EXPERIMENTAL STUDIES

THE STRUCTURAL ANALOGUE OF APELIN-12 PREVENTS ENERGY DISORDERS IN THE HEART IN EXPERIMENTAL TYPE 1 DIABETES MELLITUS

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Type 1 diabetes mellitus (T1DM) is the most severe form of diabetes, which is characterized by absolute insulin deficiency induced by the destruction of pancreatic beta cells. The aim of this study was to evaluate the effect of a structural analogue of apelin-12 ((N$^{\alpha}$Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH, metilin) on hyperglycemia, mitochondrial (MCh) respiration in permeabilized cardiac left ventricular (LV) fibers, the myocardial energy state, and cardiomyocyte membranes damage in a model of streptozotocin (STZ) diabetes in rats. Metilin was prepared by solid-phase synthesis using the Fmoc strategy and purified using HPLC. Four groups of animals were used: initial state (IS); control (C), diabetic control (D) and diabetic animals additionally treated with metilin (DM). The following parameters have been studied: blood glucose, MCh respiration in LV fibers, the content of cardiac ATP, ADP, AMP, phosphocreatine (PCr) and creatine (Cr), the activity of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in blood plasma. Administration of metilin to STZ-treated rats decreased blood glucose, increased state 3 oxygen consumption, the respiratory control ratio in MCh of permeabilized LV fibers, and increased the functional coupling of mitochondrial CK (mt-CK) to oxidative phosphorylation compared with these parameters in group D. In STZ-treated animals metilin administration caused an increase in the PCr content and prevention of the loss of total creatine (ΣCr=PCr+Cr) in the diabetic hearts, as well as restoration of the PCr/ATP ratio in the myocardium and a decrease in the activity of CK-MB and LDH in plasma to initial values. Thus, metilin prevented energy disorders disturbances in cardiomyocytes of animals with experimental T1DM.

Key words: apelin-12 analogue; streptozotocin diabetes; rat heart; mitochondrial dysfunction; myocardial energy state

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a dangerous metabolic disease that increases the risk of many long-term complications. One of the severe consequences of T1DM is the development of diabetic cardiomyopathy (DCM). Current evidence suggests that DCM is associated with changes in myocardial energy production, and mitochondrial (MCh) dysfunction may play a critical role in its pathogenesis [1]. Since mitochondria are the main cellular source of ATP, impairment of their respiratory function is considered as a key factor in the development of pathological complications of the heart in diabetic conditions [2]. MCh dysfunction in experimental diabetes and in patients is associated not only with impaired formation and transport of high-energy phosphates, but also with increased oxidative stress, impaired calcium homeostasis, endothelial dysfunction, and extracellular matrix remodeling [3]. In this context, the search for innovative strategies to protect cardiac energetics from the damaging effects of T1DM attracts much attention.

The peptide apelin is an endogenous ligand of the G protein-coupled transmembrane receptor APJ [4]. The apelin/APJ system plays an important regulatory role in the energy metabolism of various organs, including the heart [5]. In addition, apelin exhibits antidiabetic effects: it increases sensitivity to insulin, increases glucose utilization and reduces blood glucose [6]. Apelin-12 is the smallest fragment of the apelin prepropeptide, consisting of 77 amino acids; it retains effective binding to the APJ receptor and has potent cardioprotective effects [7]. We have modified this peptide and synthesized its structural analogues, which are characterized by greater stability with respect to amino- and carboxypeptidases and better solubility in water [8]. Testing of these pharmacological APJ receptor agonists in various models of myocardial ischemia/reperfusion injury (IRI) showed that the peptide (N$^{\alpha}$Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH, called metilin, exhibited the highest biological activity [9]. The effect of metilin on the heart under conditions of experimental T1DM has not been studied yet.

The aim of this study was to investigate the effect of metilin on energy metabolism disorders in the heart caused by the administration of streptozotocin (STZ) to rats. Our results show that metilin administration reduces blood glucose levels, improves MCh function and myocardial energy status, and reduces cardiomyocyte membrane damage in STZ-treated rats.

MATERIALS AND METHODS

Reagents

Fmoc-protected amino acid derivatives were purchased from Novabiochem (Germany) and Bachem (Switzerland). Reagents for peptide synthesis were purchased from Fluka Chemie GmbH (Switzerland). Enzymes and chemicals for determining metabolites and respiration parameters of myocardial fibers were purchased from Merck Life Science (Russia). Solutions were prepared using deionized water (Millipore Corp., USA).

Synthesis and Chromatography of Metilin

Metilin ([MeArg1, NLe10]-apelin12) is an analogue of natural apelin-12, in which the N-terminal part contains N^{α}-methylated Arg1. This modification increases the proteolytic stability of the peptide. The Met10 residue, which may be subjected to unwanted oxidation, has been replaced with norleucine, a natural non-protein amino acid resistant to oxidation by oxygen. Metilin was synthesized on 2-chlorotrityl chloride (CTC) resin (Iris Biotech GmbH, Germany) using a Tribute-UV peptide synthesizer (Protein Technologies Inc., USA) according to the standard single condensation of N^{α}-9-fluorenylmethyloxycarbonyl-(Fmoc)-amino acids using the uronium salt method [10]. Analytical HPLC was performed using a Knauer Smartline chromatograph (Knauer, Germany) and a Diasphere-110-5 C18 column (4.0×250 mm, 5 μm, BioChemMack S&T, Russia). Preparative HPLC was carried out using a Knauer Well-Chrom chromatograph (Knauer) and a Eurosphere C18 column (20×250 mm, 10 μm). After purification, the peptide homogeneity was 98.7%. The structure of the peptide was confirmed by proton magnetic resonance (^{1}H-NMR) and mass spectrometry. ^{1}H-NMR spectra were recorded on a WH-500 Bruker 500 MHz spectrometer (Bruker Daltonik GmbH, Germany) in DMSO-d6 at 300 K. Chemical shifts were measured relative to tetramethylsilane. Mass spectrometry analysis was carried on an Amazon instrument (Bruker Daltonik GmbH) using the electrospray ionization (ESI) method in positive ion detection mode (capillary voltage 3500 V). Mass scanning range *m/z* was 70–2200. The peptide structure, HPLC, 1H-NMR and mass spectrometry data are shown in Figures S1, S2, S3, and Table S1 (see Supplementary Materials). The characteristics of metilin are shown in Table 1.

Animals

Male Wistar rats weighing 280–290 g (n=40) were obtained from the Stolbovaya Animal House of the Scientific Center for Biomedical Technologies (Russia). Animals were kept in individual cages at 20–25°C with a natural light-dark cycle and free access to standard granulated food (Little One, Russia) and water in accordance with sanitary and epidemiological rules SR 2.2.1.3218-14 "Sanitary and epidemiological requirements for the design, equipment, and maintenance of experimental biological clinics (vivariums)" dated August 29, 2014 No. 51.

Experimental Design

Before the study, all animals were weighed. After a 24-h fast, blood was collected from the tail vein of 10 animals to determine the blood baseline glucose level and creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) activities. Then rats were anesthetized with 2,2,2-tribromoethanol (Avertin, 1 mg/kg, i.p., Merck) and the hearts were isolated to determine the parameters of energy metabolism (n=5) and MCh respiration in the LV fibers (n=5) (initial state group IS). The remaining rats were randomly assigned to three groups of 10 rats each: diabetic (D), D+metilin (DM), and control (C). T1DM was induced by a single dose administration of STZ (60 mg/kg, i.v.) [11]. The development of diabetes was assessed by an increase in the blood glucose level to 12 mM or higher three days after STZ injection; CD1 development was found in all animals. Rats of the DM group received a single injection of STZ (60 mg/kg, i.v.) and a course of metilin injections (150 nmol/kg/day, i.p.) for 2 weeks. Animals in this group were treated with metilin starting on day 3 after STZ injection. Metilin was dissolved in 0.02% DMSO in saline immediately before use. Its dose was chosen based on our pilot data. Rats in the control group (C) received a single intravenous injection of 0.1 M citrate buffer pH 4.5 (STZ vehicle) and, starting from day 3 of the experiment, 0.02% DMSO in saline for 2 weeks. The body weight and blood glucose level of animals from

Table 1. Characteristics of metilin

Peptide	Sequence	M, g/mol	Yield*, %	ESI +, *m/z*	Solubility in water, mg/ml	HPLC**	
						R_t, min	Purity, %
Metilin	H(N^{α}Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH	1418.70	65	709.97^{2+}	>100	12.5	98.7

R_t, retention time. *, The yield is given per starting amino acid attached to the polymer. **, Analytical HPLC was performed on a Diasphere-110-5 C18 column (4.0×250 mm, BioChemMack S&T, Russia) with the sorbent particle size of 5 μm; mobile phase buffer A: 0.05 M KH_2PO_4, pH 3.0; buffer B: 70% acetonitrile in buffer A. The peptide was eluted with a gradient of buffer B in buffer A from 20% to 80% in 30 min at a flow rate of 0.75 ml/min and detection at 220 nm.

the C, D, and DM groups were determined after the first week and at the end of the experiment. After the 17-day study, blood samples were collected from the tail vein to determine plasma CK-MB and LDH activity. After anesthesia with Avertin (1 mg/kg, i.p.) the hearts from five rats from each group were isolated and frozen in liquid nitrogen using Wollenberger forceps for subsequent metabolite analysis. The hearts of the remaining five animals were immediately used to determine the parameters of respiration in LV fibers. The scheme of the experimental protocol is shown in Figure 1.

MCh Respiration in Permeabilized Myocardial Fibers

Saponin-permeabilized fibers from the rat heart LV were prepared using a modified method [12]. LV fiber respiration parameters were assessed by means of an Oxygraph plus system (HansaTech Instr., UK) using 10 mM glutamate and 5 mM malate as respiratory substrates. Results were expressed as nmol O_2/min/mg dry weight. State 3 respiration was achieved by adding 2 mM ADP. The dry weight of the fibers was determined after drying overnight at 95°C. The respiratory parameters of each LV fiber sample were measured twice. State 2 respiration was assessed by the oxygen consumption (OC) rate after the addition of 10 mM glutamate and 5 mM malate without ADP. The MCh function was assessed by the respiratory control ratio (RCR), which was determined as the ratio of the OC rate at state 3 to the OC rate at state 2. The integrity of the outer mitochondrial membrane was assessed by adding 10 μM cytochrome *c* after maximal stimulation of respiration with 2 mM ADP and expressed as the ratio $V_{cyt\ c}/V_{ADP}$ as a percentage. The degree of functional coupling of MCh creatine kinase (mt-CK) with oxidative phosphorylation (OXPHOS) was assessed by adding 30 mM creatine (Cr) to fibers in the presence of a submaximal ADP concentration (0.1 mM) and was calculated as the ratio $(V_{Cr} - V_{ADP})/V_{ADP}$ as a percentage [13].

Myocardial Tissue Processing and Metabolite Analysis

Protein-free extracts from frozen hearts were prepared using cold 6% $HClO_4$ (1:10 w/v) and an Ultra-Turrax T-25 homogenizer (IKA, Germany) [9]. The dry weight of heart tissue after extraction with 6% $HClO_4$ was determined by weighing after drying overnight at 110°C. The concentrations of ATP, ADP, AMP, phosphocreatine (PCr), and Cr in neutralized heart tissue extracts were determined by modified enzymatic methods [14] using a UV-1800 spectrophotometer (Shimadzu, Japan). Fasting blood glucose was measured using an Accu-Check Instant glucometer (Boehringer Mannheim, Germany).

Assay of CK-MB and LDH Activities in Plasma

Blood samples collected at the initial state and at the end of the experiment were used to determine plasma CK-MB and LDH activities. LDH activity was determined enzymatically with pyruvate as a substrate at 340 nm using a UV-1800 spectrophotometer [15]. CK-MB activity was determined by enzyme immunoassay using standard kits from BioSystems S.A. (Spain).

Statistical Analysis

All data are expressed as means ± standard error of the mean (M±SEM). Results were analyzed by one-way analysis of variance (ANOVA) using Bonferroni's multiple test to evaluate differences between more than two groups. Student's *t*-test

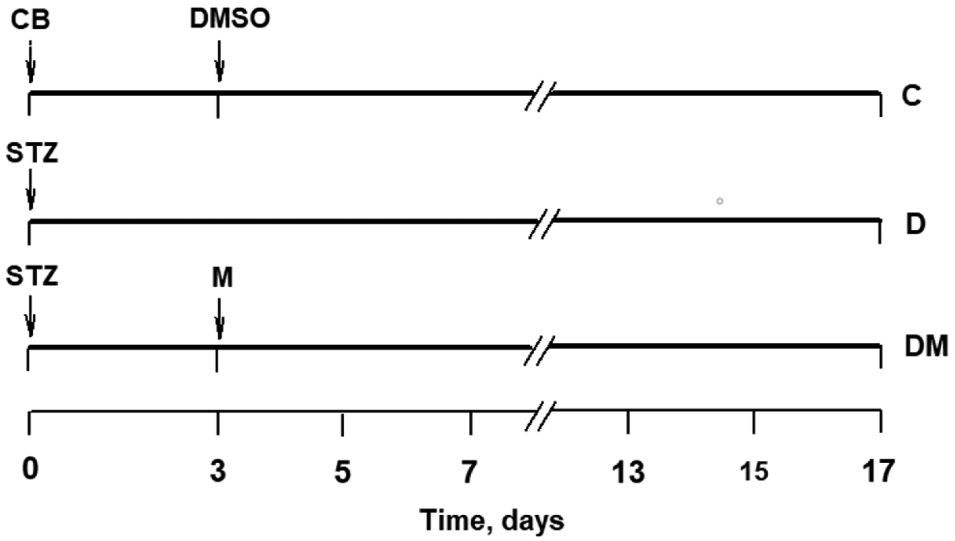

Figure 1. The scheme of the experimental protocol. C, control group; D, diabetic group (rate were intravenously (i.v.) injected with STZ (60 mg/kg) in 0.1 M citrate buffer (CB) pH 4.5); DM, group of diabetic rats (STZ 60 mg/kg, i.v.) treated with metilin (150 nmol/kg/day, intraperitoneally (i.p.)) for 2 weeks starting on day 3 after STZ injection. CB, 0.1 M citrate buffer pH 4.5; STZ, streptozotocin; M, metilin.

was used to compare two groups. Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, USA). Differences were considered statistically significant at $p<0.05$.

RESULTS

Body Mass and Blood Glucose

In the initial state, the body mass of rats insignificantly differed between the groups (Table 2). In the control group, during the observation period there was a progressive increase in the body mass of animals. In rats of group D, after the first week and at the end of the study, the average body mass was 7.4% and 8.5% lower than in the initial state ($p<0.001$), and it was also significantly lower compared to the corresponding value in the control group ($p<0.001$). Administration of metilin to STZ-treated rats only slightly increased body mass gain. Thus, no differences in this parameter were found between the initial state and the first week of the study. However, significant differences between the control and DM groups remained at the end of the first week and at the end of the study ($p<0.02$ and $p<0.01$, respectively).

The STZ administration significantly increased the blood glucose level compared to the control throughout the experiment. After the 17-day study, the blood glucose concentration in group D animals was more than 3 times higher than the initial level: 21.0±0.7 mM compared to 6.4±0.3 mM ($p<0.001$). Treatment with metilin decreased the level of experimental hyperglycemia in animals with STZ-treated diabetes. By the end of the study, this parameter in group DM was significantly lower than in group D ($p<0.05$).

Activity of CK-MB and LDH in Plasma

The activity of CK-MB and LDH assayed in plasma of STZ-treated rats after the 17-day study was 2.0-fold and 1.8-fold higher, respectively, as compared with the initial state and control (Fig. 2). Administration of metilin prevented the increase in the level of these enzymes in the blood. At the end of the experiment in group DM, these indicators did not differ from normal values.

Table 2. Changes in the body mass and blood glucose in the studied groups of animals

Group	Initial state (IS)	Day 7	Day 17
Body mass, g			
C	286.9±2.4	299.3±6.0	313.3±11.6^^
D	287.3±3.8	260.2±5.4^^^^****	234.2±8.1^^^^****++
DM	284.5±4.5	267.5±7.7**	251.9±10.9^^^***
Blood glucose, mM			
C	6.1±0.2	5.9±0.2	6.0±0.3
D	6.4±0.3	27.6±1.8^^^^****	21.0±0.7^^^^****+++
DM	5.7±0.2	25.0±2.0^^^^****	17.6±1.3^^^^****#+++

Data are presented as M±SEM for groups of 10 animals. C, control; D, rats treated with STZ; DM, rats treated with STZ and metilin. **, $p<0.02$ compared with C; ***, $p<0.01$ compared with C; ****, $p<0.001$ compared with C; ^^^^, $p<0.01$ compared with IS; ^^^^, $p<0.001$ compared with IS; #, $p<0.05$ compared with D; ++, $p<0.02$ compared with day 7; +++, $p<0.01$ compared with day 7.

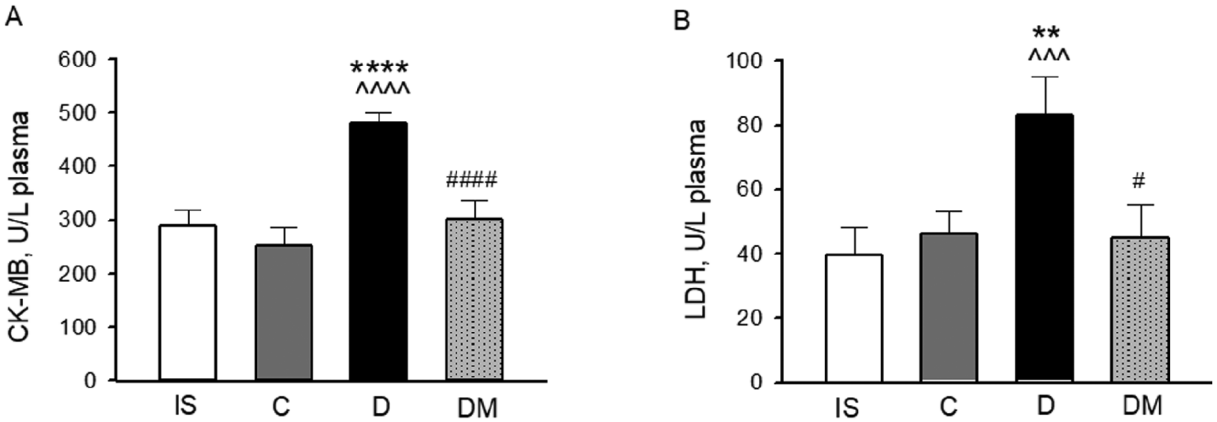

Figure 2. Activity of creatine kinase-MB (CK-MB, **A**) and lactate dehydrogenase (LDH, **B**) in rat plasma. Data represent M±SEM for groups of 10 animals. IS, initial state; C, control; D, rats treated with STZ; DM, rats treated with STZ and metilin. **, $p<0.02$ compared with C; ****, $p<0.001$ compared with C; ^^^^, $p<0.01$ compared with IS; ^^^^, $p<0.001$ compared with IS; #, $p<0.05$ compared with D; ####, $p<0.001$ compared with D.

Respiration Parameters of Saponin-Skinned Myocardial Fibers

After the 17-day experiment, there were no differences in state 2, state 3, RCR, and the degree of functional coupling of mt-CK with OXPHOS between the control group and these indicators in the initial state (Fig. 3). In STZ-treated rats, there was a decrease in state 2 and especially in state 3 respiration (by 28% and 60% compared to control, respectively, $p<0.05$ and $p<0.001$), which halved the RCR value ($p<0.001$). The degree of functional activity of mt-CK, assessed in the Cr test, decreased in animals with diabetes by 1.6 times compared to controls ($p<0.001$). Daily administration of metilin for 2 weeks to STZ-treated animals had no effect on state 2, but increased the maximum ADP-stimulated state 3 and RCR by 1.7-fold ($p<0.01$). The functional mt-CK coupling in group DM increased 2.4 times compared to group D ($p<0.02$) and did not differ significantly from the control value. Representative respiration protocols demonstrating the effects of metilin on state 3 and mt-CK functional activity are shown in Figure S4 (see Supplementary materials). Addition of 10 μM cytochrome *c* did not affect ADP-stimulated respiration in animals of diabetic groups D and DM at the end of the experiment. The percentage of $V_{cyt\ c}/V_{ADP}$ was 101.4±2.8, 98.0±2.0, and 100.2±2.4% in groups C, D and DM, respectively, thus indicating the absence of the outer mitochondrial membrane damage during administration of STZ and metilin.

Myocardial Energy State

At the end of the experiment, the myocardial ATP content did not differ significantly from the initial value in the control group and was slightly reduced ($p=0.051$) in animals with diabetes (Table 3). A short period of diabetes development had no effect on the ADP and AMP levels. The content of these adenine nucleotides was close to the values in the initial state and in the control. In the myocardium of group D animals, the total pool of adenine nucleotides (ΣAN=ATP+ADP+AMP) was significantly lower than in control ($p<0.05$). In myocardium of diabetic animals, a significant decrease in the PCr content was detected (on average by 40% compared to the initial state and control, $p<0.02$ and $p<0.001$, respectively) without changes in the Cr content. As a result, the loss of ΣCr=PCr+Cr was 22% and 16% compared to the initial state and control, respectively ($p<0.05$).

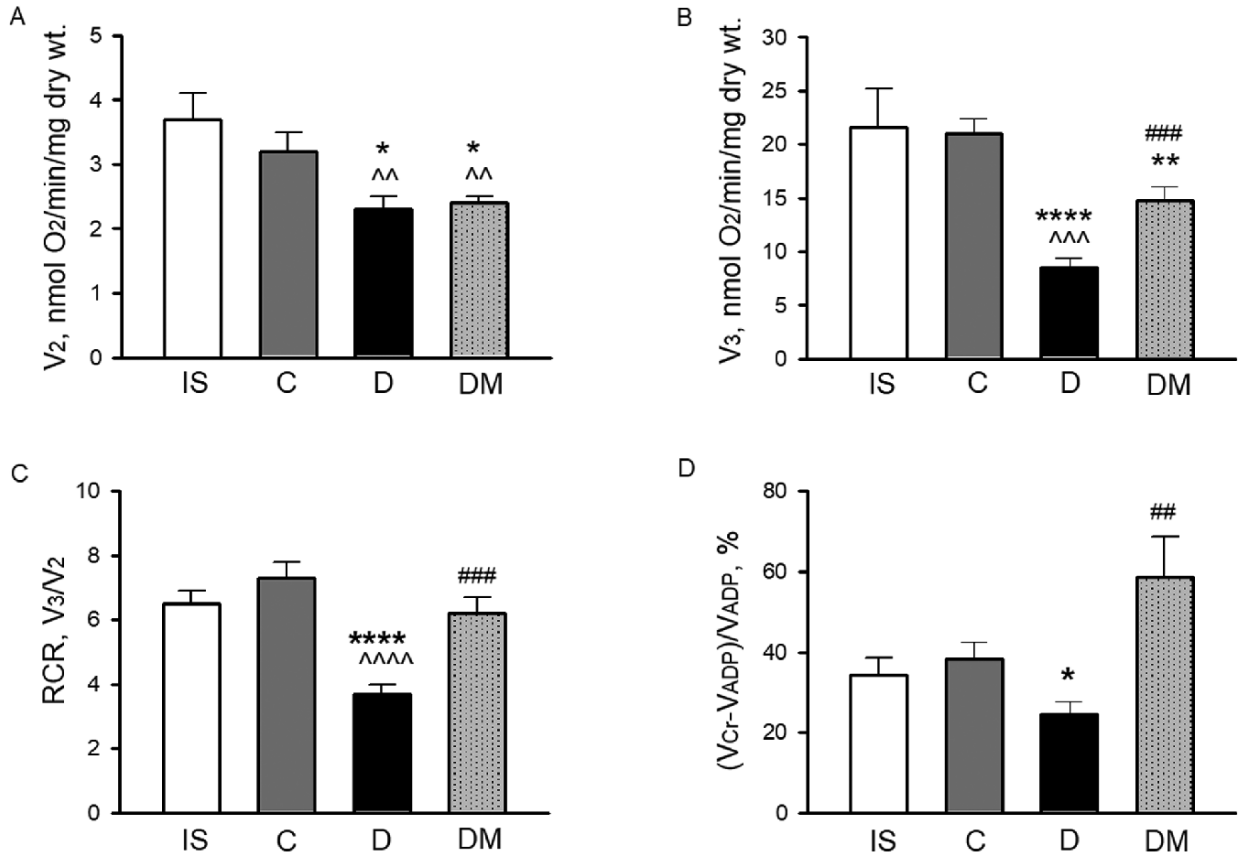

Figure 3. Parameters of mitochondrial respiration in saponin-skinned LV fibers in the presence of 10 mM glutamate and 5 mM malate. **A**, rate of oxygen consumption in state 2 (V_2); **B**, rate of oxygen consumption in state 3 (V_3); **C**, respiratory control ratio (RCR) = V_3/V_2; **D**, degree of mt-PK coupling to OXPHOS $(V_{Cr} - V_{ADP})/V_{ADP}$, %. Data represent M±SEM for groups of 5 animals. IS, initial state; C, control; D, rats treated with STZ; DM, rats treated with STZ and metilin. *, $p<0.05$ versus C; **, $p<0.02$ versus C; ****, $p<0.001$ versus C; ^^, $p<0.02$ versus IS; ^^^^, $p<0.01$ versus IS; ^^^^, $p<0.001$ versus IS; ##, $p<0.02$ versus D; ###, $p<0.01$ versus DM.

Table 3. Parameters of cardiac energy state in the studied groups

	IS	C	D	DM
ATP	19.40±1.35	17.52±0.98	14.92±0.57	16.12±0.92
ADP	5.45±0.56	5.50±0.37	5.39±0.18	5.60±0.97
AMP	1.08±0.98	1.29±0.24	1.44±0.12	1.81±0.53
ΣAN	25.40±2.80	24.31±1.12	21.39±0.52*	23.63±0.75$^{\#}$
PCr	26.89±3.61	25.09±0.30	14.96±0.66****^^	23.15±2.35$^{\#\#}$
Cr	35.08±1.67	32.75±2.76	33.62±1.84	36.18±2.82
ΣCr	60.76±3.14	57.84±2.77	48.58±1.95*^	59.33±2.40$^{\#\#\#}$
PCr/ATP	1,39±0,14	1,43±0,11	1.00±0.08**^	1.44±0.13$^{\#}$

IS, initial state; C, control; D, rats treated with STZ; DM, rats treated with STZ and metilin. ΣAN=ATP+ADP+AMP; ΣCr=PCr+Cr. Data are presented as M±SEM for groups of 5 animals. *, $p<0.05$ compared with C; **, $p<0.02$ compared with C; ****, $p<0.001$ compared with C; ^, $p<0.05$ compared with IS; ^^^, $p<0.02$ compared with IS; #, $p<0.05$ compared with D; ##, $p<0.02$ compared with D; ###, $p<0.01$ compared with D.

Metilin administration had no effect on individual adenine nucleotides or ΣAN, despite a trend to the increase in myocardial ATP in rats of group DM. Metilin had a much greater effect on the PCr-Cr system. Administration of metilin to the STZ-treated rats increased the PCr content in group DM by 1.5 times compared with group D at the end of the study ($p<0.02$). After the administration of metilin, PCr remained at the prediabetic level, while the Cr content increased slightly compared to the control; this led to an increase in ΣCr by 1.3 times compared with group D ($p<0.01$) and no difference with the initial state was observed. Thus, metilin administration completely prevented the loss of myocardial ΣCr in diabetic animals. Treatment with STZ, caused a 1.4-fold decrease in the myocardial PCr/ATP ratio as compared to the initial state and control ($p<0.05$ and $p<0.02$, respectively). Administration of metilin restored the PCr/ATP ratio to a normal value, which was significantly differed from this indicator in group D ($p<0.05$).

DISCUSSION

Myocardial Energetics and the Effects of Metilin in Rats with T1DM

Treatment of rats with STZ resulted in hyperglycemia, loss of weight gain, increased activity of CK-MB and LDH in plasma, and pronounced disturbances in cardiac energy: a decrease in the content of PCr and ΣCr and a decrease in the PCr/ATP ratio in the myocardium. In addition, significant changes in the respiratory parameters of myocardial fibers were found: a two-fold decrease in RCR in animals treated with STZ, which was due to a profound decrease in the maximum ADP-stimulated oxygen consumption (state 3). Typically, such changes are associated with limited ATP production [16] and increased formation of reactive oxygen species (ROS) [17]. In addition, diabetic animals were characterized by a decrease in the functional activity of mt-CK, assessed in a test with Cr. The latter is an indicator reflecting deterioration in the cardiomyocyte bioenergetics [18].

In this study we have demonstrated for the first time the protective effect of metilin in STZ-treated rats. Administration of this peptide to STZ-treated animals slightly increased body mass gain and reduced the level of experimental hyperglycemia by the end of the experiment. Metilin improved the energy of diabetic myocardium at the tissue and MCh level. The metilin action resulted in the increase of the PCr content, prevention of the ΣCr loss, and recovery the reduced PCr/ATP ratio in the diabetic heart. Typically, a decrease in the PCr/ATP ratio is found in the myocardium of patients with type 2 diabetes mellitus [19] and heart failure [20]. It is believed that such changes may reflect impaired MCh function in cardiometabolic diseases [21]. A direct relationship between the PCr/ATP ratio and cardiac MCh function has not been found. However, the factors such as Cr and PCr availability and mt-KK activity may influence MCh function in cardiomyocytes. Our results show that the elimination of ΣCr loss by metilin was associated with an increase in the functional coupling of mt-CK to OXPHOS in STZ-treated animals. This corresponded to an increase in MCh respiration in the ADP-stimulated state 3 and an increase in RCR. Administration of metilin to diabetic rats preserved the integrity of cardiomyocyte membranes, which was manifested in a decrease in the activity of CK-MB and LDH in plasma to initial levels. Increased levels of circulating CK-MB and LDH were found in laboratory animal models of STZ-induced DCM and in diabetic patients. They may serve as markers of cardiovascular risk and myocardial damage [22, 23]. This effect of metilin reflects a better metabolic state of the heart and, in particular, prevention of ΣCr loss, which indicates less damage to cardiomyocyte membranes [24].

Possible Mechanisms of Metilin Action in T1DM

Results of our study show that daily administration of metilin reduces blood glucose. This is consistent with earlier reports that natural C-terminal fragments of apelin exhibit glucose-lowering effects in STZ-induced diabetes [25] by increasing glucose utilization in various tissues especially in skeletal muscle, adipose tissue, and cardiac muscle [26]. These effects are associated with apelin-stimulated phosphorylation of AMP-activated protein kinase (AMPK) and endothelial NO synthase (eNOS) [26]. Some studies have shown that in addition to the decrease of blood glucose and the increase in glucose utilization, the apelin peptides may stimulate insulin production and reduce insulin resistance [27]. For example, administration of exogenous apelin-13 significantly improved pancreatic islet mass and increased serum insulin levels in T1DM models [28, 29].

The improvement in cardiac energetics in diabetic rats treated with metilin could be associated with a decrease in the ROS formation. In cultured H9c2 cardiomyoblasts and adult cardiomyocytes, this peptide attenuated IRI-induced oxidative stress by reducing the production of mitochondrial superoxide and H_2O_2 [9]. Using EPR and spin trapping 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), we have shown that metilin reduced the formation of DMPO-OH hydroxyl radical adducts during cardiac reperfusion after a period of ischemia [30]. The mechanisms by which metilin reduces ROS production are closely related to the activation of antioxidant enzymes. This assumption is well supported by a metilin-induced increase in the activity of Cu, Zn-SOD, CAT, and GSH-Px in the heart and a concomitant decrease in the formation of malondialdehyde, a secondary product of lipid peroxidation, under conditions of myocardial IRI [30]. Another possibility for reducing ROS production is the mobilization of reperfusion kinases PI3K-Akt and MEK1/2-ERK1/2 [31], leading to inhibition of mitochondrial permeability transition pore (mPTP) opening and protection from apoptosis. The resulting decrease in the release of cytochrome *c* from the MCh intermembrane space may contribute to a decrease in the formation of mitochondrial superoxide. Taken together, these results clearly demonstrate the antioxidant activity of metilin. Currently, there is increasing experimental evidence for involvement of ROS in the energy deficiency of the diabetic heart, which contributes to the development of DCM [32]. In this regard, the effect of metilin on ROS sources may be a useful therapeutic approach for the prevention and treatment of diabetes.

The system metilin/APJ may influence myocardial energetics in diabetes due to changes in MCh biogenesis. Apelin peptides increased the expression of peroxisome proliferator-activated receptor coactivator-1α (PGC1-α), a transcriptional coactivator regulating cellular energy metabolism, nuclear respiratory factors 1 (NRF1) and 2 (NRF2), and mitochondrial transcription factor A (Tfam) in skeletal muscles of insulin-resistant mice [33]. These effects were associated with an increase in the mtDNA/nuclear DNA ratio and the density of intramyofibrillar and subsarcolemmal MCh. The expression of components of complexes II, III, and V of the respiratory chain and the activity of citrate synthase, a marker of the MCh content, also increased in apelin treated mice [34]. Thus, the enhancement of MCh biogenesis by metilin may contribute to the respiratory function improvement found in skinned heart fibers of diabetic rats in our experiments. It should be noted that apelin peptides enhance the expression of PGC1-α through the AMPK pathway in cardiac cells [35]. This is the particular mechanism by which natural apelins and metilin can regulate induction of antioxidant defense, including SOD, CAT, and GSH-Px, as well as the increase the expression of sirtuin 3, a key regulator of the mitochondrial antioxidant system. This demonstrates links between mitochondrial biogenesis and the mitochondrial antioxidant capacity realized by apelin peptides.

The other important aspect of the metilin action is the reduction of cardiac IRI. We previously studied the signaling mechanisms involved in the metilin-induced limitation of the size of acute myocardial infarction in rat models of cardiac IRI [31]. It was found that the protective effect of metilin was associated with signaling via components of the PI3K and MEK1/2 kinase cascades, accompanied by activation of downstream targets — eNOS and mitochondrial K^+ATP channels, as well as Na^+/H^+ and Na^+/Ca^{2+} sarcolemma exchangers. Recent studies have shown that apelin-12 inhibits apoptosis and oxidative stress in diabetes through pathways involving PI3K and p38-MAPK and this decreased cardiac IRI [36]. In accordance with these data, administration of apelin-12 abolished muscle fiber loss, necrosis, and reduced diabetic heart dysfunction. In addition, apelin-13 decreased the expression of adhesion molecules, increased proliferation and angiogenesis through activation of the APJ receptor-nuclear factor κB (NF-κB) pathway, which reduced diabetes-induced endothelial dysfunction [37]. These data suggest that the use of metilin in patients with T1DM with myocardial IRI may be promising. Figure 4 schematically presented the discussed effects of metilin, our previous results and the effects of apelin peptides on the diabetic heart shown by other authors.

CONCLUSIONS

The results of the work indicate that the administration of STZ to rats caused impairments in cardiac energetics, including metabolic dysfunction in the early stages of diabetes development.

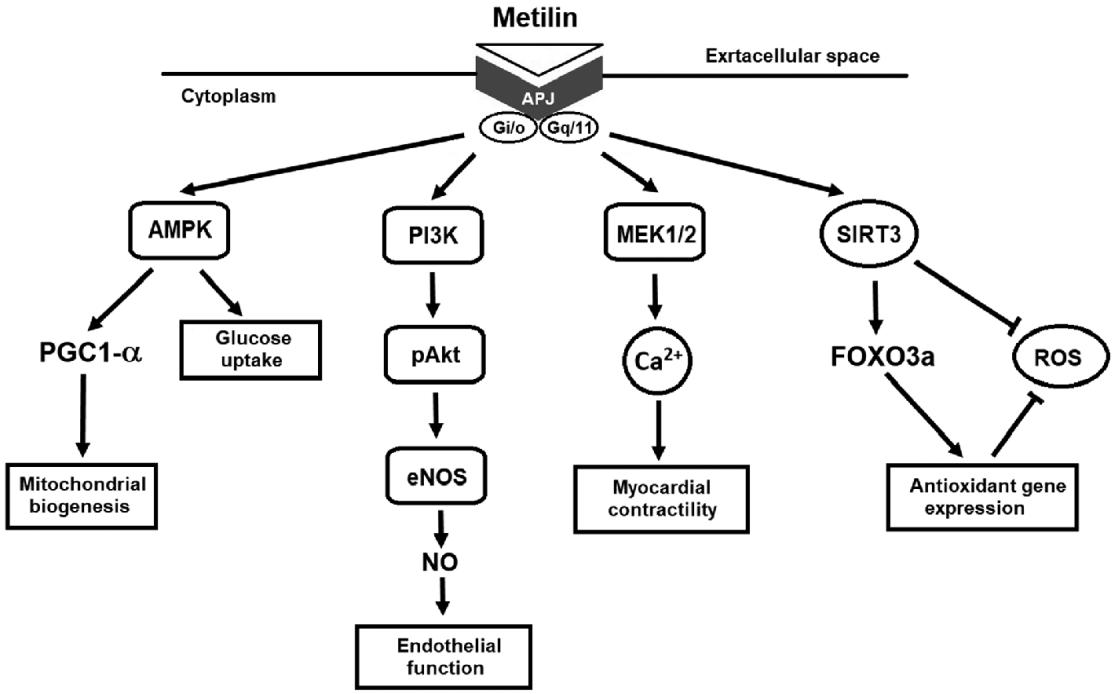

Figure 4. Activation of the APJ receptor by metilin triggers signaling cascades that exert protective effects on the diabetic heart. AMPK, AMP-activated protein kinase; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator 1-α; PI3K, phosphoinositide 3-kinase; pAkt, phosphorylated protein kinase B; eNOS, endothelial nitric oxide synthase; MEK1/2, mitogen-activated protein kinase kinases 1 and 2; SIRT3, sirtuin-3 NAD-dependent protein deacetylase; FOXO3a, member of the forkhead transcription factor (FoxO) family; ROS, reactive oxygen species.

This study also demonstrates the antidiabetic effect of metilin, a structural analogue of apelin-12, in STZ-treated rats. Administration of metilin reduced blood glucose, positively modulated cardiac mitochondrial dysfunction, thus improving the energy state of the myocardium, and reduced cardiomyocyte membrane damage. We believe that the use of proteolytically stable modified APJ receptor peptide agonists may be a promising pharmacological strategy for cardiac protection in T1DM.

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COMPLIANCE WITH ETHICAL STANDARDS

All stages of the experiment complied with the requirements of international rules for the humane treatment of animals, reflected in the sanitary rules for the selection of animals and the maintenance of experimental biological clinics (vivariums).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

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СТРУКТУРНЫЙ АНАЛОГ АПЕЛИНА-12 ПРЕПЯТСТВУЕТ НАРУШЕНИЯМ ЭНЕРГЕТИКИ СЕРДЦА ПРИ ЭКСПЕРИМЕНТАЛЬНОМ САХАРНОМ ДИАБЕТЕ 1 ТИПА

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Сахарный диабет первого типа (СД1) — наиболее тяжёлая форма диабета, которая характеризуется абсолютной недостаточностью инсулина, вызванной деструкцией бета-клеток поджелудочной железы. Целью данной работы было оценить влияние структурного аналога апелина-12 ((N$^{\alpha}$Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH, метилин) на гипергликемию, параметры дыхания митохондрий (МХ) в пермеабилизированных волокнах левого желудочка (ЛЖ) сердца, энергетическое состояние миокарда и повреждение мембран кардиомиоцитов на модели стрептозотоцинового (СТЗ) диабета у крыс. Метилин получали методом твердофазного синтеза с использованием Fmoc-стратегии и очищали с помощью ВЭЖХ. Использовали четыре группы животных: исходное состояние (ИС); контроль (К), диабет (Д) и животных с диабетом, дополнительно получавших метилин (ДМ). Оценивали уровень глюкозы в крови, дыхание митохондрий (МХ) в волокнах ЛЖ, содержание ATP, ADP, AMP, фосфокреатина (PCr) и креатина (Cr) в сердце, активность креатинкиназы-МВ (КК-МВ) и лактатдегидрогеназы (ЛДГ) в плазме крови. Введение метилина крысам, получавшим СТЗ, снижало уровень глюкозы в крови, увеличивало потребление кислорода в состоянии 3 и дыхательный контроль в МХ пермеабилизированных волокон ЛЖ, повышало функциональное связывание митохондриальной КК с окислительным фосфорилированием по сравнению с этими параметрами в группе Д. Под действием метилина у животных, получавших СТЗ, наблюдали повышение содержания PCr и предотвращение потери общего креатина (ΣCr=PCr+Cr) в диабетическом сердце, восстановление отношения PCr/ATP в миокарде и снижение активности КК-МВ и ЛДГ в плазме до исходных значений. Таким образом, метилин предотвращал нарушения биоэнергетики кардиомиоцитов при моделировании СД1.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: аналог апелина-12; стрептозотоциновый диабет; сердце крысы; дисфункция митохондрий; энергетическое состояние миокарда

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