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Inhibition of PKC-delta expression using siRNAs restores coronary dilation in rats with streptozotocin-induced diabetes

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In 2014, the prevalence of diabetes was 8,5 % among adults (aged 18 and more). The number of people with diabetes has quadrupled, from 108 million in 1980 to 422 million in 2014 [1, 2]. Diabetes will become the seventh leading cause of death by 2030 according to the prediction of WHO [3]. The complications developing as a result of the systemic metabolic disorders are the main reason of suffering in patients with diabetes mellitus. Hyperglycemia, dyslipidemia, genetic, epigenetic modulators, tissue response on the toxic metabolites lead to occlusion of the large (coronary insufficiency, atherosclerosis) and peripheral vessels (retinopathy, nephropathy, and neuropathy) [4]. The prolonged exposure of myocardium to hyperglycemia results in diabetic cardiomyopathy. The latter is characterized by the left ventricular hypertrophy, fibrosis, apoptosis and necrosis of cardiomyocytes. All these factors cause the serious impairment of the cardiac contractile function resulting in the heart failure [5, 6]. It has been suggested that coronary insufficiency in patients with both I and II type of diabetes mellitus (DM) is mainly associated with impaired endothelium-dependent relaxation of the coronary arteries probably caused by the subendothelial and endothelial fibrosis. This complication is associated with significantly reduced coronary blood flow and increased risk of myocardial infarction [7–9]. Protein kinase C (PKC) is known to play an important role in the manifestation of detrimental effects of hyperglycemia in cardiovascular system. It has been shown that the PKC activation may be a key factor in the coronary circulatory dysfunction, fibrosis and cardiomyopathy development, increase of vascular permeability, etc [10]. The expression of delta-isoform of PKC (PKC-δ) in the heart appears to be significantly increased in diabetes mellitus. However, its role in development of the above mentioned pathological processes remains to be elucidated [11, 12].

The aim of the study was to investigate the possible involvement of PKC-δ in the development of coronary circulatory and cardiac contractile malfunction in rats with experimental diabetes mellitus.

Materials and methods. *Ethical approval.* All experimental procedures conformed to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and were approved by the Ethics Committee of the Institute of Pharmacology and Toxicology of NAMS of Ukraine.

Diabetes mellitus animal model. The experiments were conducted on 42 white male rats (276 ± 15) g), which had free access to food and water and were kept in animal house at a temperature of (23 ± 2) °C with a 12-h light/dark cycle. All animals were randomly distributed into 3 groups of 14 animals each. In animals of two groups, the development of diabetes mellitus has been induced, while the third group served as the control (healthy). The animals of one of the

experimental groups were injected with siRNAs that inhibits expression of δ-isoforms PKC (PRKCD-siRNAs). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) in a dose of 65 mg/kg. STZ was dissolved in the buffer solution containing 0,9 % NaCl and 10 mmol/L citrate, pH 4,6. Rats of the control group were injected intraperitoneally with a citrate buffer used for dilution of STZ. The animals were taken into the experiment 2 months after the STZ injection. In the RNA-interference studies we used hearts from diabetic rats on the third month of diabetes, diabetic rats on the seventh day after siRNAs targeted to PKC-δ isoform administration, and age-matched control healthy rats.

Measurement of blood glucose levels in the rats. The plasma concentration of glucose was measured in all animals 1 month after STZ injection and on the day of the experiment. The plasma glucose levels were measured immediately before the administration of small interfering RNA and on the day of the experiment. The glucose levels were measured using glucometer Bionime (BIONIME Rightest GM 300, Switzerland).

Total mRNA isolation and real-time (RT) polymerase chain reaction (PCR). Total RNA was isolated from rat heart tissue using a Trizol RNA-prep kit (Isogen, Russian Federation) according to the manufacturer's protocol. RNA concentration was determined with NanoDrop spectrophotometer ND 1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). Reverse transcription was performed with RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, USA), using 200–300 ng of total RNA and a random hexamer primer. Single-chain DNA was used for real-time PCR (RT-PCR).

We performed amplification in 10 μl of SYBR Green PCR Master Mix containing 30 pmol of each primer. For amplification mRNA delta PKC and β-actin (housekeeping gene) fragments, we used the following primers: PKC delta sense 5' -CTG GAA TAG TGA GCT CCC AGA C -3' and antisense 5' -ATC ACC AGT CAC CCA CTC TTC T -3'; β-actin sense 5' -TGT TAC GTC GCC TTG GAT TTT GAG -3' and antisense 5' -AAG AGA GAG ACA TAT CAG AAG C -3'. The primers were synthesized by Metabion (Martinsried, Germany). The sample volume was driven to 20 μl with deionized water. An amplification was performed on thermocycler 7500 Fast Real-Time PCR System. Thermal cycling conditions comprised an initial denaturation and AmpliTaq Gold® DNA polymerase activation step at 95 °C for 10 min, followed by the treatments at 95 °C for 20 s, and at 65 °C for 45 s and for 50 cycles, followed by the dissociation step. For control of specificity, we performed dissociation stage-sequential increase of temperature from 65 °C to 95 °C with registration of the drop in the double-stranded DNA-SYBR Green complexes fluorescence strength. We performed calculations using the 7500 Fast System SDS software provided. The CT (cycle threshold) is defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. The expression of the target gene relative to the housekeeping gene was calculated as the difference between the threshold values of the two genes.

Inhibition of PRKCD expression using siRNA in vivo. Small interfering RNAs (siRNA) used for suppression of δ-isoform PKC gene (PRKCD) were synthesized on demand by Metabion company (Germany). siRNAs for PRKCD gene had the following sequence: PRKCD-S-5'-GGA AAG GUA CUU UGC AAU CUU -3'; PRKCD-A-5'-AGA UCU UUU GUU UCU GAG UUU -3'. Before the experiments, double-stranded RNA was obtained by annealing of single strands. For this purpose, the solutions of respective sense and antisense oligonucleotides were mixed with buffer for annealing of the following composition (in mmol/L): 30 HEPES-KOH pH 7,4, 100 KCl, 2 $MgCl_2$, 50 $NH_4C_2H_3O_2$ (Metabion, Germany). Oligonucleotides were dissolved in two steps: at first, the equal volumes of each solution of oligonucleotides were transferred into the separate test tubes, and then the same amounts of annealing buffer were added to these tubes.

After such a mixing of the sense and antisense oligonucleotide solutions in a

test tube, the 4-fold smaller volume of annealing buffer was added to the mixture. The resulting mixture was incubated for 1 min at 90 °C and then cooled for 45 min at the room temperature. Annealing was performed using thermal cycler GeneAmp System 2700 (Applied Biosystems, USA). The double-stranded siRNAs was injected intravenously in the tail vein of rats in a dose of 400 μg/kg twice with a 24-hour interval between injections. The experiments were conducted one week after siRNAs injections.

Non-invasive measurement of blood pressure. The rat tail was placed inside the tail cuff. Then, the cuff was inflated and released a few times to accustom the animal to this procedure. Systolic blood pressure values (three consecutive readings) were obtained using a tail sphygmomanometer (S-2 Hugo-Sachs Elektronik, Germany) connected to analog-to-digital converter PowerLab 4/30 (ADInstruments, Australia).

Langendorff preparation of isolated heart perfused at constant flow. The animals were anesthetized with chloralose-urethane mixture (0,2 g chloralose and 2,0 g urethane in 100 ml of distilled water) in dose 2,0 ml / 100 g b.m. after intraperitoneal injection of 500 IU heparin. Thereafter, the animal thorax was opened from bilateral axillary lines up to the first rib under artificial ventilation. Then, the ascending aorta was cannulated and the heart was carefully isolated from the body (all vessels were cut out) and transferred immediately to a constant flow Langendorff rat isolated heart set up. The isolated spontaneously beating hearts were continuously perfused with Krebs-Henseleit solution (at 37 °C, pH = 7,4, gassed with 95 % O_2 + 5 % CO_2 and perfusion flow (14,07 ± 0,52) ml/min) with the following composition (in mmol/L): NaCl, 118; KCl, 7,4; $NaHCO_3$, 25,0; KH_2PO_4, 1,2; $CaCl_2$, 2,5; $MgSO_4.7H_2O$, 1,2; glucose, 11,0. A distilled water-filled latex balloon connected to a pressure transducer was introduced in the left ventricle. Left ventricular end-diastolic pressure was initially set to 5 mm Hg by balloon inflation. The pressure transducers and ECG electrodes were connected to converter PowerLab 4/30 (ADInstruments, Australia). A 15 min period was allowed for the heart to reach a steady state prior to any treatment [13].

Due to the fact that the response of coronary arteries to acetylcholine is not sufficiently pronounced in rat, we assessed the ability of the coronary arteries to relax basing on their reaction to isoproterenol administration. Responses to isoproterenol have been shown to be mediated by coronary endothelium as well as those induced by acetylcholine [14]. Myocardial function was assessed using applications of various concentrations of beta-adrenoreceptor agonist isoproterenol (10^{-6}–10^{-8} mol/l).

Chemicals. Trizol RNA-prep kit (Isogen, Russian Federation); RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, USA); siRNAs for PRKCD gene and substances of buffer for annealing (Metabion, Germany). All other substances and constituents of the Krebs solution were obtained from Sigma-Aldrich, USA.

Statistical analysis. Recordings and data analysis were made using «Chart 5» software (ADInstruments, Australia). Data were statistically analyzed with «Statistica 8» (StatSoft Inc., USA). Data distribution was tested for normality using the Shapiro-Wilk test. In case of normal distribution, the values were presented as means ± SEM (standard error of the mean). In all other cases, the values were presented as median, 25^{th} and 75^{th} quartile. Nonparametric comparisons of multiple dependent samples were performed using Friedman ANOVA test and Kendall coefficient of concordance. Multiple comparisons were performed using Kruskal-Wallis ANOVA test with appropriate Newman-Keuls post hoc test. Difference was considered significant if $p < 0,05$.

Results and discussion. The effects of STZ-treatment on blood glucose and body mass are summarized in Table 1. In 2-months diabetic animals, blood glucose level was significantly increased and body mass was significantly lower than in control animals. It should be noted that the injection of PRKCD-siRNAs did not significantly affect the blood glucose

Table 1

General effects of STZ treatment (M ± SEM)

	Baseline	2-months healthy (n = 14)	2-months diabetic (n = 28)
Body mass,g	276 ± 15	323 ± 18 p1 = 0,09971	205 ± 17 p1 = **0,00296** p2 = **0,00010**
Blood glucose, mmol/L	6,7 ± 0,5	6,9 ± 0,2 p1 = 0,82081	30,0 ± 0,5 p1 = **0,0** p2 = **0,0**
Mean blood pressure, mm Hg	112,7 ± 1,6	113,3 ± 1,2 p1 =0,83518	126,5 ± 3,9 p1 = **0,00043** p2 = **0,02369**

Notes. p1 – vs. Baseline, p2 – vs. 2-months healthy.

concentration in the rats with experimental diabetes (28,7 ± 0,9 mmol/L; n = 14; p > 0,05). The initial systolic blood pressure in the rats was (112,7 ± 1,6) mm Hg. The development of STZ-induced hyperglycemia in the animals of experimental groups resulted in significant blood pressure increase by 12 % (up to 126,5 ± 3,9 mm Hg; p < 0,00044). The injection of PRKCD-siRNAs had no significant effect on the blood pressure as well (123,7 ± 3,9 mm Hg; p > 0,05).

The use of RT-PCR revealed the presence of the PKC-delta isoform in adult rat cardiac myocytes. It has been noted that PKC-delta expression levels in rat hearts were significantly lower compared to the aortic tissues [15]. Furthermore, there was no significant difference between PKC-delta expression in hearts of control and STZ-treated animals (0,20 ± 0,05 vs. 0,19 ± 0,05 relative units). PKC-delta gene silencing led to insignificant decrease in PKC-delta expression to (0,17 ± 0,04 n = 6, p > 0,05, Figure 1).

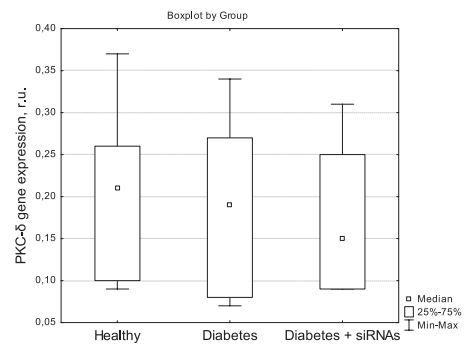

Figure 1. PKC-δ gene expression in hearts of healthy, diabetic and PRKCD-siRNA-treated diabetic rats (n = 6)

The left ventricular pressure (LVP) in the isolated hearts of diabetic rats (hDR) was lower by 25 % as compared to the hearts obtained from healthy rats (hHR). However, there was no significant difference in contractility index (CI) values. LVP in hDR was higher by 60 % in the group of animals that were injected with PRKCD-siRNAs compared to the hearts of diabetic animals non-treated with siRNAs (p < 0,01; Table 2). In contrast, CI was lowered by approximately 30 % after the PRKCD-siRNAs administration (p < 0,05; Figure 2A). The application of beta-adrenoreceptor agonist isoproterenol in concentrations of 10^{-8}, 10^{-7}, and 10^{-6} mol/L for 5 min evoked a transient increase in LVP and CI in the hearts of animals of all groups. It is worth noting that maximum LVP in hDR during this response was significantly lower in rats treated with all three concentrations of isoproterenol as compared to the control animals (p < 0,05). However, LVP values in hDR after PRKCD-siRNAs injection were not significantly different from the values in hHR (p > 0,05). In addition, CI in the hearts after PKC-δ gene expression inhibition was reduced by 40 % (p < 0,05; Table 2, Figure 2B).

There was no significant difference between initial values of coronary perfusion pressure (CPP) and coronary vascular resistance (CVR) in both hHR and hDR groups (Table 3, Figure 3A). The perfusion with increasing concentrations of isoproterenol resulted in the decrease in CPP and CVR in the hearts obtained

Table 2

Effect of various concentrations of isoproterenol on left ventricular pressure in rat isolated heart (Median, Q(25;75); n = 8)

Conditions	Parameters	Groups		
		2-months healthy	**2-months diabetes**	**2-months diabetes + siRNA**
Baseline	LVP, mm Hg	63,63 (58,68; 68,48)	67,30 (54,13; 77,86) p2 = 1,0	112,19 (109,12; 116,17) p2 = **0,00107** p3 = **0,00388**
	dP/dtmax, mm Hg • s^{-1}	1828,18 (1690,47; 2016,77)	1896,09 (1705,26; 2004,51) p2 = 1,0	2237,38 (2011,19; 2437,64) p2 = **0,03999** p3 = 0,14315
	dP/dtmin, mm Hg • s^{-1}	-1002,49 (-1083,83; -893,29)	-977,39 (-1019,62; -867,37) p2 = 1,0,	-1164,94 (-1267,43; -1058,70) p2 = 0,471898 p3 = 0,18286
Isoproterenol, 10^{-8} mol/L	LVP, mm Hg	124,61 (109,63; 128,07) p1 = **0,00044**	88,64 (69,32; 108,19) p1 = **0,05910** p2 = 0,35939	165,67 (9162,04; 178,09) p1 = **0,01169** p2 = 0,44154 p3 = **0,00796**
	dP/dtmax, mm Hg • s^{-1}	5444,01 (4649,28; 6319,08) p1 = **0,00007**	4012,00 (3189,48; 4780,85) p1 = **0,00259** p2 = 0,35939	5318,12 (3832,31; 5852,33) p1 = **0,00711** p2 = 1,0 p3 = 0,81922
	dP/dtmin, mm Hg • s^{-1}	-2860,48 (-3276,70; -2444,68) p1 = **0,00013**	-1965,62 (-2294,84; -1425,95) p1 = **0,00344** p2 = 0,13163	-2552,86 (-3030,72; -1829,65) p1 = **0,00123** p2 = 1,0 p3 = 0,86653
Isoproterenol, 10^{-7} mol/L	LVP, mm Hg	189,28 (176,65; 208,40) p1 = **0,00003**	90,19 (67,82; 113,0) p1 = **0,05255** p2 = **0,00267**	164,49 (159,58; 172,62) p1 = **0,00003** p2 = 0,35939 p3 = 0,23130
	dP/dtmax, mm Hg • s^{-1}	11291,03 (10069,93; 12700,15) p1 = **0,00005**	4511,54 (3355,54; 7149,36) p1 = **0,00264** p2 = **0,00054**	8019,34 (7091,38; 8927,43) p1 = **0,00001** p2 = 0,14315 p3 = 0,23130
	dP/dtmin, mm Hg • s^{-1}	-4573,36 (-4832,28; -4303,85) p1 = **0,00001**	-2248,68 (-2724,00; -1767,78) p1 = **0,00896** p2 = **0,00708**	-3780,85 (-4134,69; -3299,64) p1 = **0,00006** p2 = 0,31163 p3 = 0,47190
Isoproterenol, 10^{-6} mol/L	LVP, mm Hg	199,63 (170,19; 219,65) p1 = **0,00006**	110,21 (80,76; 121,49) p1 = **0,01619** p2 = **0,01566**	166,03 (132,62; 192,95) p1 = **0,02217** p2 = 0,77370 p3 = 0,28972
	dP/dtmax, mm Hg • s^{-1}	12236,51 (10119,34; 14556,20) p1 = **0,00004**	6393,61 (5057,39; 7138,34) p1 = **0,00319** p2 = **0,01566**	7457,61 (6550,61; 8108,14) p1 = **0,00097** p2 = 0,11095 p3 = 1,00000
	dP/dtmin, mm Hg • s^{-1}	-4845,90 (-5246,18; -4463,55) p1 = **0,00000**	-2824,69 (-3337,42; -2042,16) p1 = **0,00151** p2 = **0,01002**	-3710,52 (-4271,13; -3584,09) p1 = **0,00003** p2 = 0,24960 p3 = 0,68800

Notes. 1 – vs. baseline (Friedman ANOVA and Kendall coefficient of concordance); p2 – vs. healthy (Kruskal-Wallis ANOVA test); p3 – vs. diabetes (Kruskal-Wallis ANOVA test).

Table 3

Effect of various concentrations of isoproterenol on mean coronary perfusion pressure (mm Hg) in rat isolated heart (Median, Q(25;75); n = 8)

Conditions		Groups		
		2-months healthy	2-months diabetes	2-months diabetes + siRNA
Baseline		78,77 (75,09; 81,94)	82,46 (78,48; 86,68) p2 = 0,95050	86,97 (84,99; 93,05) p2 = **0,03191** p3 = 0,31075
Isoproterenol, 10^{-8} mol/L	10^{-8}	59,51 (53,13; 63,92) p1 = **0,01333**	74,43 (65,25; 83,37) p1 = 0,28884 p2 = 0,07665	58,29 (42,05; 64,71) p1 = 0,22067 p2 = 1,00000 p3 = 0,15406
	10^{-7}	60,85 (53,44; 64,05) p1 = **0,01332**	67,05 (60,27; 76,91) p1 = **0,01333** p2 = 0,37070	54,08 (39,20; 63,97) p1 = **0,04122** p2 = 1,0 p3 = 0,09455
	10^{-6}	57,62 (49,28; 60,41) p1 = **0,01333**	62,16 (57,92; 68,77) p1 = **0,01333** p2 = 0,39969	56,51 (40,83; 58,25) p1 = **0,04123** p2 = 1,0 p3 = 0,28166

Notes. p1 – vs. baseline (Friedman ANOVA and Kendall coefficient of concordance); p2 – vs. healthy (Kruskal-Wallis ANOVA test); p3 – vs. diabetes (Kruskal-Wallis ANOVA test).

from all animal groups. Maximal CVR decrease in hDR was only 16 % of initial values vs. 29 % in hHR. It is important to note that these changes were statistically insignificant in hDR. In contrast, the most pronounced decrease of CVR in response to isoproterenol was observed in the hearts of diabetic animals injected with PRKCD-siRNAs (up to 41 %). In this group, the tone of coronary arteries was significantly lower during isoproterenol challenge than in the hearts of the non-treated diabetic animals (Table 3, Figure 3B).

The deleterious effects of hyperglycemia on the blood vessels may be associated with various pathways. The cellular mechanisms include the polyol metabolism activation [16], non-enzymatic glycosylation and advanced glycosylation end products pathways [17], enhanced reactive oxygen species production [18], activation of the diacylglycerol (DAG)-protein kinase C (PKC) pathway [19]. In general, hyperglycemia-induced intra- and extracellular changes lead to alterations of signal transduction pathways, affecting gene expression and protein function to cause cellular dysfunction and damage. The DAG-PKC pathway is one of the most studied pathways in cellular signaling induced by diabetes [20]. The attention is mainly focused on evaluation of role of the conventional isoforms (PKC-α, -β1, -β2, and -γ) [4, 21]. Moreover, the clinical trials have already provided the data on the positive effect of PKC-β1/2 inhibition on diabetes-related endothelial dysfunction, retinopathy, and nephropathy [22–26]. However, there are evidences of PKC-δ involvement in development of diabetic vasculopathy [27–29]. The diabetes-related heart diseases are manifested in the form of coronary heart disease, autonomic neuropathy, diabetic cardiomyopathy. It has been suggested that activation of PKC-δ plays an important role in the pathogenesis of the latter [30].

It is known that diabetes mellitus leads to significant changes in the regulation of coronary artery tone. This vascular disorder is expressed in blunted endothelium-dependent relaxation and

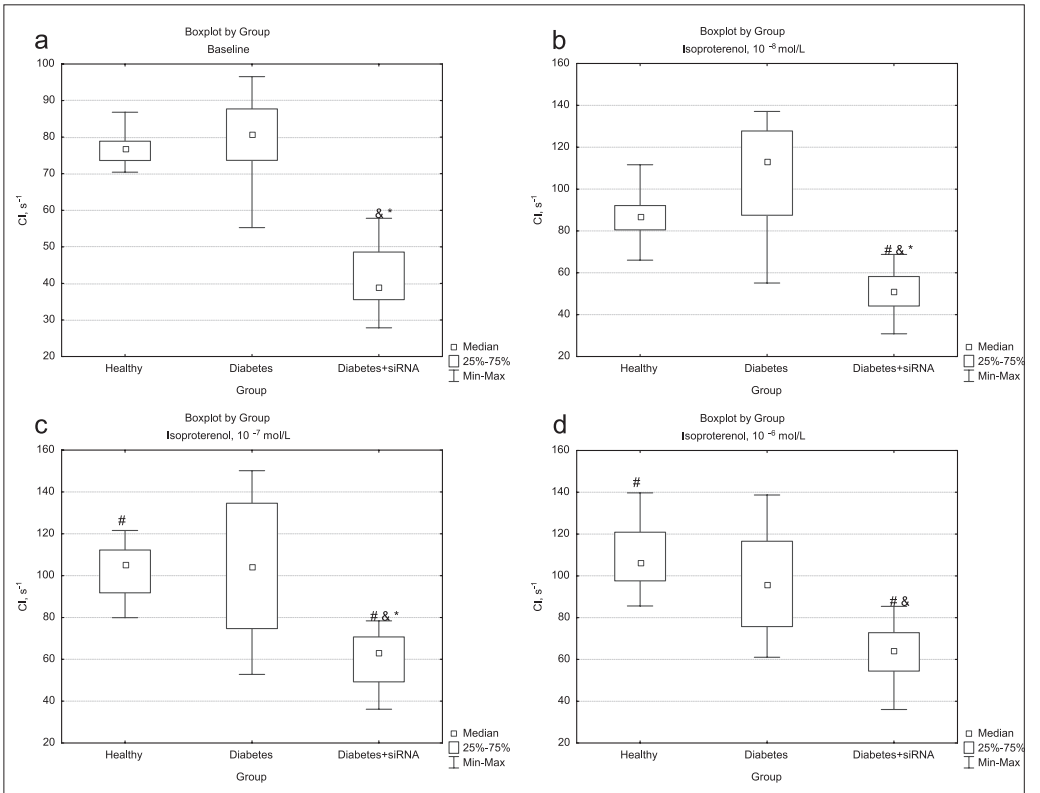

Figure 2. Changes in contractility index of isolated hearts in healthy, untreated diabetic and PRKCD-siRNA-treated diabetic rats (n = 8)

Notes. a – baseline values; b, c, d – effects of isoproterenol in different concentrations (10^{-8}, 10^{-7}, 10^{-6} mol/L respectively) on contractility index.

*#p < 0,05 compared to baseline values; &p < 0,05 compared to corresponding values in hHR; *p < 0,05 compared to corresponding values in hDR.*

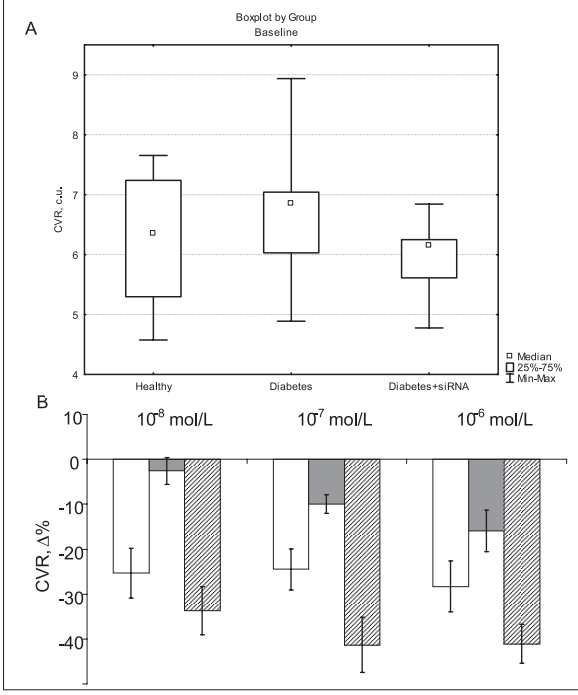

Figure 3. Changes in coronary vascular resistance in isolated heart of healthy, untreated diabetic and PRKCD-siRNA-treated diabetic rats (n = 8)

Notes. A – The baseline (in absolute values, Med Q(25,75); B – Effect of various concentrations of isoproterenol on coronary vascular resistant (in delta percent from initial level, M ± SE).

*Notes. #p < 0,05 compared to the baseline values; &p < 0,05 compared to the corresponding values in hHR; *p < 0,05 compared to the corresponding values in hDR.*

White column – 2-months healthy; Grey column – 2-months diabetes; Dashed column – 2-months diabetes + siRNA.

increased response of vascular tissues to vasoconstrictors. For instance, it has been shown that in patients with diabetes mellitus, unlike in healthy individuals, the coronary arteries respond to acetylcholine with vasoconstriction [31, 32].

The previously obtained data indicate that PKC-δ is involved in the regulation of vascular tone [15, 33]. The fact that Ca^{2+} sensitivity of aorta myocytes from diabetic rats is restored by suppression of PKC-δ gene expression supports this assumption [34]. These data taken together suggest that inhibition of PKC-δ may improve the cardiac metabolism in diabetes.

However, no significant changes in PKC-δ gene expression have been revealed in this study both in induced pathology and after injection of PRKDC-siRNA.

The data obtained demonstrate a significant decrease in the myocardium sensitivity to stimulation of beta-adrenoreceptors in hDR. This conclusion is based on the significant attenuation of the positive inotropic and vasodilatory effects of isoproterenol. The mentioned phenomena were also observed in other studies and probably indicate the development of diabetic cardiomyopathy in the experimental animals. It has also been shown previously that changes in the myocardium sensitivity to beta-adrenoreceptor agonists in diabetes mellitus are associated with abnormality in the Ca^{2+} release from the sarcoplasmic reticulum of cardiomyocytes [35–37].

In our study the injection of siRNA led to significant changes in the responses of hDR. First of all, the treatment with PRKCD-siRNAs significantly potentiated the relaxation of coronary arteries in isolated hearts induced by isoproterenol. The maximum values of CPP during the response were not statistically different from those in hHR.

The decrease in CVR in response to isoproterenol (10^{-8} mol/L) was 1.5 c.u. in hHR, while the corresponding value in hDR was only 0.1 c.u. Considering these results, we suggest that endothelium-dependent relaxation is significantly blunted in hDR. Under the conditions of increased oxygen demands this may result in development of myocardial ischemia in diabetes mellitus [38]. The siRNAs injection completely restored the relaxation of coronary arteries to the beta-adrenoreceptor agonist in diabetic rats. In this experiments, CVR was lower by 1.5–1.9 c.u., depending on the isoproterenol concentration, than in hDR without PRKCD-siRNAs treatment.

Despite the absence of significant changes in PKC-δ gene expression in the myocardium of diabetic rats, the myocardial contractility after administration PRKCD-siRNAs was decreased. This is supported by significant decrease in CI compared to other groups. These changes can be attributed to the fact that PKC-δ plays an important role in the regulation of myocardial contractility via mediation of the positive inotropic effect by activation of Ca^{2+}-ATPase in the cardiomyocyte sarcoplasmic reticulum [4, 39]. Perhaps, there is a threshold level of δPKC activity, which is necessary to maintain the integrity of the cardiomyocyte cytoskeleton [40] and normal development of the heart [41]. We should also mention the important role of the PKC-δ activity in the regulation of glucose level and lipid utilization in cardiomyocytes [30, 42].

Conclusion

In summary, the obtained data demonstrate that the development of type 1 experimental diabetes in rats led to decrease in the endothelium-dependent relaxation capacity of coronary vessels and accompanied by the compensated impairment of heart contractile activity. The dilatory responses of coronary arteries were almost completely restored by administration of PRKCD-siRNAs in diabetic rats. Our results suggest that PKC-δ activity may be involved in reactivity of coronary arteries in rats, which can possibly play a role in coronary dysfunction in diabetes.

Thus, the use of PRKCD-siRNAs appears to be a good therapeutic approach to normalize coronary circulation in diabetic rats. However, we need to keep in mind that treatment with PRKCD-siRNAs leads to the significant decrease in myocardial contractility.

Abbreviations

c. u. – convention unit
CI – contractility index, s^{-1}
CPP – mean coronary perfusion pressure mm Hg
CVR – coronary vascular resistance, r.u.
DM – diabetes mellitus
dP/dt – rate of LVP change, mm Hg • s^{-1}
ECG – electrocardiogram
hDR – hearts of the rats with streptozotocin-induced diabetes
hHR – hearts of healthy rats
LVP – pressure in the heart left ventricle, mm Hg
r.u. – relative unit

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Inhibition of PKC-delta expression using siRNAs restores coronary dilation in rats with streptozotocin-induced diabetes

It is well known that expression of PKC delta-isoform (PKC-δ) in mammalian heart and aorta is increased in diabetes mellitus (DM). However, its role in coronary circulation dysfunction and cardiomyopathy remains

unclear. *The aim of this study* was to evaluate the involvement of PKC-δ in coronary circulatory and cardiac contractile dysfunction in rats with streptozotocin-induced DM. In diabetic animals, a severe hyperglycemia was accompanied by increased blood pressure. DM resulted in some alterations of myocardial contractile activity and in significant decreased dilatory response to isoproterenol (10^{-8}–10^{-6} mol/L) in coronary arteries. PKC-δ gene silencing in diabetic rats using siRNAs had no effect on blood pressure and glucose levels. In contrast, PKC-δ gene silencing completely restored the coronary dilatory response in isolated rat hearts, but led surprisingly to decrease in myocardial contractility.

Key words: diabetes mellitus, streptozotocin, protein kinase C, siRNAs, coronary flow, myocardial contractility

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Інгібування експресії PKC-дельта з використанням siRNAs відновлює дилатацію коронарних артерій у щурів зі стрептозотоцин-індукованим діабетом

Добре відомо, що в артеріальних судинах ссавців за умов цукрового діабету (ЦД) збільшується експресія дельта-ізоформи протеїнкінази С (PKC-δ). Проте її значення в порушеннях коронарного кровообігу та розвитку кардіоміопатії залишається нез'ясованим.

Мета дослідження – з'ясування ролі PKC-δ у регуляції коронарного кровообігу та скоротливої функції серця в щурів зі стрептозотоцин-індукованим діабетом.

У діабетичних тварин важка гіперглікемія (> 30 ммоль/л) супроводжувалася підвищенням артеріального тиску. Розвиток ЦД призводив до деяких змін скоротливої активності міокарда та значно зменшував дилатаційну відповідь коронарних артерій на ізопротеренол (10^{-8}–10^{-6} моль/л). Сайленсинг гена PKC-δ з використанням siRNAs у щурів з ЦД не супроводжувався достовірними змінами вмісту глюкози та величини артеріального тиску. Разом з тим, спостерігалося повне відновлення дилатаційної відповіді коронарних артерій ізольованих сердець щурів і несподівано зменшувалася скоротливість міокарда.

Ключові слова: цукровий діабет, стрептозотоцин, дельта-ізоформа протеїнкінази С, малі інтерферуючі РНК, коронарний кровоток, скоротливість міокарда

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Ингибирование экспрессии PKC-дельта с использованием siRNAs восстанавливает дилатацию коронарных артерий у крыс со стрептозотоцин-индуцированным диабетом

Хорошо известно, что в артериальных сосудах млекопитающих при сахарном диабете (СД) увеличивается экспрессия дельта-изоформы протеинкиназы С (PKC-δ). Однако ее роль в нарушениях коронарного кровообращения и развитии кардиомиопатии остается неясной.

Цель исследования – выяснение роли PKC-δ в регуляции коронарного кровотока и сократительной функции сердца у крыс со стрептозотоцин-индуцированным диабетом.

У диабетических животных тяжелая гипергликемия сопровождалась повышением артериального давления. Развитие СД приводило к некоторым изменениям сократительной активности миокарда и значительно уменьшало дилатационный ответ коронарных артерий на изопротеренол (10^{-8}–10^{-6} моль/л). Сайленсинг с использованием siRNAs гена PKC-δ у крыс с СД не сопровождался достоверными изменениями содержания глюкозы и величины артериального давления. Вместе с тем, наблюдалось полное восстановление дилатационного ответа коронарных артерий изолированных сердец крыс и неожиданно уменьшалась сократимость миокарда.

Ключевые слова: сахарный диабет, стрептозотоцин, дельта-форма протеинкиназы С, малые интерферирующие РНК, коронарный кровоток, сократимость миокарда

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