Hyperglycemia aggravates atrial interstitial fibrosis, ionic remodeling and vulnerability to atrial fibrillation in diabetic rabbits

Hiperglisemi diyabetik tavşanlarda atriyal interstisiyel fibrosis, iyonik remodeling ve atriyal fibrilasyon duyarlılığını arttırmaktadır

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ABSTRACT

Objective: The purpose of this study was to investigate the effects of hyperglycemia on atrial interstitial fibrosis, ionic remodeling and vulnerability to atrial fibrillation (AF) in alloxan-induced diabetic rabbits.

Methods: Sixty Japanese rabbits were randomly assigned to alloxan-induced diabetic group (n=30) and control group (n=30). Ten rabbits in each group were respectively used to electrophysiological and histological study, patch-clamp study and Western blotting analysis. Langendorff perfusion was used to record inter-atrial conduction time (IACT), atrial effective refractory period (AERP) and dispersion (AERPD) and vulnerability to AF. Histological study was measured by Sirius-red stain. Patch-clamp technique was used to measure action potential duration (APD) and atrial ionic currents (INa and ICaL). Western blotting was applied to assess atrial protein expression of transforming growth factor beta 1 (TGFβ1).

Results: Compared with control group, electrophysiological studies showed IACT was prolonged (37.91±6.81 vs. 27.43±1.63ms, p<0.01), AERPD was increased (30.37±8.33 vs. 14.70±5.16ms, p<0.01) in diabetic group. Inducibility of AF in diabetic group was significantly higher than in controls (8/10 vs. 1/10 of animals, p<0.01). Collagen volume fraction was increased (6.20±0.64% vs. 2.15±0.21%, p<0.01) in diabetic group. Patch-clamp studies demonstrated APD90 and APD50 were prolonged in diabetic rabbits (p<0.05 vs. control). The densities of INa were reduced and the densities of ICaL were increased (p<0.01 vs. control). Protein expression of TGFβ1 was increased in diabetic group (p<0.001 vs. control).

Conclusion: Our study suggests that hyperglycemia contributes to atrial interstitial fibrosis, ionic remodeling and vulnerability to AF in diabetic rabbits, resulting in atrial structural remodeling and electrical remodeling for the development and perpetuation of AF.

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Key words: Hyperglycemia, atrial interstitial fibrosis, ionic remodeling, atrial fibrillation, animals

ÖZET

Amaç: Bu çalışmanın amacı, alloksan etkisi ile oluşturulmuş diyabetik tavşanlarda atriyal intersitisyel fibrosis, iyonik remodeling ve atriyal fibrilasyona (AF) duyarlılığa hipergliseminin etkisini araştırmaktır.

Yöntemler: Altmış Japon tavşanı alloksan etkisi ile oluşturulmuş diyabetik grup (n=30) ve kontrol grup (n=30) olarak gelişi güzel belirlendi. Herbir grupta 10 tavşan sırasıyla elektrofizyolojik ve histolojik çalışma, "patch-klemp" çalışma ve Western blotting analizi kullanıldı. İnter-atriyal iletim zamanı (İAİZ), atriyal efektif refraktör dönem (AERP), dağılım (AERD) ve AF duyarlılığını kaydetmek için Langendorff perfüzyonu kullanıldı. Histolojik çalışma Sirius-kırmızı boyama ile değerlendirildi. Aksiyon potansiyel süresi (APS) and atriyal iyonik akımları (INa and ICaL) ölçmek için "patch-klemp" tekniği kullanıldı. Dönüştürücü büyüme faktörü beta 1 (DBFβ1)'in atriyal protein ekspresyonunu değerlendirmek için Western blotting uygulandı.

Bulgular: Kontrol grubu ile karşılaştırıldığında, diyabetik grupta elektrofizyolojik çalışmalar İAİZ'nın uzadığını (37.91±6.81 ve 27.43±1.63 ms, p<0.01), AERD'nin yükseldiğini gösterdi (30.37±8.33 ve 14.70±5.16 ms, p<0.01). Diyabetik grupta AF'nin indüklenebilmesi kontrol grubundan önemli derecede yüksekti (hayvanların 8/10 ve 1/10'u, p<0.01). Diyabetik grupta kollajen hacim fraksiyonu artmıştı (%6.20±0.64 ve %2.15±0.21, p<0.01). "Patch-klemp" çalışmaları, diyabetik tavşanlarda APS90 ve APS50'lerin uzadığını gösterdi (p<0.05 ve kontrol). INa yoğunluğu azaldı, ICaL yoğunluğu arttı (p<0.01 ve kontrol). DBFβ1'nin protein ekspresyonu diyabetik grupta arttı (p<0.001 ve kontrol).

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Sonuç: Çalışmamız, hipergliseminin diyabetik tavşanlarda AF gelişmesi ve tekrarlamasında etkili atriyal yapısal ve elektriksel remodeling'le sonuçlanan AF duyarlılığı, atriyal intersitisyel fibrozis ve iyonik remodelinge katkıda bulunduğunu düşündürmektedir.
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Anahtar kelimeler: Hiperglisemi, atriyal intersitisyel fibrozis, iyonik remodeling, atriyal fibrilasyon, hayvanlar

Introduction

Atrial fibrillation (AF) is known to induce atrial electrical remodeling (AER), which includes shortening, maladaptation, and increased dispersion of atrial effective refractory period (AERP) as well as decreased atrial conduction velocity (1, 2). Intracellular calcium overload and inhibition of the sodium pump have been regarded as the major ion mechanisms of the initiation and maintenance of AF (3, 4). Short-term atrial remodeling primarily involves functional changes of L-type Ca^{2+} channel and subsequent inactivation of voltage-dependent L-type Ca^{2+} current (ICaL) (5). The slowing of intra-atrial conduction is considered to be one of the key factors for the formation of reentry, which is necessary for the induction of AF. Also, Na^{+} current (INa) is essential for conduction of the electrical impulses (6).

Diabetes mellitus (DM) is one of the strongest independent risk factors for subsequent AF (7, 8) and has pathophysiological links with AF (9), but the exact mechanisms that underlying the relationship between DM and AF remain speculative. A recent meta-analysis (10) indicated that individuals with DM had an approximate 40% greater risk of AF compared with unaffected individuals. The pathophysiology of diabetic cardiomyopathy is multifactorial, and major reason has been attributed to persistent hyperglycemia (11). Hayden et al. (12) showed that high glucose levels have been shown to induce myocardial fibrosis in rats.

Molecular mechanisms for DM-induced electrical and structural remodeling is still unknown.

The aim of this study was to expound the effects of hyperglycemia on atrial interstitial fibrosis, ionic remodeling and vulnerability to AF.

Methods

Study design and preparation of the rabbit DM model

This study was designed as randomized experimental study. Sixty Japanese rabbits of either sex, weighing between 1.7 and 2.5kg were randomly assigned to alloxan- induced DM group (n=30) and control group (n=30). Ten rabbits in each group were respectively used to electrophysiological and histological study, patch-clamp study and Western blotting analysis. Experimental animal application approvals by the Experimental Animal Administration Committee of Tianjin Medical University and Tianjin Municipal Commission for Experimental Animal Control were obtained, which followed the guidelines established by the U.S. National Institutes of Health.

In DM group, alloxan monohydrate (Sigma, Saint Louis, USA) was dissolved in sterile normal saline to achieve a concentration of 5% (W/V), and 150mg/kg was immediately administered intravenously via the marginal ear vein. The diabetic state was examined 48h later by quantitative determination of blood glucose levels of ≥14mmol/L (once) or ≥11mmol/L (twice). Then, blood glucose concentration had been monitored weekly with glucometer Optium Xceed (Abbott, Bedford, USA) for 8 weeks.

Hemodynamic and echocardiographic examination

At 8 weeks, the right carotid artery of the rabbit was isolated surgically with a median incision in the neck. Cannula was inserted into the right carotid artery to monitor and record aortic systolic and diastolic blood pressure (SBP and DBP). The cannula was moved through the aortic valve into left ventricle to measure left ventricle end-diastolic pressure (LVEDP). All the rabbits (n=20) for electrophysiological and histological studies underwent transthoracic echocardiography to measure left atrial diameter (LAD) and left ventricular ejection fraction (LVEF) with standard method.

Electrophysiological studies

A median sternotomy was performed, and the heart was quickly excised and placed in perfusion fluid (4°C). The aorta was cannulated and connected to Langendorff perfusion system filled with Tyrode's solution saturated with 95% O_2 and 5% CO_2. The heart was perfused at 25 mL/min and the perfusion pressure was maintained at 80 mmHg. Tyrode's solution contained (mmol/L) NaCl 130, KCl 5.6, $NaHCO_3$ 24.2, $CaCl_2$ 2.2, $MgCl_2$ 0.6, NaH_2PO_4 1.2, and Glucose 12, pH 7.40 (adjusted with NaOH). Four sets of silver bipolar electrodes were placed in the high right atrium (HRA), high left atrium (HLA), low left atrium (LLA) and right ventricle (RV). Wenckebach cycle length of atrial-ventricular conduction was measured by right atrial incremental pacing. The inter-atrial conduction time (IACT) was measured during 250 ms pacing of HRA. The atrial effective refractory period (AERP) was evaluated using programmed extra-stimuli, which defined as the longest S1S2 interval that failed to capture. AF vulnerability was tested by the burst pacing (50 ms) for 1s, 5 times at 30s interval. The appearance of AF was defined as rapid, irregular atrial response longer than 1000 ms.

Histological examination

Following electrophysiological study, isolated left atrium (LA) was placed in 4% paraformaldehyde, embedded in paraffin, and cut into 4μm cross-sections. Sirius-red (SR) (Sigma) stain was used to evaluate interstitial fibrosis (n=10 for each group). Micrographs were digitized using Photoshop 7.0 (Adobe, San Jose, CA), and areas of fibrosis were analyzed using Image Pro Plus 4.5 Scion image software (Scion Co., Frederick, MD).

Whole-cell patch-clamp studies

In our study, experimental procedures were complied with previously developed methods (13-15). Another twenty rabbits were used for whole-cell patch-clamp studies. LA was used for the isolation of single myocyte by 15-min perfusion with the solution containing collagenase (315 U/mL, CLS II, Worthington Biochemical) and 0.2% bovine serum albumin (BSA) (Sigma). The whole-cell patch-clamp technique was used with an Axopatch 200B amplifier (Axon Instruments) to record ionic currents in voltage-clamp mode and AP in current-clamp mode. Membrane capacitance averaged 63.76±10.49 pF in the cells from control group (n=35 cells), 65.75±12.58 pF in DM group (n=37 cells). To control for cell-size variability, currents were expressed as densities (pA/pF). Voltage command pulses were generated by a 12-bit digital-to-analog (D/A) converter (Digidata 1200, Axon Instruments) controlled by pClamp 10.0 software. The action potentials (APs) were recorded at cycle lengths 60 bpm, 120 bpm, 180 bpm, 240 bpm and 300 bpm. APD stabilized within 5 action potentials at each cycle length, and steady state APD was measured to 50% (APD50) and 90% (APD90) of full repolarization. The recordings were low pass-filtered at 0.5kHz and the sampling frequency was 0.5 Hz for recording INa, and was 0.2 Hz for recording ICaL. The current-voltage relation was determined in the extracellular solution over a voltage range of -80 to +60 mV increased in 10-mV steps from a hold potential (HP) of -90mV for INa and -40 to +60 mV increased in 10-mV steps from a HP of -50mV for ICaL.

Western blotting analysis

Ten rabbits from each group were prepared for Western blotting analysis. Protein of LA tissue was extracted by total protein extraction buffer. An equal amount of protein was loaded onto a 15% SDS denaturing polyacrylamide gel, separated by electrophoresis, transferred onto PVDF membrane (Merck Millipore, USA) and incubated with the specific primary antibody overnight at 4˚C. Protein levels of transforming growth factor beta 1 (TGFβ1) were expressed as ratio to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The membranes were then washed and subsequently incubated with the secondary antibody conjugated to horseradish peroxidase (HRP). Protein was visualized using enhanced chemiluminescence. The anti-GAPDH and anti-TGFβ1 antibodies were purchased from Abcam Inc. (Cambridge, UK). The resulting bands were quantified using GeneTools software (Gene, USA).

Statistical analysis

Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean±1 SD and categorical variables are presented as percentages. Kolmogorov-Smirnov test was used to test the distribution of numeric variables and variables with normal distribution were compared with Student t-test and those without normal distribution were compared with Mann-Whitney U test. The incidence of AF was analyzed by Fisher's exact test. A two-tailed p<0.05 was considered significant.

Results

Hemodynamic and echocardiographic parameters

As shown in Table 1, there are no significant differences in heart rate or SBP between both groups at baseline (p>0.05). The DBP and LVEDP were slightly increased in DM group, however, no significant differences were found in each group (p>0.05). LAD was significantly increased in DM group (p<0.001), but there are no significant differences in LVEF between both groups (p>0.05).

Electrophysiological studies

As shown in Table 2, IACT in DM group was significantly prolonged compared with controls (p<0.01). AERPs in DM group were slightly prolonged, but no significant differences were observed between both groups. AERPD was significantly increased in DM group (p<0.01). Figure 1 shows the inducibility of AF in both groups. AF was induced in 8/10 (80%) of animals in

Table 1. Baseline characteristics

Variables	Control (n=10)	DM (n=10)	*p
Males/females, n	5/5	5/5	1.00
Weight, kg	2.17±0.15	2.29±0.33	0.30
Glucose, mmol/L	5.96±0.71	18.47±2.01	<0.001
Insulin, mU/L	21.59±8.05	13.78±3.12	<0.05
HR, bpm	167.20±15.44	156.45±26.05	0.27
SBP, mmHg	126.1±8.68	118.75±7.50	0.06
DBP, mmHg	96.60±6.60	100.40±6.53	0.21
LVEDP, mmHg	-8.50±2.80	-5.80±4.91	0.15
LAD, mm	6.89±0.53	8.21±0.67	<0.001
LVEF, %	72.80±6.08	70.21±6.34	0.36

Values are presented as mean±SD
*Student t-test, Mann-Whitney U test
DBP - diastolic blood pressure, DM - diabetes mellitus, HR - heart rate, LAD - left atrial diameter, LVEDP - left ventricular end-diastolic pressure, LVEF - left ventricular ejection fraction, SBP - systolic blood pressure

Table 2. Electrophysiological studies measurements

Variables	Control (n=10)	DM (n=10)	*p
AVWCL, ms	167.90±7.32	181.82±27.40	0.138
IACT, ms	27.43±1.63	37.91±6.81	<0.01
HRAERP, ms	81.50±4.10	92.40±21.08	0.125
HLAERP, ms	84.25±6.64	93.70±18.01	0.136
LLAERP, ms	85.40±5.94	94.13±19.02	0.182
AERPD, ms	14.70±5.16	30.37±8.33	<0.01

Data are presented as mean±SD
*Student t-test
AERPD - atrial effective refractory periods dispersion, AVWCL - Wenckebach cycle length of A-V conduction, DM - diabetes mellitus, HLAERP - high left atrium effective refractory period, HRAERP - high right atrium effective refractory period, IACT - interatrial conduction time, LLAERP - low left atrium effective refractory period

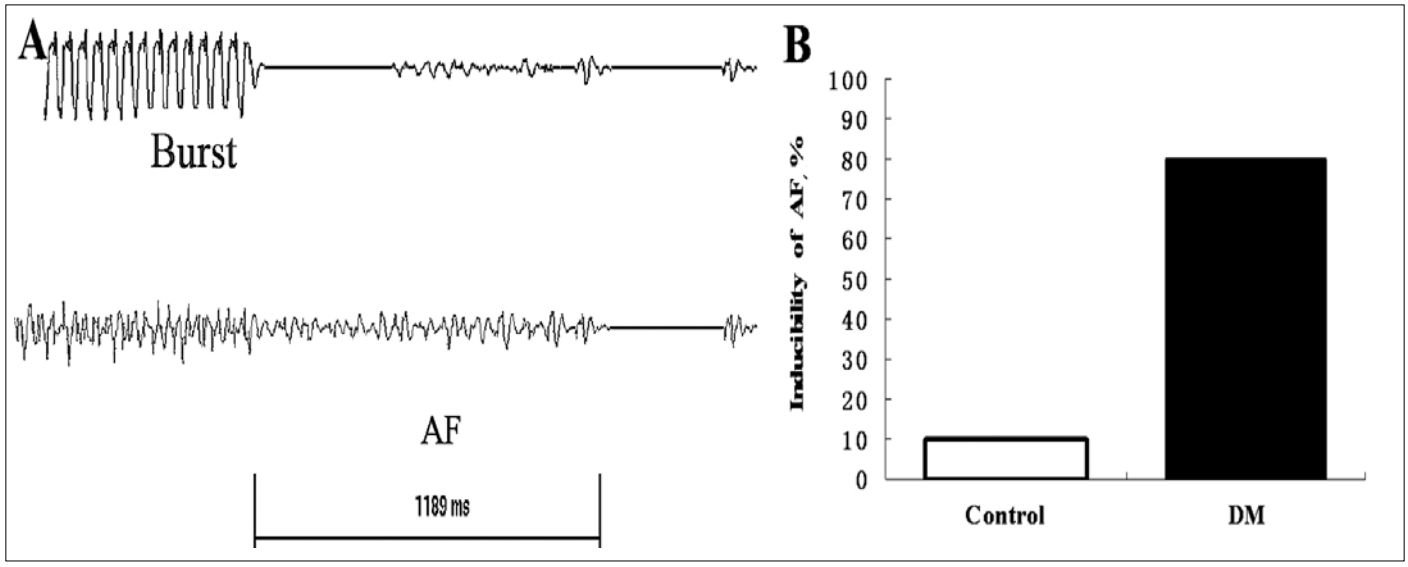

Figure 1. AF inducibility in both groups: A- AF episode induced by burst pacing in DM group, B- inducibility of AF in both groups, bars indicate percentage of inducibility of AF

*p<0.01 vs. corresponding value in control group

AF - atrial fibrillation, DM - diabetes mellitus

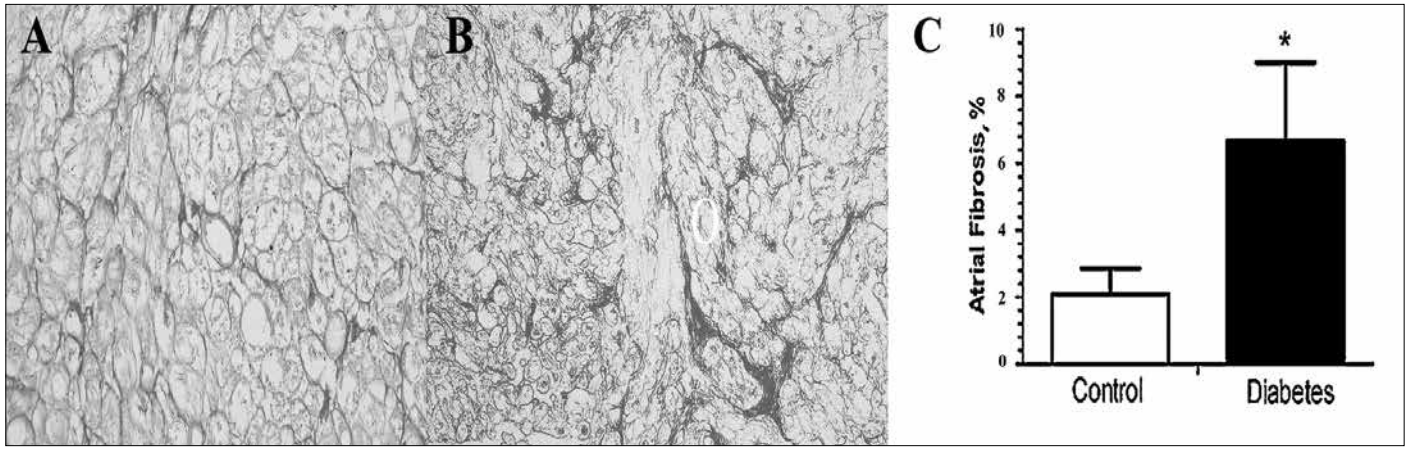

Figure 2. Histological examinations on atrial fibrosis in both groups. A) SR stain of control group, B) SR stain of DM group, C) comparison of the mean percentage of atrial fibrosis

*p<0.01 vs. corresponding value in control group

DM - diabetes mellitus, SR - Sirius-red

DM group, whereas 1/10 (10%) of animals in control group (p<0.01). Average duration of AF was 1180.63±93.78ms.

Atrial interstitial fibrosis

Figure 2 shows the representative histological sections of the LA free wall from control group (A) (×200; SR stain) and DM group (B) (×200; SR stain). Connective tissue was shown crimson in DM group, atrial tissue from control group appeared normal. Mean percentage of left atrial interstitial fibrosis shows 2.15±0.21% in control group vs. 6.20±0.64% in DM group (p<0.01); Data are presented as mean±SD (C).

Action potential changes

AP measurements were begun 5 min after cell rupture. In cells used for AP studies, the resting potential averaged respectively -62.8±2.4 mV in controls (n=10 cells) and -60.3±2.2 mV in DM (n=10 cells). In both groups, APD90 and APD50 decreased as stimulation frequency increased. DM substantially altered APD90 and APD50 compared with controls, mean values of APD are shown for two groups in Table 3. In addition to reducing APD90, DM caused no significant reductions in rate-dependent APD90 changes.

ICaL and INa changes (Figures 3, 4)

Figure 3A illustrates overall results for ICaL densities, peak ICaL density is shown as a function of test potential (TP). DM was associated with a increase in ICaL density. Table 4 shows maximum peak ICaL density averaged 9.60±3.59 pA/pF in DM group (n=10 cells) compared with 4.79±1.28 pA/pF in control group (n=10 cells) (p<0.01). In Figure 4A, B, representative recordings of ICaL from a cell of each group are displayed.

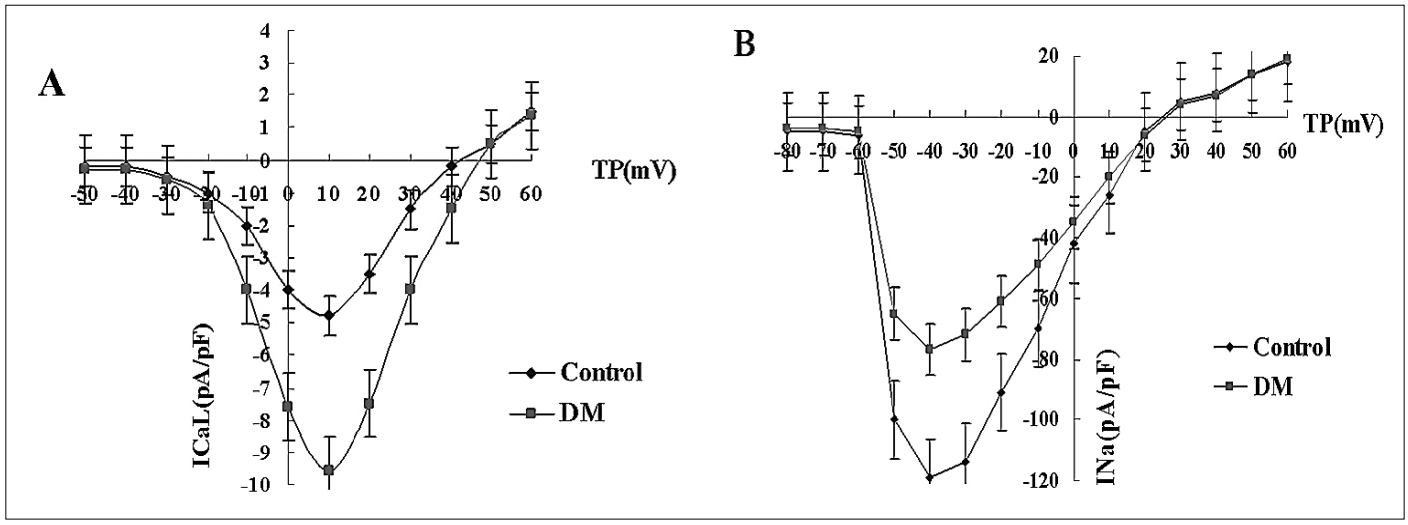

Figure 3. A-I-V curves of ICaL obtained from both groups, B-I-V curves of INa obtained from both groups, current densities as function of test potential (TP)

DM - diabetes mellitus, ICaL - ionic calcium L type current, INa - ionic sodium current

Table 3. Changes in APD90 and APD50 in cells from both groups

Variables		Control (n=10)	DM (n=10)	*p
60 bpm	APD90, ms	140.13±27.17	210.03±54.30	<0.01
	APD50, ms	43.94±10.15	88.08±22.65	<0.01
120 bpm	APD90, ms	132.30±22.41	206.69±45.89	<0.05
	APD50, ms	41.65±15.32	74.38±33.56	<0.05
180 bpm	APD90, ms	130.67±16.33	190.20±30.20	<0.05
	APD50, ms	36.23±12.39	65.99±20.20	<0.05
240 bpm	APD90, ms	123.60±13.09	168.02±29.73	<0.05
	APD50, ms	31.49±7.42	62.43±18.64	<0.01
300 bpm	APD90, ms	115.21±7.68	150.10±20.26	<0.05
	APD50, ms	27.33±6.46	53.24±15.37	<0.01

Values are presented as mean±SD
*Student t-test
60, 120, 180, 240 and 300 bpm - stimulating frequency
APD - action potential duration, DM - diabetes mellitus

Depolarizing upon 200-ms pulses from an HP of -50mV to voltages ranging from -40 mV to +60 mV elicited typical ICaL (Fig. 4E).

Figure 3B illustrates overall results of INa densities, peak INa density is shown as a function of TP. DM was associated with a decrease in INa density. Table 4 shows maximum peak INa density averaged 76.90±12.53 pA/pF in DM group (n=17 cells) compared with 119.33±17.58 pA/pF in control group (n=15 cells) (p<0.01). Although INa density decreased in DM group, the form of the I-V curve did not change. INa was significantly reduced at voltages ranging from -60mV to +20mV. In Figure 4C-D, representative recordings of INa from a cell of each group are displayed. INa was measured upon 50-ms pulses from an HP of -90mV to voltages ranging from -80mV to +60mV (Fig. 4F).

Western blotting analysis

Figure 5 shows a product obtained by western blotting for TGFβ1. Figure 5A shows the upper band in lines corresponds to

Table 4. Current densities of ICaL and INa

Variables	Control (n=10)	DM (n=10)	*p
ICaL, pA/pF	-4.79±1.28 (10 cells)	-9.60±3.59 (10 cells)	<0.01
INa, pA/pF	-119.33±17.58 (15 cells)	-76.90±12.53 (17 cells)	<0.01

Data are presented as mean±SD
*Student t-test
DM - diabetes mellitus, ICaL - ionic calcium L type current, INa - ionic sodium current

TGFβ1 protein product, and the lower band is GAPDH protein product. Figure 5B shows TGFβ1 protein expression was increased significantly in DM group (p<0.001).

Discussion

The major findings of our study are as follows: (i) Electrophysiological changes in DM group included prolonged IACT and increased AERPD; (ii) Increased inducibility of AF and LA interstitial fibrosis are evident and may constitute a substrate for the development of AF; (iii) APD90 and APD50 of atrial myocytes were prolonged in diabetic rabbits. The densities of reduced INa and increased ICaL in the atria were associated with DM ionic remodeling; (iv) DM increased fibrosis -related TGFβ1 proteins in rabbit atria. Thus, this study provides pathophysiological insights for the mechanisms of atrial electrical and structural remodeling in the setting of DM.

Recent epidemiological studies have shown that DM may exert a pro-arrhythmic substrate of AF (16-18). Type-1 diabetes, chronic hyperglycemia and insulin resistance might be the mechanisms responsible for the observed increased risk of AF. Hyperglycemia and AF have been studied by others in more detailed experiments in mice, but not in rabbits. In keeping with previous findings (19), we showed that atrial electromechanical function were associated with increased IACT measured in Langendorff perfused rabbit hearts. Kato et al. (20) showed that

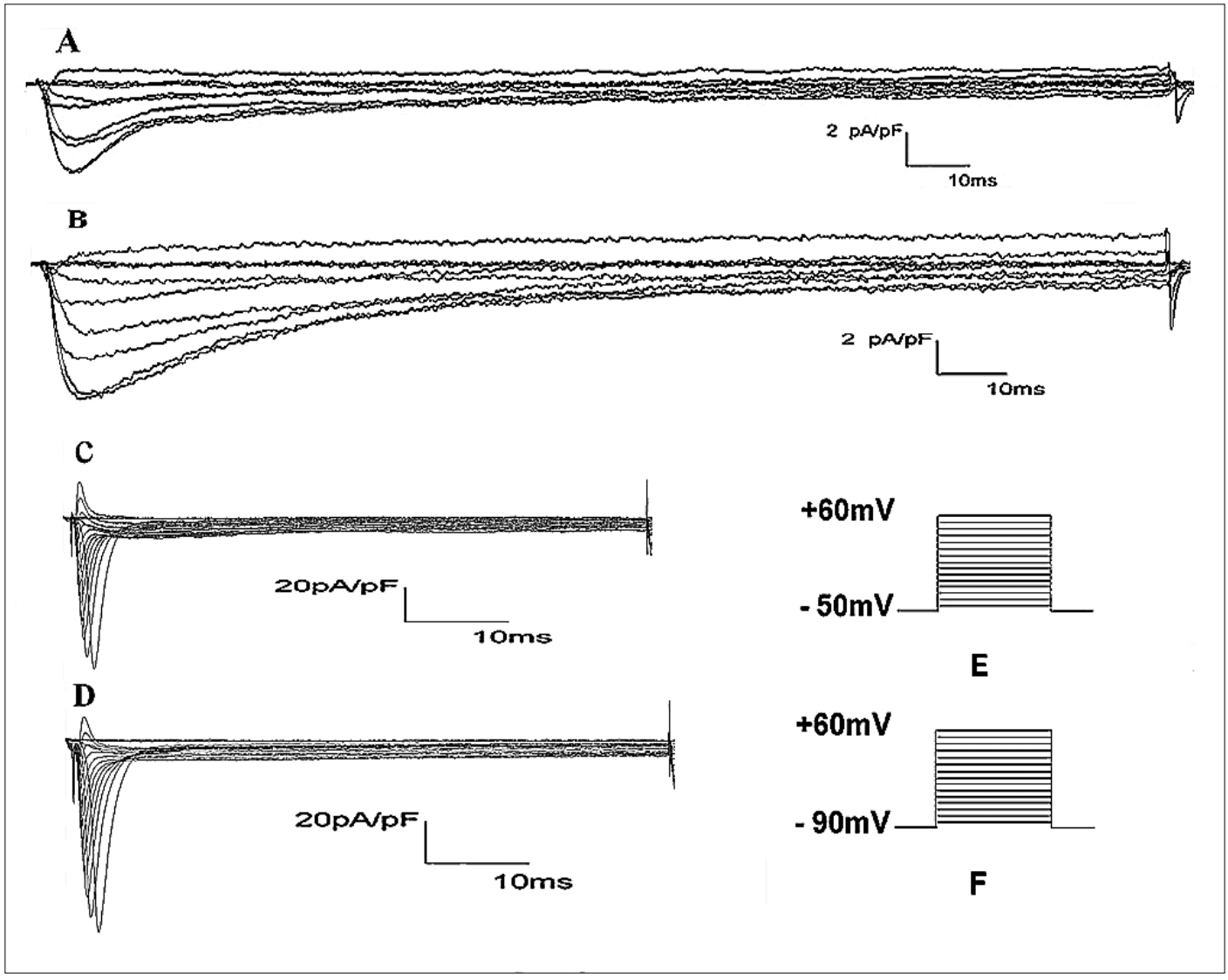

Figure 4. Voltage-dependent ICaL and INa races recorded in a representative myocyte, A) ICaL in control group, B) ICaL in DM group, C) INa in DM group, D) INa in control group, E) voltage protocol for recording ICaL, F) voltage protocol for recording INa

DM - diabetes mellitus, ICaL - ionic calcium L type current, INa - ionic sodium current

inter-atrial conduction delay and increased fibrotic deposition in atrium play a major role in producing atrial arrhythmogenicity, which indicates that atrial structural remodeling characterized by extensive interstitial fibrosis may be one of the major mechanisms of AF development in DM. The present study showed that atrial fibrosis along with increased IACT and LAD was related to increased inducibility of AF. AERPD was increased in DM rabbits, which suggesting that heterogeneity of repolarization is an important electrophysiological change in this setting.

One of the hallmarks of diabetic cardiomyopathy is the extracellular matrix (ECM) remodeling leading to the increased fibrosis of myocardium. TGFβ1 cascade is most probably involved in this process, which includes disproportionate increase in collagen and excessive ECM deposition due to enhanced expression of TGFβ1 (21). We hypothesize that TGFβ1 might involve in the pathogenesis of AF. Western blotting results revealed that TGFβ1 elevated in LA tissue of DM rabbits had positive correlation with atrial fibrosis. However, the precise nature of molecular components activated by TGFβ1 leading to fibrosis is not known and needs further studies.

Shortening of APD is the major characteristic in atrial myocytes during AF. By promoting the formation and maintenance of multiple wavelets, the reductions in refractory period and APD are associated with the onset and maintenance of AF (1, 22). Both clinical (23, 24) and experimental (25) studies consistently point to an important down-regulating effect of AF on ICaL, also down-regulation of ICaL as one of the arrhythmogenic AP abnormalities was associated with AF maintenance. ICaL plays a significant role in maintaining the plateau in atrial myocytes (13), therefore, it is a candidate to underlie the APD changes caused by DM. Depression of ICaL was responsible for much of the APD shortening in DM rabbits, and blockade of INa caused little fur-

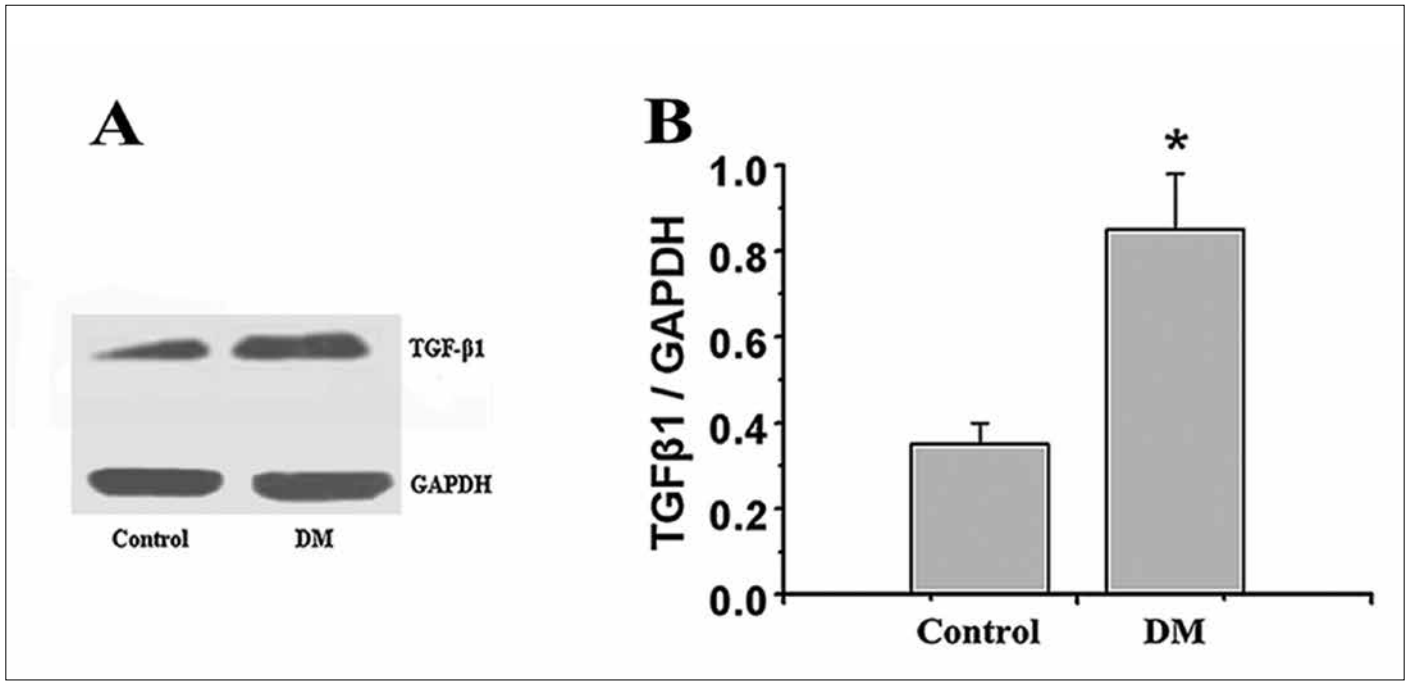

Figure 5. DM increases TGFβ1 protein expression in rabbit atria
***p<0.001 vs. corresponding value in control group**
DM - diabetes mellitus, TGFβ1 - transforming growth factors β1

ther change in APD (26). In our study, based on the presence of atrial ion current changes, eight weeks of hyperglycemia is sufficient to cause atrial ionic remodeling.

Cardiac voltage-gated Na^+ channel (Nav) conducts the inward INa which is an important determinant of conduction velocity (CV) in cardiac cells. INa plays a central role in the generation of AP and contributes to control APD (27). The Nav1.5 α subunit is the principal component of INa channel forming the pore and all essential gating elements, which is sufficient by itself for generating INa in heterologous expression systems. Previous studies showed prolongations of IACT implying slowed atrial conduction in different animal's models (27, 28) and patients (29, 30) with AF. The slowing of IACT is considered to be one of the most important players for the initiation of reentry in addition to the reduction in AERP and AERP rate adaptation, also the induction of AF. A significantly wider zone of IACT delay was observed in patients with paroxysmal AF compared with controls (31). The decrease of INa density and gene expression may relate to intra-cellular calcium overload which is regarded as a major mechanism of electrical remodeling (3, 32). Increased cytosolic Ca^{2+} can decrease INa density by means of down-regulating mRNA expression encoding Nav (33). The number of wavelets in atrium is strongly affected by the wavelength which is defined as the distance traveled by the activation wave during functional refractoriness and which is determined by the local AERP and CV. Slowing conduction results in shorter wavelength and increases the number of wavelets that could coexist in the given atrial dimension, which increases the likelihood that AF would sustain itself.

Further studies on atrial electrophysiological function may own special value to assess the impact of novel therapeutic drugs of anti-remodeling effects, such as pioglitazone (34, 35) or candesartan (36), on prevention and treatment of AF.

Study limitations

One of limitations of the study is that only the LA in whole cell electrophysiological study was analyzed, the response of the right atrium to hyperglycemia is still unknown. Furthermore, we did not assess the possible paradoxical response of AERP to increased heart rate, which is another characteristic of electrical remodeling. Finally, the molecular mechanisms for DM-induced electrical and structural remodeling should be further investigated in future studies.

Conclusion

Our study demonstrated that, hyperglycemia contributes to aggravate atrial interstitial fibrosis, ionic remodeling and vulnerability to AF in diabetic rabbits, which resulting in effects on atrial structural remodeling and electrical remodeling for the development and perpetuation of AF. Hence, counterbalancing the hyperglycemia actions may represent a novel pathway to prevent atrial remodeling, and perhaps an important therapeutic approach to the prevention of AF.

Conflict of interest: None declared.

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Authorship contributions: Concept - G.L.; Design - T.L.; Supervision - C.L.; Resource - G.L.; Materials - J.L.; Data collection&/or Processing - C.L., H.F.; Analysis &/or interpretation - H.F.; Literature search - W.Y.; Writing - C.L.; Critical review - T.L.; Other - L.C.

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