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Diabetes mellitus attenuates the repolarization reserve in mammalian heart

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Abstract

Objective: In diabetes mellitus several cardiac electrophysiological parameters are known to be affected. In rodent experimental diabetes models changes in these parameters were reported, but no such data are available in other mammalian species including the dog. The present study was designed to analyse the effects of experimental type 1 diabetes on ventricular repolarization and its underlying transmembrane ionic currents and channel proteins in canine hearts.

Methods and results: Diabetes was induced by a single injection of alloxan, a subgroup of dogs received insulin substitution. After the development of diabetes (8 weeks) electrophysiological studies were performed using conventional microelectrodes, whole cell voltage clamp, and ECG. Expression of ion channel proteins was evaluated by Western blotting. The QT_c interval and the ventricular action potential duration in diabetic dogs were moderately prolonged. This was accompanied by significant reduction in the density of the transient outward K^+ current (I_{to}) and the slow delayed rectifier K^+ current (I_{Ks}), to 54.6% and 69.3% of control, respectively. No differences were observed in the density of the inward rectifier K^+ current (I_{K1}), rapid delayed rectifier K^+ current (I_{Kr}), and L-type Ca^{2+} current (I_{Ca}). Western blot analysis revealed a reduced expression of Kv4.3 and MinK (to 25±21% and 48±15% of control, respectively) in diabetic dogs, while other channel proteins were unchanged (HERG, MiRP1, α_{1c}) or increased (Kv1.4, KChIP2, KvLQT1). Insulin substitution fully prevented the diabetes-induced changes in I_{Ks}, KvLQT1 and MinK, however, the changes in I_{to}, Kv4.3, and Kv1.4 were only partially diminished by insulin.

Conclusion: It is concluded that type 1 diabetes mellitus, although only moderately, lengthens ventricular repolarization, attenuates the repolarization reserve by decreasing I_{to} and I_{Ks} currents, and thereby may markedly enhance the risk of sudden cardiac death.

Keywords: Diabetes mellitus; QT interval; Potassium channels; Channel proteins; Repolarization reserve

1. Introduction

It has been reported that both type 1 and type 2 diabetes mellitus are associated with increased risk of sudden cardiac death [1,2], which can not easily be attributed to general pathophysiological changes such as atherosclerosis, hyperlipidaemia, hypertension or heart failure. In type 1 diabetes mellitus prolongation of the QT_c interval and increased QT_c dispersion were observed [3,4]. It was speculated that this increased QT_c interval, similarly to non-diabetic subjects with QT_c prolongation, can be related to the excess risk of mortality [5–7]. Studies in identical twins also showed that QT_c was longer in type 1 diabetic than in non-diabetic

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subjects further suggesting that prolongation of QT_c was likely due to diabetes rather than genetic factors [8]. The cellular mechanisms of lengthening of repolarization and increased susceptibility to ventricular arrhythmia and sudden cardiac death in type 1 diabetes mellitus are poorly understood. Earlier studies designed to elucidate the cellular mechanisms of diabetes-induced repolarization abnormalities were performed in rats [9–12]. These studies suggested that the decreased amplitude of the transient outward K^+ current (I_{to}) and the reduced expression of Kv4.3 and Kv4.2 channel proteins were responsible for the elongation of repolarization in diabetic rats [13,14]. However, ventricular repolarization in rats involves distinctly different mechanisms than in other mammals including dogs and humans, since it lacks a prominent plateau phase and has a short duration. Therefore, these results obtained in rats have limited value in understanding the repolarization abnormalities observed in insulin-dependent patients. Realizing this limitation, in this study the electrophysiological changes and alterations in expression of the underlying ion channel proteins induced by experimental type 1 diabetes were investigated in the dog, *i.e.* in a species having cardiac repolarization and pattern of cardiac ion currents more resembling those of the human heart.

2. Methods

2.1. Experimental protocol

Adult mongrel dogs of either sex, weighing 21–32 kg ($n=60$), were used in these experiments. Altogether in 51 dogs infusion of a single intravenous dose of alloxan was applied in order to induce diabetes mellitus (560 μmol/kg alloxan monohydrate, Sigma, St Louis, MO, USA). 12 dogs died within 72 h after the alloxan infusion, and diabetes was not induced in 11 dogs. In the diabetes period 5 dogs died in the untreated and 1 dog in the insulin-treated groups. Another 7 dogs (1 in control group, 4 in diabetes group and 2 in insulin-treated diabetes group) were omitted from the experiments because of technical reason. This attrition rate is expected and were reported previously [15].

The diabetes-inducing agent alloxan is reported to cause destruction of pancreatic islet beta-cells. The destruction is associated with pancreatic beta-cell DNA injury related to free radicals. The pancreatic islets are relatively susceptible to active oxygen, and are more easily damaged than other tissues. This is because the activities of antioxidant enzymes such as superoxide dismutase are low in pancreatic islet beta-cells [16].

Development of diabetes was confirmed by intravenous glucose tolerance test 1 week later. 8 from the 16 alloxan-treated dogs received insulin substitution (0.5–1.0 IU/kg day^{-1} s.c., Humulin N, Eli Lilly, USA) to maintain normoglycaemia. Insulin treatment was initiated 1 week after the injection of alloxan and was maintained throughout the entire experiment. The untreated animals were used as controls.

Body weight and plasma glucose levels were determined in each animal at the beginning as well as at the end of the experiment (8 weeks after the alloxan injection). The plasma potassium and urea nitrogen values were also determined. These data, verifying the diabetic state of the animals, are presented in Table 1. The thyroid hormone levels (free T3 and T4) were measured by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics). At the end of the experiment, the animals were sacrificed under anaesthesia induced by intravenous infusion of 0.25 mg/kg midazolam plus 10 mg/kg ketamine. The hearts were rapidly removed and tissues for cellular electrophysiological measurements and for Western blot studies were excised.

All experiments were carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication NO 85–23, revised 1985). The protocols were approved by the Review Board of the Committee on Animal Research of the University of Szeged (54/1999 Oej).

2.2. Electrocardiography

Conventional ECG recordings were taken from each animal under anaesthesia at the beginning and the end of the experiment. All leads were sampled by an ECG signal processing system (Haemosys, Experimetria Ltd, Budapest, Hungary) under the control of a personal computer. Following analogue-to-digital conversion, the data were digitally stored and analyzed off-line. ECG parameters (RR, PQ, QRS, QT and QT_c intervals) were determined manually using cursors. QT intervals were measured on lead II from the QRS onset to the end of the T wave. QT_c intervals were derived according to the Fridericia formula [$QT_c = QT/RR^{1/3}$] [17]. ECG parameters were averaged from 3 consecutive complexes analyzed always by a single observer.

2.3. Recording of action potentials from ventricular myocardium

After opening the chest the heart was quickly removed. Thin papillary muscles were excised from the right ventricle and immersed in oxygenated modified Locke's solution containing (in mM): NaCl 120, KCl 4, $CaCl_2$ 1.0, $MgCl_2$ 1, $NaHCO_3$ 22 and glucose 11. The pH of this solution was set to 7.4 ± 0.05 when saturated with the mixture of 95% O_2 and 5% CO_2 at 37 °C. Transmembrane action potentials were recorded using the conventional microelectrode technique. Recordings were taken from 3 distinct impalements in the case of each animal. The preparations were stimulated at a frequency of 2 Hz using rectangular constant current pulses of 2 ms duration delivered through a bipolar platinum electrode. At least 1 h was allowed for each preparation to equilibrate before beginning of the measurements. Microelectrodes, filled with 3 M KCl and having tip resistances of 5–20 MΩ, were connected to the input of a high impedance electrometer (HSE, type 309 microelectrode amplifier). Data acquisition was performed with an ADA 3300 analogue-to-digital board (Real Time Devices Inc., State College, PA, USA.) using a maximum sampling rate of 40 kHz.

Table 1
Changes in body weight and plasma constituents during the experimental period in dogs

Dog	Control ($n=8$)		Diabetic ($n=8$)		Insulin-treated ($n=8$)	
	Initial	Final	Initial	Final	Initial	Final
Body weight (kg)	25.1±2.0	25.9±2.2	27±1.2	22.2±2.1*†	24.6±1.3	23.6±0.7
Glucose (mM)	4.7±0.2	4.8±0.1	4.8±0.3	19.4±2.8*†	4.3±0.3	6.4±0.7*†
Potassium (mM)	4.5±0.1	4.6±0.1	4.5±0.1	4.9±0.1*†	4.4±0.2	4.6±0.2
Urea nitrogen (mM)	3.9±0.3	3.8±0.3	4.0±0.8	6.8±0.9†	4.7±0.4	6.9±0.5*†
Free T3 level (pM)		2.75±0.18		3.55±0.45		4.23±0.26
Free T4 level (pM)		9.62±0.66		9.98±1.03		11.09±1.37

The experimental period lasted for 8 weeks. Data represent means±SEM, asterisks denote significant changes between the initial and final states determined using Student's *t*-test for paired data, † indicates significant differences comparing to the control group calculated with Student's *t*-tests for unpaired data ($p<0.05$).

2.3.1. Voltage clamp

Canine left midmyocardial ventricular myocytes were enzymatically dissociated using the segment perfusion technique, as described earlier in detail [18]. One drop of cell suspension was placed within a transparent recording chamber mounted on the stage of an inverted microscope, and individual myocytes were allowed to settle and adhere to the bottom of the chamber for at least 5 min before superfusion was initiated. HEPES buffered Tyrode's solution was used as the normal superfusate. This solution contained (in mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, Glucose 5.5, and HEPES 5.0 at pH of 7.4. All experiments were performed at 37 °C.

Patch-clamp micropipettes were fabricated from borosilicate glass capillaries using a P-97 Flaming/Brown micropipette puller (Sutter Co, Novato, CA, USA). These electrodes had resistances between 1.5 and 2.5 MΩ after filling with pipette solution containing (in mM): K-aspartate 100, KCl 25, ATP 3, $MgCl_2$ 1, EGTA 10 and HEPES 5, or KCl 110, KOH 40, EGTA 10, HEPES 10, TEACl 20, MgATP 5, GTP 0.25, when measuring K^+ and Ca^{2+} currents, respectively. The pH of the pipette solution was adjusted to 7.2 by KOH.

Membrane currents were recorded with Axopatch-1D and 200B patch-clamp amplifiers (Axon Instruments, Union City, CA, USA) using the whole cell configuration of the patch-clamp technique. The series resistance was typically 4–8 MΩ before compensation (50–80%, depending on the voltage protocols). Membrane currents were digitized using a 333 kHz A/D converter (Digidata 1200, Axon Instruments) under software control (pClamp 8, Axon Instruments, Union City, CA, USA) after low-pass filtering at 1 kHz. Membrane currents were normalized to cell capacitance, determined by applying 10 mV hyperpolarizing pulses at − 10 mV. Voltage protocols used for measuring specific ion currents are described in the appropriate section of results.

2.3.2. Western blotting

Ion channel proteins from control, diabetic, and insulin-treated canine hearts ($n=8$ in each group) were obtained using a method modified after Han et al. [19]. Briefly, tissues were pulverized in liquid N_2 and suspended in ice-cold TE buffer (20 mM Tris, 1 mM EDTA, and 1:100 dilution of Protease inhibitor cocktail containing at final concentrations: 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 0.8 μM aprotinin, 20 μM leupeptin, 40 μM bestatin, 15 μM pepstatin A, and 14 μM E-64) (all purchased from Sigma–Aldrich, St. Louis, MO, USA). The suspension was disrupted by sonication on ice (3×20 s) and was centrifuged at 100,000 g for 90 min at 4 °C. The resulted pellet was then resuspended in ice-cold TE buffer containing 2% Triton X-100 (Sigma) and was again centrifuged at 100,000 g for 45 min at 4 °C. The protein content of the resulted supernatant (membrane fraction) was measured by a modified BCA protein assay (Pierce, Rockford, IL, USA). Samples were then mixed with SDS-PAGE sample buffer, subjected to SDS-PAGE according to Papp et al. [20] (8% gels were loaded with vigorously equal amounts of 40 μg protein per lane) and transferred to nitrocellulose membranes (BioRad, Wien, Austria). Membranes were blocked (1 h, room temperature) with 5% nonfat dry milk in phosphate-buffered saline (PBS) and incubated overnight with the following primary antibodies (1:50–1:100 dilution in milk-PBS containing 0.1% Tween 20, PBST); polyclonal rabbit anti-Kv 1.4, anti-α_{1C}, anti-MinK, anti-HERG, anti-MIRP1 (all form Alomone Labs, Jerusalem, Israel), polyclonal goat anti-KChIP2 and anti-KvLQT1 (from Santa Cruz Biotech, Santa Cruz, CA, USA). After 3 washes in PBST, membranes, in the cases of the rabbit primary antibody staining, were incubated with an anti-rabbit ABC kit (Vector Laboratories, Burlingame, CA, USA), as suggested by the manufacturer. In the case of the goat anti-KChIP2 labelling, membranes were first incubated with a rabbit anti-goat secondary antibody (1:1000 dilution in milk-PBST, BioRad) and then with the above anti-rabbit ABC kit. Immunoreactive bands were visualized by an ECL Western blotting detection kit (Pierce) on light sensitive film (AGFA, Brussels, Belgium). For obtaining negative controls, samples were also probed with primary antibodies preincubated with antigenic (control) peptides; in all cases, the specific staining was suspended by the presence of the control peptide (data not shown). Band density was quantified using a GelDoc instrument (BioRad) on films exposed and processed equally, as described before [20]. Densitometric values of the individual lanes were normalized to those obtained for the control

animals in several independent experiments and were expressed as percentage of control (100%).

2.4. Statistical analysis

Results were compared using Student's *t*-tests for paired or unpaired data as appropriate. Differences were considered significant when $p<0.05$. Data are expressed as mean± SEM.

3. Results

3.1. Effect of diabetes on ventricular repolarization

Diabetes induced a moderate, but statistically significant, lengthening of ventricular repolarization, which was prevented by insulin substitution. Results obtained from ECG recordings (QT_c) and from isolated papillary muscle preparations (APD) are summarized in Table 2. Lengthening of repolarization was the single diabetic alteration observed, since neither the heart rate (RR interval), nor the velocity of atrio-ventricular (PQ interval) and intraventricular (QRS duration) conduction was significantly modified by alloxan or insulin. The angles of the QRS and T-wave vectors were also unchanged in diabetic and insulin-treated diabetic dogs (Table 2). Action potential configuration was also characteristically altered in diabetic dogs: phase-1 repolarization became attenuated (Fig. 1). Similar to changes in repolarization, this effect was prevented by insulin treatment.

3.2. Diabetes-induced changes in ionic currents

Steady-state current–voltage relationship of the membrane was determined by applying 400 ms long voltage pulses to test potentials ranging from −120 mV to +60 mV, arising from the holding potential of −90 mV. Membrane currents measured at the end of these pulses were plotted against their respective test potentials. The negative branch of the I–V curve is associated with I_{K1}. As shown in Fig. 2A, no significant differences were observed in the steady-state current–voltage relationship, and consequently, in the density of I_{K1}, in ventricular myocytes obtained from control, diabetic, and insulin substituted dogs.

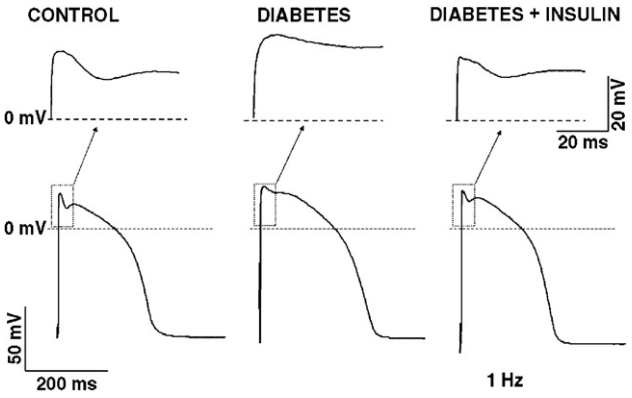

Fig. 1. Representative action potential recordings measured in ventricular papillary muscle originating from control (left panel), diabetic (middle panel) and insulin-treated diabetic (right panel) dogs. Insets show a magnification of the early repolarization phase of the action potential. Stimulation frequency was 1 Hz. Dotted lines indicate zero potential level.

I_{Kr} was activated by 1 s long depolarizing test pulses to membrane potentials ranging from −20 mV to +50 mV at frequency of 0.05 Hz. The amplitude of the tail current, measured upon returning to the holding potential of −40 mV, was plotted as a function of the activation voltage and was used to define the magnitude of I_{Kr}. These experiments were performed in the presence of 1 μM nisoldipine and 30 μM chromanol 293B in order to suppress I_{Ca} and I_{Ks}, respectively. Results displayed in Fig. 2B indicate that neither the amplitude of I_{Kr}, nor the voltage-dependence of its activation was altered in the alloxan-induced diabetes or by the applied insulin substitution.

I_{Ca} was recorded in the presence 3 mM 4-aminopyridine in order to block I_{to}. The current was evoked by 400 ms long depolarizing test pulses to voltages between −40 to +55 mV, arising from the holding potential of −40 mV. Peak values of I_{Ca} were plotted against the respective test potentials (Fig. 2C). The density of I_{Ca} was not significantly different in the control, diabetic and insulin-treated groups, having also similar voltage-dependence of activation.

I_{to} was evoked in the presence of 1 μM nisoldipine by applying 400 ms long test depolarizations to voltages ranging from −20 to +50 mV. These depolarizations were arising from the holding potential of −90 mV, and were separated by 3 s long interpulse intervals. The amplitude of I_{to} was defined

Table 2
Changes in ECG parameters and action potential duration during the 8 weeks experimental period

	Dog	RR (ms)	PQ (ms)	QRS (ms)	QT (ms)	QT_c (ms)	QRS-vector (degree)	T-wave vector (degree)	APD_{50} (ms)	APD_{90} (ms)
Initial	Control	335±20.0	106±3.9	53±2.4	196±3.8	283±2.9	81.7±3.0	85.6±0.5		
	DM	342±16.5	105±3.6	55±1.9	198±5.7	284±4.5	81.4±3.0	83.0±1.4		
	DM+insulin	319±16.5	104±5.0	57±2.7	193±5.7	283±4.6	82.8±1.3	84.4±1.2		
Final	Control	346±24.4	106±1.9	54±2.3	199±6.3	284±4.2	84.3±1.7	86.7±0.8	191.2±4.0	225.0±4.0
	DM	330±19.2	102±3.6	55±2.2	208±6.4	302±8.8*	85.1±0.9	83.0±1.6	205.2±4.7†	242.8±5.6†
	DM+insulin	304±17.8	105±3.7	54±2.5	187±3.2	280±5.4	84.0±1.2	85.7±1.1	180.7±3.1$^{\dagger\#}$	213.1±3.6$^{\dagger\#}$

Data represent means±SEM. Asterisks denote significant changes between the initial (0 week) and final states (8 week) determined using Student's *t*-test for paired data ($p<0.05$). † indicates significant differences comparing to the respective value in the control group calculated with Student's *t*-tests for unpaired data ($p<0.05$). $^{\#}$ indicate significant differences comparing to the respective value in the diabetes group (DM) calculated with Student's *t*-tests for unpaired data ($p<0.05$). ECG and action potential measurements (19–25 impalements) were made from 8 dogs in each group.

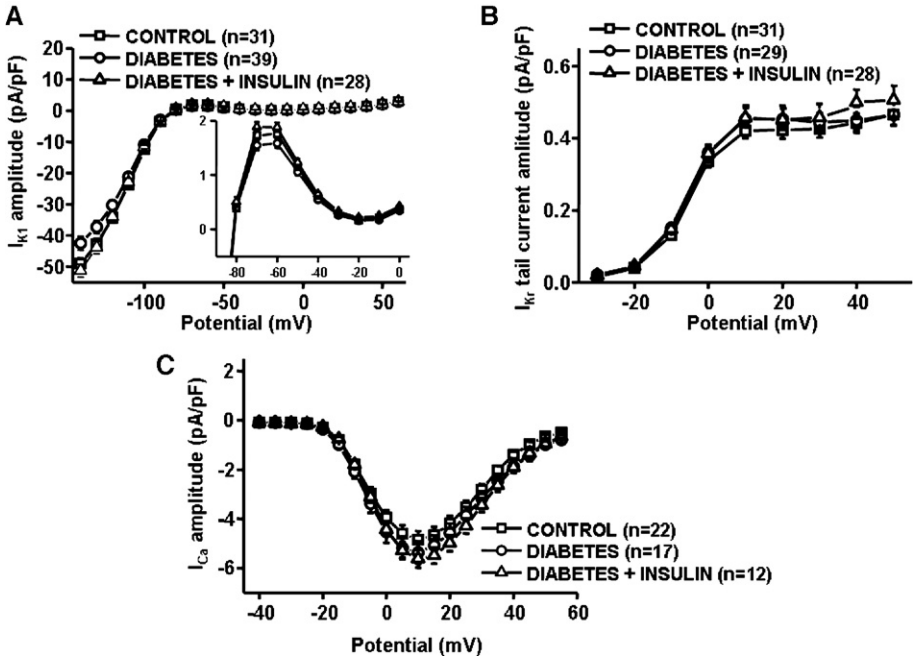

Fig. 2. Effect of diabetes and insulin substitution on the current–voltage (I–V) relationships obtained for I_{K1} (A), I_{Kr} (B) and I_{Ca} (C) in canine ventricular myocytes. A segment of the I–V curve between −80 and 0 mV is enlarged in the inset of panel A. Symbols and bars represent mean values±SEM; *n* indicates the number of cells.

at each membrane potential as a difference of the peak outward current and the steady-state current (recorded at the end of the test pulse). Amplitude of I_{to} was significantly smaller in diabetic myocytes than in cells isolated from the control animals (9.54±1.11 pA/pF *versus* 17.39±1.92 pA/pF at +50 mV, $p<0.05$, $n=35$ and 32, respectively), however, the voltage-dependence of activation remained unaltered (Fig. 3). Similarly, the inactivation kinetics of I_{to} was not modified by diabetes (the decay time constant was 5.2± 0.11 ms in control, $n=29$; and 5.5±0.21 ms in diabetes, $n=27$). The diabetes-induced suppression of I_{to} could be only partially prevented by insulin substitution.

I_{Ks} was activated by depolarizing test pulses of 5 s duration clamped to membrane potentials ranging between −20 mV and +50 mV and applied at frequency of 0.1 Hz. The amplitude of the tail current measured upon repolarization to the holding potential of −40 mV was plotted against the activation voltage and was used to define the magnitude of I_{Ks}. In these experiments the external solution was supplemented with 1 μM nisoldipine and 1 μM E-4031 in

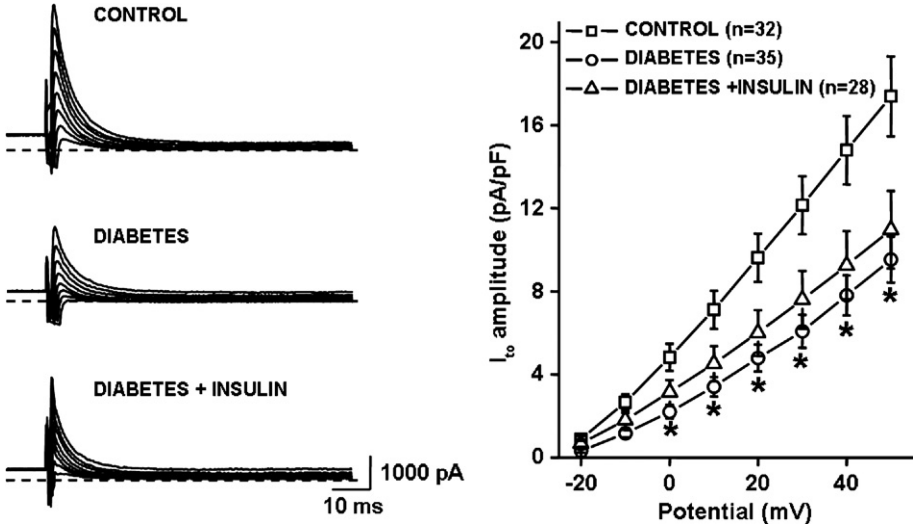

Fig. 3. Diabetes-induced changes in the amplitude of I_{to} in dog ventricular myocytes. I_{to} families and current–voltage relationships are presented in the left and right panels, respectively. Symbols and bars represent mean values±SEM, dashed lines indicate zero current level, and n=number of cells. Asterisks denote significant differences ($p<0.05$) from control.

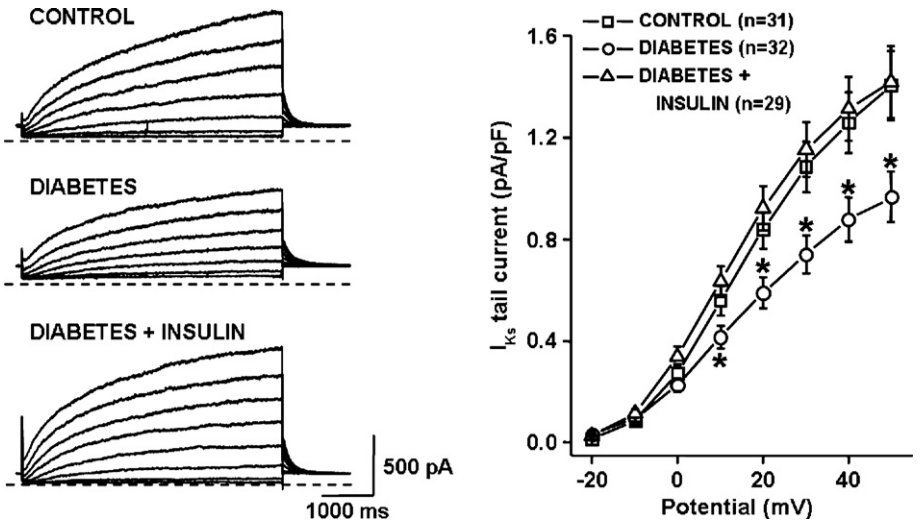

Fig. 4. Suppressive effect of diabetes on the amplitude of I_{Ks}. Note the full prevention of diabetic alterations in myocytes obtained from insulin-treated hearts. Symbols and bars represent mean values±SEM; n=cell number. Asterisks denote significant differences ($p<0.05$) from control.

order to eliminate I_{Ca} and I_{Kr}, respectively. As displayed in Fig. 4, the amplitude of I_{Ks} was significantly less in diabetic than in non-diabetic dogs (0.97±0.1 pA/pF *versus* 1.40± 0.13 pA/pF at +50 mV, $p<0.05$, $n=32$ and 31, Fig. 4) Insulin substitution fully prevented the development of the diabetes-induced reduction in I_{Ks}, *i.e.* identical I_{Ks} values were recorded in the insulin-treated and control canine myocytes within the entire voltage range.

3.3. Effect of diabetes on expression of ion channel proteins

Electrophysiological studies revealed significant reduction in the density of I_{to} and I_{Ks} in diabetic myocytes. Therefore, expression of the underlying channel forming proteins (α-subunits), together with some of their important regulatory subunits, was compared in left ventricular myocardial samples excised from control, diabetic, and insulin-treated animals ($n=8$ in each group). Since our experimental technique allowed only comparison of paired samples — instead of determination of absolute density of channel proteins — the optical densities obtained in the diabetic and insulin-treated hearts were normalized to those measured in the non-diabetic controls and expressed as percentage. As is shown in Fig. 5, expression of α_{1C} (pore-forming subunit of the L-type Ca^{2+} channel), HERG and MiRP1 (pore-forming and regulatory subunits of the I_{Kr} channel, respectively) were

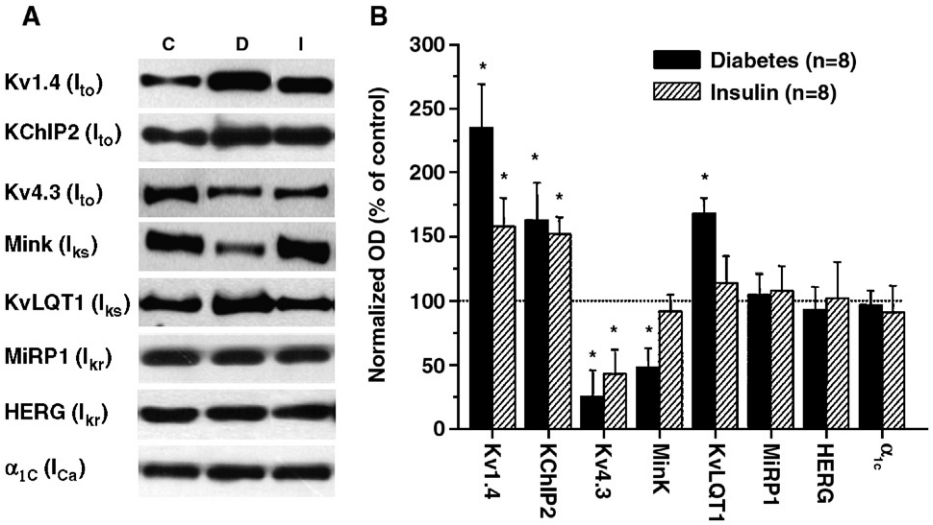

Fig. 5. Expression of ion channel proteins (α-subunits and regulatory proteins) in dog ventricular myocardium determined by Western blotting. Series of representative Western blots are presented in the left panel (A). Samples were obtained from non-diabetic control (C), diabetic (D), and insulin-treated (I) hearts. In the right side diagram (B) relative optical density (OD) values, generated by normalizing the results obtained from diabetic and insulin-treated preparations to control ones, are given in the ordinate. Dotted line indicates 100%. Columns and bars represent mean±SEM values obtained from 8 independent experiments for each antigen. Asterisks denote significant differences ($p<0.05$) from control.

not significantly altered by diabetes. In contrast, expression of KvLQT1 and MinK (pore-forming and regulatory subunits of the I_{Ks} channel) was inversely affected by diabetes: the density of MinK was reduced to 48±15%, while that of KvLQT1 was elevated to 168±12% of the control. Similar asymmetry was observed in distribution of channel proteins responsible for mediation of I_{to}: the expression of Kv4.3 (the major pore-forming subunit in dog) was decreased to 25± 21% of the control, while the density of Kv1.4 (less dominant pore-forming subunit) and KChIP2 (regulatory subunit) was significantly elevated in diabetic myocardium (to 235±34% and 163±29 5%, respectively). In accordance with results of the voltage clamp studies, the diabetes-induced changes in MinK and KvLQT1 expression were fully prevented by insulin substitution. In the case of Kv4.3 and Kv1.4 only partial prevention could be achieved, while the concomitant change in KChIP2 density was not affected by insulin at all.

4. Discussion

To the best of our knowledge this is the first report describing diabetes-induced changes in cardiac ion currents and the underlying ion channel proteins in the dog, *i.e.* in a species having ventricular repolarization very similar to that of the human [18]. The major finding of this study is that experimentally induced diabetes mellitus was associated with only moderate lengthening of action potential duration and QT_c interval in canine heart, while causing marked reduction of K^+ currents (I_{to} and I_{Ks}) involved in ventricular repolarization. Although the role of these currents in normal repolarization (*i.e.* in healthy individuals) was questioned, their importance under certain pathophysiological conditions seems to be strongly increased [18,21–27]. Cardiac repolarization is determined by several ion channels, including numerous outward transmembrane K^+ currents. Under normal conditions block of one type of K^+ current does not necessarily cause excessive lengthening of action potential duration, since other types of K^+ current may provide sufficient repolarization. This concept was termed by Roden as "*repolarization reserve*" [28–30]. These K^+ currents may compensate for each other to secure the repolarization process [28–32]. If repolarization is excessively lengthened due to the blockade of I_{Kr}, hypokalaemia, genetic abnormality, or bradycardia, the subsequent increase in action potential duration would facilitate the activation of I_{Ks} and provide a negative feedback mechanism to limit further lengthening [18,31–34]. Deficiency of such a mechanism in case of inheritance (*e.g.* long QT syndromes) or acquired disease (e.g. ion channel remodeling caused by heart failure, acute myocardial infarction, or diabetes mellitus), may lead to excessive prolongation of repolarization and increased propensity for development of early afterdepolarizations in response to a relatively weak inhibition of a K^+ current [35–39]. Therefore, it is likely that the decreased I_{Ks} in diabetes mellitus would attenuate the repolarization reserve, and as such, it would increase the proarrhythmic risk especially when I_{to}, another repolarizing potassium current, is also diminished. Consequently, diabetic patients may carry an increased proarrhythmic risk due to their compromised repolarization reserve capacity even if their QT_c interval is close to normal. This must be born in mind when designing pharmacotherapy for diabetic patients.

Several studies in the rat showed that experimentally induced type 1 diabetes mellitus lengthened cardiac action potential duration and decreased the amplitude of I_{to} [9–12]. This is consistent with our finding in the dog where both I_{to} and Kv4.3 protein (the dominant pore-forming subunit in canine, human and even in rabbit ventricle [40]) were found to be downregulated. In previous experiments it was also found that the density of Kv4.3 protein was decreased, while the density of Kv1.4 protein was increased in type 1 diabetes mellitus [13,14]. These results are congruent with our data obtained in canine heart. The way by which the rapidly inactivating I_{to} may contribute to repolarization is controversial. It has to be emphasized, however, that following inactivation of the fast component of I_{to} a second, slower component of inactivation is also evident having a time constant of 25–35 ms and amplitude of 200–300 pA. This component of I_{to} is operating probably during the early phase of plateau, and its possible reduction due to diabetes may result in lengthening of repolarization.

The decreased density of I_{Ks} and expression of the underlying regulatory β-subunit channel protein, MinK in the diabetic heart is a new and important finding. It is surprising, however, that this reduction of I_{Ks} was associated with an increased expression of the pore-forming α-subunit, KvLQT1. On the other hand there may be more direct interactions between the KvLQT1 and HERG α-subunits of I_{Ks} and I_{Kr}. KvLQT1 modulates the distribution and biophysical properties of HERG. It means that HERG current density was approximately doubled by co-expression with KvLQT1 [41]. It can be speculated, therefore, that downregulation of MinK is the primary consequence of diabetes mellitus, and the concomitant upregulation of KvLQT1 can be a secondary compensatory process, which may partially oppose the consequences of the diabetes-induced downregulation of MinK. The opposite mechanism might operate in the case of I_{to}, resulting in reduction of the Kv4.3 expression associated with the upregulation of Kv1.4 and KChIP2. Patel et al. [42] proposed a Kv4.3 gating model wherein KChIP2 isoforms accelerate recovery from inactivation, slow closed-state inactivation, and promote open-state inactivation. Therefore, the upregulated KChIP2 may slow the inactivation at depolarized potentials increasing the current, the repolarizing force in the plateau phase, and may promote reopening of the I_{to} current upon membrane repolarization, which may contribute to the late repolarization [42].

Deschenes and Tomaselli [43] reported that when MinK was co-expressed with Kv4.3, the current density increased about five-fold compared to Kv4.3 expressed alone, and the inactivation and the recovery from inactivation kinetics of

Kv4.3 were slowed by MinK [43]. Therefore, the alteration of MinK by diabetes might at least partly explain the attenuation of I_{to} density in dog. It is also worthy of note that insulin substitution was able to fully prevent the diabetes-induced reduction of I_{Ks} (and the concomitant changes in the expression of the underlying channel proteins), but had only a limited protective effect in the case of I_{to}. The reason for this discrepancy remains unclear, however, it can be concluded that patients with type 1 diabetes — even when they are properly treated with insulin — may carry an increased proarrhythmic risk.

The mechanism by which diabetes mellitus can influence expression of cardiac ion channels is not fully understood. Elevation of the plasma free fatty acids in diabetes increases the rate of β-oxidation and the subsequent formation of amphiphillic metabolites, such as palmitoylcarnitine and palmitoyl-CoA, which was shown to directly suppress I_{to} in the rat heart [44], but the effects of these metabolites on ion channel expression is unknown. Activation of the renin–angiotensin system was also demonstrated in insulin-dependent diabetic rats, and the increased level of angiotensin II attenuated I_{to} [45]. It was found that inhibition of the formation or action of angiotensin II reversed the attenuated I_{to} in both type 1 and type 2 diabetes. This observation can be explained by the fact that angiotensin II has numerous and diverse cellular effects mediated by protein kinase A, protein kinase C, and tyrosine kinases, which may be linked to the inhibition of certain transmembrane ionic channels [46]. Further studies are required to elucidate these details.

4.1. Limitations of the study

Several studies indicate that in terms of the densities and kinetics of cardiac ion channels involved in ventricular repolarization canine ventricular cells can best approximate human ventricular myocytes. We propose, therefore, that present observations using diabetic canine myocardium may yield important conclusions. Previous results obtained mostly from rat models are difficult to extrapolate to the diabetic changes of human cardiac tissues at the level of ion channels. However, the present results can be implicated to diabetic patients with caution. First of all, the diabetic model used in this study can relate only to type 1 diabetes, which represents only the minority of the diabetic population. Furthermore, human diabetes has a long lasting time course extending for several decades after its diagnosis and insulin-replacement therapy is applied in these patients. Therefore, it is still uncertain how the changes in ionic currents and protein densities observed in our experiments would actually influence the repolarization reserve and proarrhythmic risk in diabetic patients in general. The possible attenuation of the repolarization reserve should be studied in patients and — if confirmed — should be taken into account as an enhanced proarrhythmic risk factor before pharmacotherapy is initiated.

Besides the importance of the decreased repolarization reserve in diabetes, regional differences in APD and the underlying ionic currents existing throughout the left ventricular wall [47–49] may be differentially affected by diabetes as it was reported in rat [50,51]. Also the electrophysiological properties of the right and left ventricles are somewhat different [52]. Therefore, in future studies it would be important to address whether and to what extent the transmural and interventricular gradients of the various ionic currents are altered by diabetes and how they influence repolarization.

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