or inversion of the T wave, commonly observed in diabetic patients (Airaksinen 1985). Such changes may be responsible for the increased incidence of cardiac reentry arrhythmias, especially following abrupt changes in cycle length, when rate-dependent changes in APD can be expected to occur (Surawicz 1992).

We examined the impact of insulin treatment on papillary mechanic and electrical activity in healthy control rats and in rats with STZ-induced DM.

Materials and Methods

Animal Handling and Treatment Protocol

Twenty-four male Wistar-albino rats (250–320 g, 8 weeks old) were obtained from the Animal House of the School of Medicine, Çukurova University. Animals were acclimatized for 2 weeks under standard conditions of temperature 22 ± 2 °C with a 12:12 h light/dark cycle. Rats were housed in specific cages (4 rats). Rats were fed with standard pellets and water ad libitum and kept in a controlled environment following the standard operating procedures of the animal house with the approval of the Animal Ethics Committee of Çukurova University. Animal care and experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health, and approval of the ethics committee of our institution was obtained before the commencement of the study.

The animal model of diabetes used in our experiments was based on partial damage of pancreatic beta-cells resulting from single administration of STZ (45 mg/kg) in adult rats. This model of experimental diabetes is associated with partial deficits in insulin secretion and consequential hyperglycemia, without changes in peripheral insulin resistance (Algenstaedt et al. 1997).

We randomly divided the animals into three groups for this study: control-group (C, $n = 8$) rats were orally fed with standard rat nutrient and water; diabetic-group (D, $n = 8$) rats were treated with a single intramuscular injection of STZ (45 mg/kg body weight), dissolved in 0.01 M sodium citrate, pH adjusted to 4.5; and insulin-treated diabetic–group (D + INS, $n = 8$) rats were treated with subcutaneous injections of 1 IU/l INS twice a day after a single intramuscular injection of STZ. Animals were fed with standard rat nutrient and water without restriction throughout the experiment. Blood glucose levels of STZ-injected animals were measured using a glucometer (Accu-Check; Roche, Mannheim, Germany) 1 week after the injection, and those with at least three times higher blood glucose levels than the control were accepted as diabetic.

Biochemical Analysis

Measurements of HbA_{1c} and Lipid Parameters

Blood plasma HbA_{1c} was determined immune-turbidimetrically. Triglycerides (TGs), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were analyzed by glycerol-3-phosphate/phenol + aminophenazone enzymatic colorimetric, cholesterol oxidase/phenol + aminophenazone (CHOD/PAP) enzymatic colorimetric and direct CHOD/PAP enzymatic colorimetric methods, respectively. The very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were determined according to the equation described by Friedewald et al. (1972). All these parameters were determined using the Cobas Integra 800 biochemical analyzer (Roche).

Isolation of Left Papillary Muscles and Contraction Experiments

Wistar albino rats were anesthetized with ether, and the hearts were quickly excised. The papillary muscles were dissected from the left ventricle and placed in the experimental chamber. The muscle was mounted in a Petri dish (about 2 ml volume) and perfused continuously with oxygenated (95 % O_2 and 5 % CO_2) Krebs (constituents in mmol/l: 113 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 7 H_2O, 1.9 $CaCl_2$, 2 H_2O, 1.2 KH_2PO_4, 25 $NaHCO_3$ 11.5 glucose, pH 7.4) solution at a constant flow. Papillary muscle strips were suspended in organ baths containing Krebs solution, with a gas mixture of 95 % O_2 and 5 % CO_2 at 30 °C and pH 7.35–7.45. After thermoregulation and attainment of optimal muscle length, the muscles were subjected to direct supramaximal stimulation with 0.1-Hz square pulses for

periods of 0.5 ms to obtain control values [Nihon Kohden (Tokyo, Japan) Stimulator, FT.03 force displacement transducer and Hitachi (Tokyo, Japan) Digital Storage Oscilloscope, VC-6045].

Electrophysiology

Following cervical dislocation, the hearts were rapidly removed and left ventricular papillary muscles were excised. The preparations were fixed in a Plexiglas chamber, allowing for continuous superfusion (6–8 ml min^{-1}) with oxygenated (95 % O_2 and 5 % CO_2) Krebs solution at a constant flow. The temperature of this superfusate was 30 ± 0.4 °C. Preparations were stimulated using rectangular current pulses (duration 1 ms and amplitude twice the diastolic threshold) at a constant pacing frequency of 1 Hz. These stimuli were delivered to the preparations through a pair of AgCl agar-jelly electrodes. Transmembrane potentials were recorded with conventional glass microelectrodes (tip resistance 5–15 MΩ), filled with 3 mol/l KCl. Electrodes were electrically coupled to the input of a high impedance amplifier (Nihon Kohden) equipped with capacitance compensation facilities. Records were digitized and analyzed using a computer-based data BİSİP system (Cukurova University School of Medicine, Department of Biophysics), allowing for online determination of the resting membrane potential, action potential amplitude, maximum rate of depolarization and repolarization (±dp/dt), duration of action potentials and level of half-repolarization (½RT). After thermoregulation of papillary muscles, they were subjected to direct stimulation with 0.1 Hz frequency square pulses for periods of 0.5 ms to obtain control values (Nihon Kohden Stimulator and Hitachi Digital Storage Oscilloscope, VC-6045) (Kavak et al. 2008).

Statistical Analysis

Statistical analysis performed using SPSS 11.5.1 software (Lead Technologies, Charlotte, NC). All data represent means ± standard error of the mean (SEM) of n observations. For all experiments, statistical analysis was performed by one-way ANOVA, followed by post hoc analysis with the Bonferroni test to detect differences between control and experimental groups. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of Insulin Treatment on Body Weight and Blood Glucose Levels

Treatment of diabetic rats with INS caused a time-dependent decrease in blood glucose. The reduction in blood glucose started to be significant by week 4 of the treatment compared to the level of the diabetic groups ($p < 0.05$) (Fig. 1). At the end of the study period, the D group had lower body weight than the C group ($p < 0.05$). Treatment of diabetic rats with INS for 8 weeks resulted in a significant increase (28.5 %) in body weight compared to the D group ($p < 0.05$) (Fig. 2).

Effect of Insulin Treatment on Lipid Profile Levels

Lipid profiles and HbA_{1C} levels of the study groups are presented in Figs. 3 and 4. HbA_{1C}, TC, TG and VLDL levels were significantly increased in the D group compared with the C group ($p < 0.05$). LDL-C levels were not significantly different between the groups. INS significantly reduced the HbA_{1C} level compared to the D group (Fig. 3) and lipid profiles in diabetic rats ($p < 0.05$). INS also decreased lipid levels compared to the D group with the exception of LDL-C level.

Effect of Diabetes and Insulin Treatment in Parameters of Mechanical Activity of the Papillary Muscle

Isometric contraction properties of the papillary muscle in three groups (C, D, D + INS) are presented in Tables 1 and 2. The amplitudes of twitch to peak tension were reduced in the D group compared to the C group, while insulin treatment caused an increase compared to the D group ($p < 0.05$) (Table 1). Contraction time (CT), the full duration of a single contraction and relaxation performance, was significantly longer in diabetic papillary muscle compared to that in the control (Table 2) ($p < 0.05$). The ½RT values were also lower in diabetic animals. INS

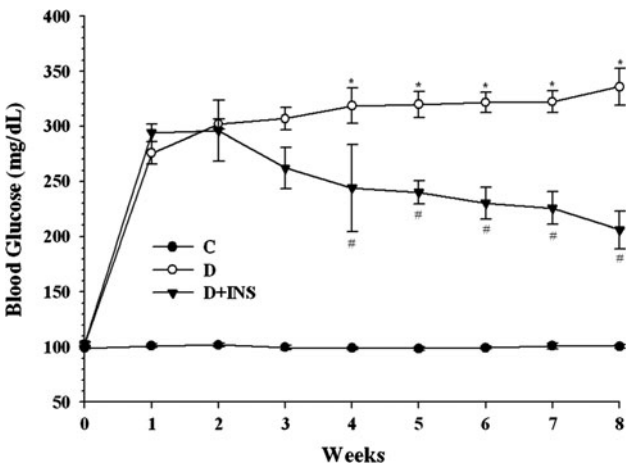

Fig. 1 Time-dependent effects of insulin on blood glucose. Rats were treated with insulin for 8 weeks. Blood glucose was determined once a week. Data are presented as means ± SD. *C* control, *D* diabetic, *D + INS* diabetic + insulin. $^{*}p < 0.05$ compared with C and $^{\#}p < 0.05$ compared with D, paired Student's *t* test

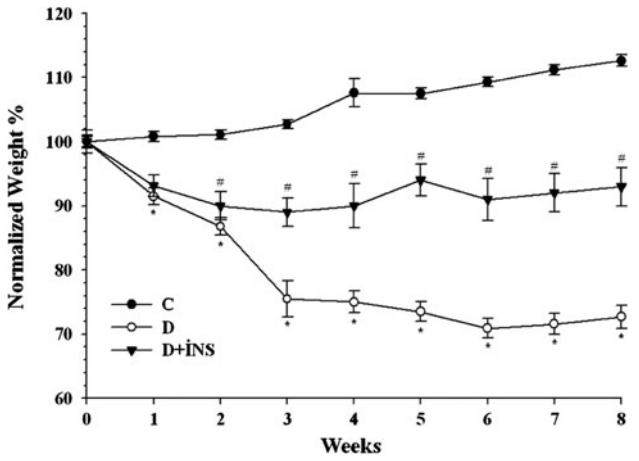

Fig. 2 Effects of insulin on body weight. Rats were treated with insulin for 8 weeks. Body weight was determined once a week. Data are presented as means ± SD. *C* control, *D* diabetic, *D* + *INS* diabetic + insulin. $^{*}p < 0.05$ compared with C and $^{\#}p < 0.05$ compared with D, paired Student's *t* test

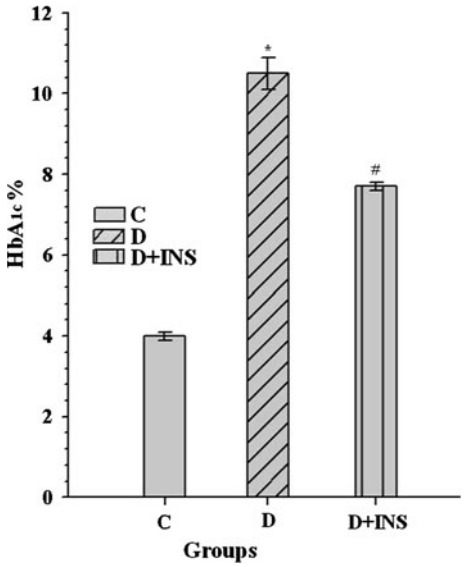

Fig. 3 Plasma HbA_{1c} levels of experimental and control groups. Data are expressed as means ± SEM. *C* control, *D* diabetic, *D* + *INS* diabetic + insulin. $^{*}p < 0.05$ compared with C and $^{\#}p < 0.05$ compared with D and C

significantly shortened both CT and ½RT compared the D group.

Effects of Insulin Treatment on Action Potential Characteristics

The effects of insulin on action potential parameters of the papillary muscle are presented in Table 3. The papillary muscles of diabetic rats had more depolarized resting membrane potentials ($p < 0.05$). There was no significant difference in resting membrane potential between diabetic

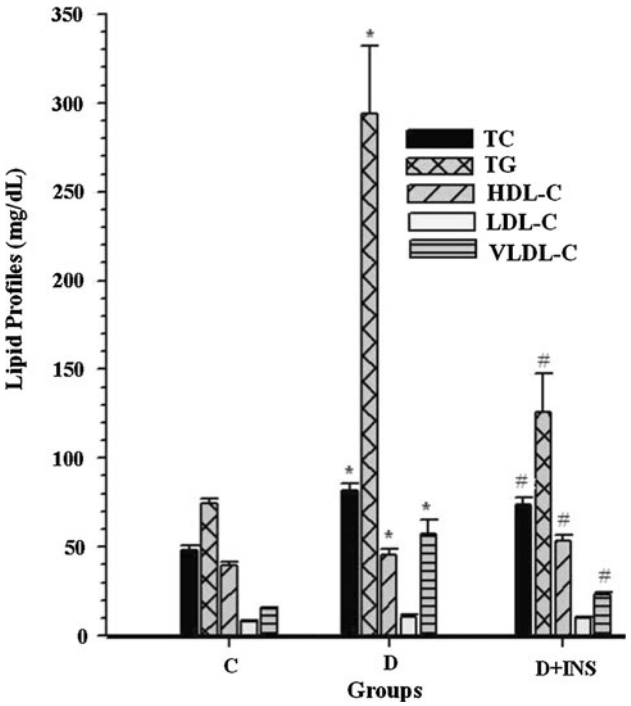

Fig. 4 Plasma lipid metabolic profiles of experimental and control groups. *C* control, *D* diabetic, *D* + *INS* diabetic + insulin. Data are expressed as means ± SEM. $^{*}p < 0.05$ compared with C and $^{\#}p < 0.05$ compared with D and C

and insulin-treated rats. Depolarization time and ½RT were significantly prolonged in the D group compared to the C group ($p < 0.05$). However, insulin supplementation to diabetic animals almost completely reversed the prolongation in APD (Table 3).

Discussion

STZ-induced diabetes is a well-established model of diabetes in rats, which is also considered to be a model for diabetic cardiomyopathy. DM patients have an increased prevalence of cardiovascular diseases, which have become the primary cause of morbidity and mortality in this group. High glucose, high lipids, insulin resistance and a number of inflammatory cytokines contribute to endothelial dysfunction in DM; and endothelial dysfunction may be implicated in the pathogenesis of diabetic vascular complications (Scheen et al. 1993). Our data show that administration of insulin to diabetic rats can partially improve the anomalous mechanical and electrical activities in isolated heart preparations.

Golay et al. (1995) indicated that treatment with insulin did not change the plasma level of lipid parameters, suggesting that beneficial effects of insulin on the vascular endothelium were not the result of improving lipid metabolism in STZ-induced diabetic rats.

Table 1 Effects of insulin on isometric contraction of the papillary muscle

Groups	TPT (mg)	+dp/dt (mg/ms)	–dp/dt (mg/ms)
C ($n = 8$)	908.1 ± 7.7	7.0 ± 0.2	4.5 ± 0.2
D ($n = 8$)	724.7 ± 11.4*	5.7 ± 0.4	2.9 ± 0.2*
D + INS ($n = 8$)	1,026.8 ± 36.0**	6.3 ± 0.4	5.1 ± 0.3**

Values are expressed as means ± SEM

C control, *D* diabetic, *D + INS* diabetic + insulin, *TPT* twitch to peak tension, *+dp/dt* peak rate of tension rise, *–dp/dt* peak rate of tension fall

*$p < 0.05$ compared with C, **$p < 0.05$ compared with D

Table 2 Effects of insulin on isometric contraction and relaxation times in the papillary muscle

Groups	CT (ms)	½RT (ms)
C ($n = 8$)	198.0 ± 4.6	144.8 ± 2.2
D ($n = 8$)	248.0 ± 6.3*	228.8 ± 5.8*
D + INS ($n = 8$)	222.5 ± 3.1**	146.3 ± 4.5**

Values are expressed as means ± SEM

C control, *D* diabetic, *D + INS* diabetic + insulin, *CT* contraction time, *½ RT* half-relaxation time

*$p < 0.05$ compared with C, **$p < 0.05$ compared with D

Table 3 Effects of insulin on action potential parameters of the papillary muscle

Groups	VR (mV)	APA (mV)	OS (mV)	DT (ms)	½RT (ms)
C ($n = 8$)	–70.2 ± 0.7	76.1 ± 0.8	9.1 ± 0.6	12.1 ± 0.4	17.5 ± 0.6
D ($n = 8$)	–66.0 ± 1.1*	72.1 ± 1.5*	10.9 ± 0.9	27.5 ± 0.9*	59.9 ± 1.0*
D + INS ($n = 8$)	–64.3 ± 0.5	77.7 ± 0.9**	12.5 ± 0.8	16.9 ± 0.5**	51.9 ± 2.4**

Values are expressed as means ± SEM

C control, *D* diabetic, *D + INS* diabetic + insulin, *VR* resting membrane potential, *APA* action potential amplitude, *OS* overshoot, *DT* depolarization time, *½RT* half-repolarization time

*$p < 0.05$ compared with D, **$p < 0.05$ compared with D

It was reported that insulin therapy improved HDL-C, TG, TC and LDL-C in some studies and decreased HDL-C levels (Scheen et al. 1993; Golay et al. 1995). Patients who were receiving 1 IU/l daily insulin showed no decrease in TG or LDL-C levels and a minute increase in HDL-C (Scheen et al. 1993; Golay et al. 1995). It was reported that 12-week insulin therapy caused a 13 % increase in HDL-C and a 19 % decrease in TG levels (Golay et al. 1995).

In our study, we found that the TG, TC, HDL-C, LDL-C and VLDL-C levels in D + INS rats were similar to the levels of C rats at the end of the treatment period (Fig. 4). In previous studies, it was indicated that the effects of treatment with insulin on body weight in diabetic rats are nonsignificant, but treatment of diabetic rats with insulin (1 IU/l daily) showed a significant increase compared with diabetic rats at the end of the treatment period (Scheen et al. 1993). In our study, we found that the body weight in D + INS rats was statistically increased compared to that in D rats, while blood glucose levels decreased (Figs. 1, 2).

Negative Inotropic Effect of Diabetes

STZ-treated rats developed diabetes with all characteristic clinical symptoms: increased blood glucose, decreased insulin plasma concentration, stagnant body weight. Eight-week diabetes in our conditions led to a considerable reduction of cardiac contraction force. In addition to that, both contraction and relaxation were slowed down in chronic diabetes. These findings are in good agreement with earlier studies (Brown et al. 2001). With regard to the basic mechanisms of the negative inotropic effect of diabetes, a considerable amount of work has been done on the cellular and molecular levels. A number of diabetes-induced changes were found at the level of Ca^{2+} handling: reduced ICaL (Lee and Downing 1976); decreased number of SR ryanodine receptors, decreased mRNA and protein levels of SERCA2 (Kim et al. 2001); further inhibition of SERCA2 by increased level of unphosphorylated phospholamban (Kim et al. 2001); and decreased mRNA and

protein levels of the Na^+/Ca^{2+} exchanger (Hattori et al. 2000). Significant alterations were also described for contractile proteins: diminished Ca^{2+} sensitivity, shifts in myosin isoenzymes (from V1 myosin with high ATPase activity to V3 myosin with low ATPase activity). However, the treatment with insulin in diabetes led in our conditions to a considerable increase of cardiac contraction force. In addition, both contraction and relaxation were slowed down in chronic diabetes (Tables 1, 2).

An alternative explanation may lie in the effects of diabetes on the electrical characteristics of papillary muscle because changes in contractile force can be associated with changes in membrane potential (Ren et al. 2000). However, in the present study the enhanced force production cannot be ascribed to changes in resting membrane potential because this was unchanged. Membrane potentials have not been measured previously in papillary muscles. There is little agreement in the reported effects of diabetes on papillary muscle resting membrane potential. In rats, STZ causes membrane potential depolarization of the papillary muscle.

In this study, at the end of week 8, action potential amplitude in the papillary muscle of the D group was significantly smaller than that the C group. Similar findings were reported by Fein et al. (1980), who showed that action potential amplitude declined in diabetic muscle. In the D + INS group treatment with insulin increased the action potential amplitude in papillary cardiac muscle. This could be explained by an increase in Na/Ca exchange kinetics. APD in diabetic rat papillary muscle was significantly prolonged within 4–6 days after induction of diabetes. Other investigators (Fein et al. 1983; Magyar et al. 1992; Jourdon and Feuvray 1993), however, have described similar electrophysiological changes on a time scale of several weeks, while Aomine et al. (1990) showed that at least 8 weeks of sustained diabetes was required to obtain significant prolongation of APD. This variation of observations suggests that diabetic changes may strongly be influenced by the experimental conditions (such as dose of STZ, duration of diabetes, origin of myocytes). It is not surprising therefore that differences were found between our papillary muscle preparations regarding the rate of development and the magnitude of diabetic changes. In addition, the prolongation of APD was most pronounced at earlier phases of papillary muscle preparations. Although the ionic background of these differences cannot be identified from the present experiments, repolarization in rat papillary muscle is influenced by several ionic currents. The early phase of ventricular repolarization is mainly a result of activation of I_{to} and I_K (Apkon and Nerbonne 1991), while activation of the inward Na^+/Ca^{2+} exchange current during the late phase is responsible for elongation of final repolarization (Mitchell et al. 1984). Lengthening of the early phase of repolarization is probably caused by suppression of K^+ currents (Magyar et al. 1992; Shimoni et al. 1994), while prolongation of final repolarization is attributed to enhancement of the Na^+/Ca^{2+} exchange current caused by Ca^{2+} overload of diabetic papillary muscle (Lagadic-Gossmann et al. 1996).

The results of the current study are consistent with the hypothesis that insulin's inotropic effect is due to increases in intracellular Ca^{2+}. A PI-3-kinase-dependent, reverse-mode Na^+/Ca^{2+} exchange activation with consequent increased trans-sarcolemmal Ca^{2+} entry during the early phase of the action potential may be one of the mechanisms by which insulin increases Ca^{2+} transients, SR Ca^{2+} content and twitch force. Nevertheless, the mechanisms that couple PI-3-kinase to Na^+/Ca^{2+} exchange remain to be determined. We observed moderate effects of insulin on AP, APD, ½RT, depolarization and repolarization velocities in untreated left ventricular papillary muscles with 0.5 Hz stimulation. However, the papillary muscle differences in development of diabetic changes suggest that impairment of various ion channels may be different in STZ-induced DM. Although the electrophysiological parameters affected by insulin are not a major therapeutic target, this effect may constitute one of the mechanisms responsible for its antidiabetic action. Thus, we raise the possibility that electrophysiological parameters are important pharmacological targets for the therapeutic action of insulin.

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