PROLONGATION OF EXPERIMENTAL DIABETES MELLITUS INCREASED SUSCEPTIBILITY TO REPERFUSION VENTRICULAR TACHYARRHYTHMIAS

Ekaterina Pershina[1], Jan Azarov[2], Marina Vaykshnorayte[2], Olesya Bernikova[2], and Alexey Ovechkin[2]

[1]Institute of Physiology Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences
[2]Institute of Physiology, Komi Science Center, Ural Branch, Russian Academy of Sciences

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Abstract

Introduction. Diabetes mellitus (DM) is associated with increased risk of sudden cardiac death, but its role in arrhythmogenesis is not clear. We evaluated contributions of DM duration and hyperglycemia level to development of proarrhythmic electrophysiological changes in the experimental ischemia/reperfusion model. Methods and Results: Ventricular epicardial 64-lead mapping and arrhythmia susceptibility burst-pacing testing were performed in 43 healthy and 55 diabetic (alloxan model) anesthetized rabbits undergoing 15-min left anterior descending coronary artery occlusion, followed by 15-min reperfusion. During ischemia, arrhythmia inducibility did not differ between the groups, but the number of reperfusion ventricular tachycardias and/or fibrillations (VT/VFs) was higher in the DM group (14 out of 55) as compared to control (3 out of 43, p=0.017). In the diabetic animals, both DM duration and glucose concentration were associated with reperfusion VT/VF development in univariate logistic regression analysis (OR 1.058; 95% CI 1.025-1.092; $p < 0.001$; and OR 1,119; 95% CI 1,045-1,198; $p = 0.001$; respectively). However, only the DM duration remained an independent predictor of reperfusion VT/VF in multivariate logistic regression analysis (OR 1.060; 95% CI 1.006 1.117; $p = 0.029$). Among mapping parameters, DM duration was associated with the prolongation of total ventricular activation duration (B 0.152; 95% CI 0.049-0.255; p=0.005) and activation-repolarization intervals (ARIs) (B 0.900; 95% CI 0.315-1.484; p=0.003). The prolonged ARI was the only mapping characteristic predicting reperfusion VT/VF development (OR 1.028; 95% CI 1.009-1.048; $p = 0.004$). Conclusions: The DM duration-dependent prolongation of ventricular repolarization presents a link between DM development and reperfusion VT/VF inducibility.

Prolongation of experimental diabetes mellitus increased susceptibility to reperfusion

VEntricular tachyarrhythmias

Ekaterina Pershina[1,2], Jan Azarov[1,3], PhD, Marina Vaykshnorayte[1], PhD, Olesya Bernikova[1], PhD, Alexey Ovechkin[1,2], PhD

[1]Department of Cardiac Physiology, Institute of Physiology, Komi Science Center, Ural Branch, Russian Academy of Sciences, 50 Pervomayskaya Street, 167982 Syktyvkar, Russia;

[2]Department of Therapy, Institute of Medicine, Pitirim Sorokin Syktyvkar State University, Syktyvkar, Russia;

[3]Department of Biochemistry and Physiology, Institute of Medicine, Pitirim Sorokin Syktyvkar State University, Syktyvkar, Russia

Correspondence

Alexey O. Ovechkin, Department of Cardiac Physiology, Institute of Physiology, Komi Science Center, Ural Branch, Russian Academy of Sciences, 50 Pervomayskaya Street, 167982 Syktyvkar, Russia;

E-mail: *alexlena@inbox.ru* Tel: +79042337013

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Conclusions : The DM duration-dependent prolongation of ventricular repolarization presents a link between DM development and reperfusion VT/VF inducibility.

KEYWORDS

Diabetes, arrhythmias, activation – recovery interval, reperfusion, repolarization.

INTRODUCTION

Cardiovascular pathologies often complicate diabetes mellitus (DM). [1] Specifically, DM increases risk of ventricular arrhythmias and sudden cardiac death in patients with coronary artery disease as well as those with no signs of heart problems. [2-4] However, experimental studies often yield inconsistent results concerning arrhythmic outcomes in DM. Susceptibility to arrhythmias in the diabetic hearts has been reported to be either increased [5-7] or decreased. [8-13]

These conflicting data obtained in clinical and experimental studies present a significant problem and warrant some unifying explanation for the changes of the arrhythmic risk in the diabetic heart. The differences concerning arrhythmia susceptibility in the above-mentioned observations may be related to concomitant pathologies, effects of therapy in clinical investigations, characteristics of the models used in the experimental studies (different species, in vivo vs in vitro preparations), etc. Among other causes, duration of exposure to diabetic conditions can influence arrhythmic outcomes of experimental DM. The duration of diabetic conditions is obviously shorter in experimental as compared to clinical studies. There are data showing that prolongation of the experimental DM duration promotes proarrhythmic changes in the myocardium. [14-16] Similarly, the level of hyperglycemia affects vulnerability of myocardium to ischemia/reperfusion injury, [17]

and its effect on susceptibility to ischemia/reperfusion arrhythmias cannot be excluded. However, the electrophysiological mechanisms of these changes are not clear.

We hypothesized that DM-related electrical remodeling develops gradually and does not immediately become arrhythmogenic. The aim of this work was to evaluate the role of the DM duration and level of hyperglycemia in the development of ventricular electrophysiological changes and the risk of ischemic and reperfusion arrhythmias in an experimental type 1 DM model.

METHODS

Experimental model

The study conformed to the Guide for the Care and Use of Laboratory Animals, 8th Edition published by the National Academies Press (US), 2011, the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, and was approved by the ethical committee of the Institute of Physiology of the Komi Science Centre, Ural Branch of Russian Academy of Sciences. The experiments were carried out on 98 rabbits of both sexes of the Chinchilla breed aged 6-8 months. For the induction of type 1 diabetes mellitus, 72 rabbits were given a single dose of alloxan at a dose of 120 mg / kg body mass by intravenous injection. Venous plasma glucose concentration was measured once a week with an OneTouch glucometer (LifeScan). Subsequently, 55 animals became diabetic, and DM did not develop in 17 rabbits (fasting venous plasma glucose level being <7.0 mmol/L). As a result, the electrophysiological measurements were carried out in 55 rabbits (28 males) with uncontrolled DM and 43 (22 males) control animals (the animals that have not been administered alloxan and animals that have not developed diabetes after administration of alloxan). DM duration was from 28 to 76 days [median 42 days, interquartile range (IQR) 36-54 days].

For electrophysiological study, the rabbits were anaesthetized by intramuscular injection of zoletil (15 mg/kg body mass). The animals were intubated and mechanically ventilated, and midsternal thoracotomy was performed. The temperature of the heart was maintained at 37-38 °C by irrigation with warm saline and heating ambient room air. A 64-lead sock array (3.0-5.0 mm inter-electrode distance) was placed on ventricular epicardium. Recordings of unipolar electrograms with reference to Wilson‘s central terminal were done under spontaneous sinus rhythm by means of a custom-designed mapping system (16 bits; bandwidth 0.05-1000.0 Hz; sampling rate 4000 Hz). After recording, the left anterior descending coronary artery was ligated to develop ischemia for 15 min followed by 15 min period of reperfusion provided by loosening the ligature. To assess arrhythmogenicity we used arrhythmia induction by burst stimulation (600 impulses per minute, twice diastolic threshold) of the left ventricular apex at the end of ischemic exposure and reperfusion.

Data processing and analysis

In each epicardial lead, activation time (AT) and end of repolarization time (RT) were determined as dV/dt minimum during QRS-complex and dV/dt maximum during T-wave, respectively, in respect to a QRS-complex onset. As a surrogate for action potential duration, activation-repolarization interval (ARI) was calculated as the difference between RT and AT. Average duration of ARI from all leads was used in analysis. A time of epicardial activation breakthrough reflecting the duration of conduction via conduction system was taken as the minimal AT throughout all epicardial leads. A duration of epicardial activation serving as an indirect estimate for activation of ventricular contractile myocardium was calculated as the difference between the maximal and minimal ATs throughout all epicardial leads. The maximal epicardial AT was used as a measure of total duration of ventricular activation. Dispersion of repolarization (DOR), a prerequisite for the unilateral conduction block, was taken as the difference between the maximal and minimal values of RTs. The inducibility of episodes of ventricular tachycardia and/or ventricular fibrillation (VT/VF) induced by burst pacing was analyzed.

Statistical analysis

IBM SPSS Statistics 23.0 was used for the statistical analysis. Data are presented as a median and interquartile interval (IQR). Nonparametric criteria (Mann – Whitney U test, Kruskal-Wallis and Dunn's test)

were used according to nonparametric data distribution confirmed by the Kolmogorov-Smirnov normality test. In order to evaluate the role of DM duration, the DM group was further divided by the median value of 42 days into short (28-41 days) and prolonged (42-76 days) DM groups. Pearson correlation, logistic and linear regression analyzes were used for search of associations between VT/VF inducibility and physiological parameters. The differences were considered statistically significant at p [?] 0.05.

RESULTS

As expected, the rabbits from DM group had a higher median venous plasma glucose level [25.6 (IQR 18.4-30.5) vs 6.0 (IQR 5.6-6.5) mmol/l, p<0.001]. In the DM group, glucose concentration and DM duration had weak significant correlation with each other (r = 0.308, p = 0.025).

First, we tested, if DM duration and/or plasma glucose level had any associations with arrhythmia inducibility. Diabetic conditions were associated with arrhythmogenesis during reperfusion but not ischemia. Table 1 shows that the diabetic animals did not differ from the controls concerning ischemic arrhythmias, but the number of arrhythmias induced at reperfusion was higher in the DM group. This increase in reperfusion arrhythmogenicity developed only in the prolonged DM group (Table 1). In the diabetic animals, both DM duration and glucose concentration were associated with reperfusion VT/VF in univariate logistic regression analysis (OR 1.058; 95% CI 1.025-1.092; p < 0.001; and OR 1,119; 95% CI 1,045-1,198; p = 0.001; respectively). In multivariate logistic regression analysis, only DM duration remained an independent predictor of reperfusion VT/VF (OR 1.060; 95% CI 1.006-1.117; p = 0.029). No associations were found between ischemic VT/VF on one hand and neither DM duration, nor glucose concentration on the other hand.

Then, to find out which electrophysiological parameters were affected by DM duration, we compared electrophysiological parameters in the control group, and groups with short and prolonged DM (Fig. 1). The groups did not differ in DOR, time of epicardial breakthrough, duration of epicardial and total ventricular activation. Among the studied electrophysiological variables obtained by ventricular epicardial contact potential mapping, only duration of ARIs differed between the groups being increased in the prolonged DM group.

Within the diabetic group (any DM duration), we tested which electrophysiological mapping parameters were associated with DM duration and/or glucose concentration in multivariate linear regression analysis (Table 2). Duration of epicardial activation was associated with glucose concentration, whereas total ventricular activation and ARI duration were associated with DM duration. Epicardial isochronal maps show prolongation of ARIs particularly in the apical areas of ventricular epicardium in the animals with DM, especially at the long follow-up (Fig. 2). DOR demonstrated no associations with neither DM duration, nor glucose concentration.

We established electrophysiological determinants of ischemic and reperfusion VT/VF development. Table 3 shows results of univariate logistic regression analysis of arrhythmia predictors. It demonstrates association of VT/VF development during ischemia with activation time of epicardial breakthrough, DOR and average ARI duration. On the other hand, reperfusion VT/VFs were associated with only average ARI duration. ROC curve analysis demonstrated significant association of prolonged ARI with reperfusion VT/VF inducibility (AUC 0.781, p = 0.001). The optimal cut-off for the ARI [?] 120 ms predicted reperfusion VT/VF with sensitivity 0.80 and specificity 0.75 (OR 8.119 95%CI 2.334-28.247, p = 0.001 in logistic regression analysis).

DISCUSSION

The objective of the present study was evaluation of arrhythmogenesis in the diabetic animals in respect to two factors that can “quantify” the progress of untreated DM, i.e. the time lapsed from DM induction and the plasma glucose concentration. It was found that reperfusion VT/VF development was associated with DM duration. Electrophysiological property associated with DM duration and reperfusion arrhythmogenesis and possibly linking the former with the latter appeared to be prolonged ARIs. Glucose concentration did not demonstrate independent association with reperfusion VT/VF inducibility, and VT/VFs arising during occlusion period were not associated with neither DM duration, nor glucose concentration.

In general, the diabetic state in our study was associated with prolongation of activation and repolarization processes. Specifically, it concerned the duration of total and epicardial activation and average duration of ARI. These findings correspond to our previous observations in rabbits and mice[18, 19] and may be related to down-regulation of sodium[20] and potassium[21-25] currents. Both delay in activation (implying decrease of conduction velocity) and prolongation of ARIs (implying increase of action potential duration) might have been arrhythmogenic. However, only the prolonged ARIs were associated with VT/VF development and only in the reperfusion state.

The relationship between reperfusion VT/VF inducibility and prolonged duration of repolarization seen in the present study was also demonstrated in the previous reports from our group concerning reperfusion arrhythmogenesis in nondiabetic rats[26] and cats[27]. It is noteworthy, that in these cited studies as well as in the present work, it is repolarization of the perfused myocardium that plays a crucial role in development of reperfusion VT/VFs. Here, we evaluated the electrophysiological parameters only in the baseline state preceding ischemia, and previously[26, 27] we tested ARIs both in ischemic and nonischemic regions, and only nonischemic ARIs demonstrated associations with VT/VF inducibility. Mechanisms of the arrhythmogenic role of long action potential duration in the nonischemic myocardium may concern increase of DOR (considering repolarization shortening in the affected myocardium) and facilitation of early afterdepolarizations serving as triggers for reentrant arrhythmias. Since DOR was directly tested as a VT/VF predictor and did not show association with reperfusion arrhythmias in the present study and previously [26, 27], we believe that the afterdepolarization-related mechanism is more probable. The plausible explanation for the absence of association between DM and ischemic arrhythmias is that the latter had several electrophysiological predictors, namely ARI duration, DOR and time of epicardial breakthrough (an estimate for duration of conduction via His-Purkinje system), and only ARIs were associated with DM conditions, specifically DM duration.

The present study demonstrated that the increase in duration augments arrhythmogenic potential of DM via influences on action potential duration assessed here as ARI. Also, the longer DM duration was associated with the longer total ventricular activation time, which however was not related to VT/VF inducibility. Previous works demonstrated the role of DM duration in alterations of cardiac autonomic innervation[16, 28] and ischemic preconditioning[14]. Both effects may be related to the changes of the electrophysiological properties, but our study was the first, to our knowledge, to report direct electrophysiological myocardial parameters in respect to DM duration and arrhythmogenesis.

It was an unexpected finding that VT/VF development was not related to the glucose concentration, which can be considered as the extent of severity of DM. Probably, this result is due to that glucose concentration might not be stable during the time-course of DM development. However, it was observed that duration of epicardial activation (an indirect measure of intramyocardial conduction) was associated with the plasma glucose concentration. These data taken together with the association between DM duration and total ventricular activation supports the notion that DM is associated with conduction disturbances[19, 20, 29]. These disturbances observed in baseline can underlie DM-induced reduction of conduction reserve in ischemic conditions[29], which however may not necessarily be arrhythmogenic.

CONCLUSION

In this study, we demonstrated that the progress of DM was associated with arrhythmogenesis during reperfusion but not ischemia. The link between development of DM-related disturbances and VT/VF inducibility was a DM duration-dependent prolongation of repolarization duration, which was a common predictor for ischemia and reperfusion arrhythmias. The presence of other, independent of DM, electrophysiological predictors for ischemic VT/VFs made them unrelated to DM progress.

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Table 1. The inducibility of ischemic and reperfusion arrhythmias in the control and DM groups (chi-square test).

	Ischemic Arrhythmias Absent	Ischemic Arrhythmias Present	Ischemic Arrhythmias p value vs control	Reperfusion Arrhythmias Absent	Reperfusion Arrhythmias Present	Reperfusion Arrhythmias p value vs control
Control	36	7		40	3	

Short DM (28-41 days)	16	3	0.961	17	2	0.636
Prolonged DM (42-76 days)	25	11	0.132	24	12	**0.003**
DM, any duration (28-76 days)	41	14	0.272	41	14	**0.017**

Table 2. The dependence of electrophysiological parameters on the glucose level and duration of diabetes (multivariate linear regression analysis)

Parameter	Duration DM	Duration DM	Duration DM	Glucose	Glucose	G
	B	95% CI for B	p	B	95% CI for B	p
Epicardial activation breakthrough time	0.086	(-0.016) -0.189	0.096	-0.119	(-0.287) – 0.049	0
Epicardial activation duration	0.066	(-0.049) – 0.180	0.255	**0.263**	**0.075 – 0.451**	**0**
Total ventricular activation duration	**0.152**	**0.049 – 0.255**	**0.005**	0.144	(-0.025) – 0.313	0
DOR	0.104	(-0.569) – 0.777	0.758	-0.281	(-1.387) – 0.825	0
ARI average	**0.900**	**0.315 – 1.484**	**0.003**	0.254	(-0.706) – 1.213	0

Table 3. Electrophysiological predictors of ischemia and reperfusion arrhythmias (univariate logistic regression analysis).

Parameter	Ischemia VF/VT	Ischemia VF/VT	Ischemia VF/VT	Reperfusion VF/VT
	OR	95% CI	p	OR
Epicardial activation breakthrough time	**1.119**	**1.006-1.244**	**0.038**	1.083
Epicardial activation duration	0.948	0.862-1.043	0.275	1.021
Total ventricular activation duration	1.023	0.947-1.105	0.570	1.064
DOR	**1.027**	**1.008-1.046**	**0.005**	1.009
ARI average	**1.031**	**1.013-1.050**	**0.001**	**1.028**

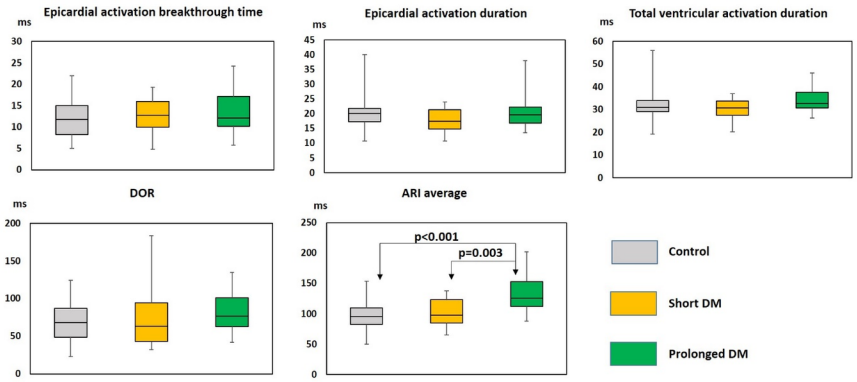

Fig.1. Comparison of electrophysiological characteristics of the control group and groups with short and prolonged diabetes mellitus (DM)(Kruskal-Wallis and Dunn's test).

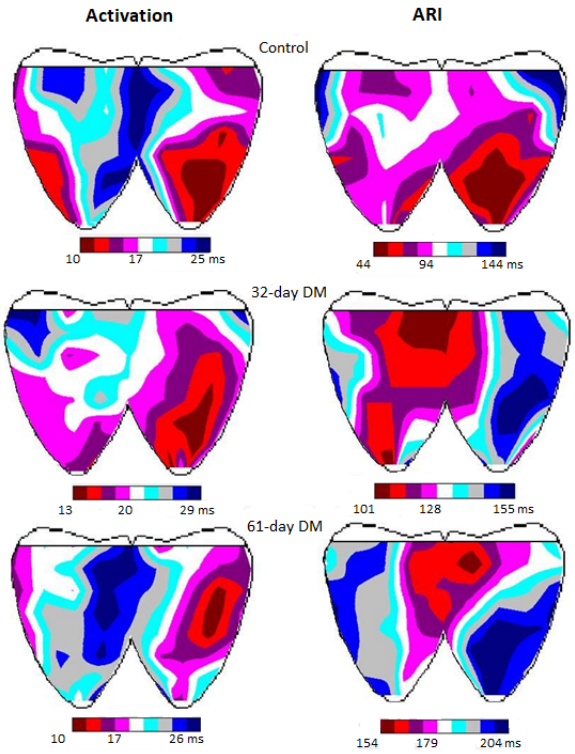

Fig.2. Representative isochronal maps of activation time (left panel) and activation-repolarization interval (ARI, right panel) in the control and two diabetic animals with the different duration of DM prior to coronary occlusion. Numbers on the scales indicate time in milliseconds from the QRS onset. The left, and right sides of each map correspond to the anterior and posterior surface of the ventricle, respectively. See progressive prolongation of repolarization in the apex area in the diabetic animals.