THE PRECONDITIONING EFFECT OF A STRUCTURAL ANALOGUE OF APELIN-12 IN A RAT MODEL OF ACUTE MYOCARDIAL INFARCTION

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The use of pharmacological agents to trigger preconditioning mechanisms may improve the prevention and treatment of coronary heart disease. The aim of this study was to evaluate the ability of a structural analog of apelin-12 ((N$^{\alpha}$Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH, metilin) to reproduce the effect of ischemic preconditioning (IP) of rat hearts *in vivo*. Control rats were exposed to 40-min occlusion of the left descending coronary artery (LDCA) followed by 60-min restoration of coronary blood flow (reperfusion). IP was modeled by three cycles of 5-min occlusion/5-min reperfusion of the LDCA before prolonged regional myocardial ischemia and reperfusion. Metilin (5 mg/kg) was administered to rats intravenously by bolus injection 30 min before LDCA occlusion. IP or metilin had a significant impact on the studied parameters. The size of necrotic damage to the left ventricle, expressed as the percentage ratio of myocardial infarction/myocardial area at risk (MI/AAR, %), at the end of reperfusion was 26.9±2.0% and 29.3±2.6%, respectively, compared with 43.8±1.2% in the control ($p < 0.01$). The activity of creatine kinase-MB (CK-MB) in blood plasma decreased to 1026.1±93.9 IU/ml and 1195.2±142.0 IU/ml, respectively, compared with 1986.3±193.7 IU/ml in the control ($p < 0.02$). Administration of metilin, as well as IP, increased the reduced content of ATP, total adenine nucleotide pool (ΣAN) and phosphocreatine (PCr) in the AAR at the end of reperfusion compared to the control ($p < 0.05$–0.01). In the metilin group, the content of total creatine (ΣCr) in AAR was higher than in the control ($p < 0.05$). Intravenous administration of 5 mg/kg 5-hydroxydecanoate (5HD), an inhibitor of mitochondrial ATP-dependent K^+ channels (mitoK_{ATP}), abolished the preconditioning effect of metilin, and increased the MI/AAR, %, and plasma CK-MB activity to values that insignificantly differed from the control (39.4±2.8% and 2258.2±179.1 IU/ml, respectively). Simultaneously, 5HD significantly reduced the ATP and ΣAN levels in AAR compared to those in the metilin group and the ATP, ΣAN, and PCr levels compared to the IP group. The results indicate that pharmacological preconditioning by metilin reduced cardiac ischemia/reperfusion injury via the involvement of mitoK_{ATP} in the mechanism of metilin action.

Keywords: metilin; rat heart; preconditioning; myocardial infarction; creatine kinase-MB; energy state of the myocardial area at risk

DOI: 10.18097/PBMCR1622

INTRODUCTION

Ischemic preconditioning (IP) is a protective adaptive response of the heart to prolonged ischemic stroke and subsequent reperfusion [1]. IP can reduce myocardial injury caused, for example, by percutaneous coronary intervention or aortic cross-clamping during coronary artery bypass grafting. However, its use, which requires cessation of blood supply, is difficult or impractical in many clinical situations [2]. Pharmacological preconditioning (PP), which triggers intracellular signaling involved in cardiac protection, may overcome these problems. Experimental studies have shown that administration of adenosine, opioid agonists, bradykinin, nitric oxide donors, or other pharmacological agents before the onset of sustained myocardial ischemia can activate signaling pathways and initiate the cardioprotective effects seen in IP [3]. However, most mimetic agents, reducing ischemia/reperfusion injury (IRI) in animal models, generally fail to exhibit cardioprotective effects in human clinical trials [4, 5]. The reasons for this discrepancy are related to comorbidities, as well as poor solubility, proteolytic instability, and a number of side effects of the tested compounds. In this context, the search for new therapeutic targets for pharmacological intervention on ischemic myocardium still remains relevant.

The apelin peptide is an endogenous ligand of the G-protein-coupled transmembrane receptor APJ [6]. The apelin/APJ receptor system is involved in the regulation of cardiac and vascular function in health and disease. Apelin-12 is the smallest fragment of the 77-amino acid apelin prepropeptide, which retains effective binding to the APJ receptor and exhibits cardioprotective action [7]. We modified apelin-12 and synthesized its structural analogs with greater storage stability and reduced proteolytic degradation [8]. Testing of these peptides has shown

that (N$^{\alpha}$Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH (metilin) demonstrated the highest biological activity [9]. Metilin reduced apoptosis and necrosis, decreased formation of reactive oxygen species (ROS), and improved metabolic and functional state of the myocardium in IRI, doxorubicin-induced cardiomyopathy and type 1 diabetes mellitus [9–11]. In IRI, metilin caused activation of reperfusion kinases PI3K/Akt and MEK1/2-ERK1/2, acting on the mitochondrial permeability transition pore (mPTP). The key link in the protective effect of metilin on ischemic myocardium is phosphorylation of endothelial NO synthase (eNOS) due to activation of the PI3K/Akt/eNOS signaling pathway [12]. These facts indicate that the metilin/APJ receptor system may serve as a promising target in cardiac PP. Studies in this area have not been conducted yet.

Thus, the aim of this study was to investigate the preconditioning properties of metilin using an *in vivo* model of acute myocardial infarction (MI) in rats. For this purpose, we selected an optimal metilin preconditioning regimen and assessed the effect of metilin on the MI size, cardiomyocyte membrane damage, and the energy status of the myocardial area at risk (AAR). The obtained data indicate that the cardioprotective effects of metilin are associated with the activation of mitochondrial ATP-dependent K^+ channels (mitoK_{ATP}).

MATERIALS AND METHODS

Synthesis of Metilin

Automated synthesis of metilin was performed by our optimized method on a Tribute-UV synthesizer (Protein Technologies Inc., USA) using a polymer support (Iris Biotech, Germany) with a 2-chlorotrityl chloride anchor group and 20% cyclic secondary amine pyrrolidine in N,N-dimethylformamide to remove the Fmoc protection [13]. Upon completion of the synthesis, the peptide was cleaved from the polymer with simultaneous deprotection of the amino acid functional groups by using trifluoroacetic acid with scavengers for 1.5 h. The resultant product was analyzed and purified by HPLC.

Analytical HPLC was performed on a Knauer instrument (Germany) using a Diaspher 110-C-18 column (BioChemMack ST, Russia) (4.0×250 mm), sorbent particle size of 5 μm. The mobile phase included: buffer A (0.05 M KH_2PO_4, pH 3.0) and buffer B (70% acetonitrile in buffer A). Elution was carried out at a flow rate of 0.75 ml/min using a concentration gradient of buffer B in buffer A from 20% to 80% in 30 min, (detection at λ = 220 nm). Preparative HPLC of metilin was performed on a Gilson instrument (France) using a Kromasil-C18 column (Kromasil, Sweden) (30×250 mm), sorbent particle size — 10 μm. The eluents used were buffer A, 0.01 M aqueous ammonium acetate solution, pH 4.5, containing 3–5% acetonitrile, and buffer B, 70% acetonitrile. Elution was performed from 100% buffer A in a concentration gradient of 0.5%/min buffer B at a rate of 20 ml/min. Fractions corresponding to the target substance were pooled, concentrated *in vacuo*, and lyophilized. The structure of the peptide was confirmed by proton magnetic resonance (^{1}H-NMR) on a WH-500 Bruker spectrometer (Bruker Daltonik GmbH, Germany) and mass spectrometry on an Amazon instrument (Bruker Daltonik GmbH) using electrospray ionization (ESI) in the positive ion recording mode (capillary voltage of 3500 V). The total yield of metilin based on the starting amino acid attached to the polymer carrier was 72.1%, the peptide purity (according to HPLC) was 98.7% (retention time on the above-mentioned sorbent was 12.5 min). ESI$^+$: found *m/z* — 355.44^{4+}, 473.82^{3+}, 710.05^{2+}; calculated for $C_{66}H_{107}N_{21}O_{14}$ — 355.47^{4+}, 473.62^{3+}, 709.92^{2+}.

Animals

Male Wistar rats weighing 300–320 g were obtained from the Stolbovaya Breeding Center of the Scientific Center for Biomedical Technologies (Moscow Region). Animals were housed in individual cages at 20–25°C with a natural light/dark cycle and free access to standard pelleted food and water.

Experimental Design

After 24 h fasting, plasma creatine kinase-MB (CK-MB) activity was measured in 5 rats. They were then anesthetized with 2,2,2-tribromoethanol (1 mg/kg intraperitoneally (i.p.), Avertin, Merck, Russia), and their hearts were isolated for metabolic measurements (n = 5, Initial state (IS) group). Forty rats were then randomly subdivided into four groups of 10 rats each (Fig. 1):

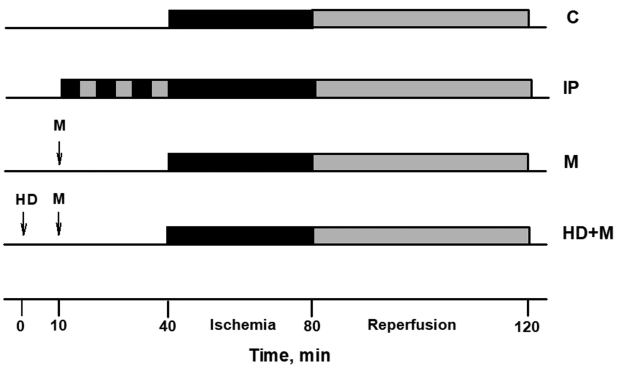

Figure 1. The scheme of the experimental protocol. C – control (40 min LDCA occlusion and 60 min reperfusion), IP – ischemic preconditioning (three cycles of 5-min occlusion / 5-min reperfusion of LDCA before ischemia), M – metilin administration (5 mg/kg, i.v., b) before ischemia, HD+M – 5-hydroxydecanoate (5 mg/kg, i.v., b) and metilin (5 mg/kg, i.v., b) before ischemia.

1) Control (C). Cardiac IRI was modeled by occlusion of the left descending coronary artery (LDCA) for 40 min, followed by restoration of coronary blood flow for 60 min [10].

2) Cardiac ischemic preconditioning (IP). Three cycles of 5 min occlusion / 5 min LDCA reperfusion were performed before 40 min of regional ischemia [12].

3) Metilin (M) preconditioning. Metilin (5 mg/kg) was administered to rats intravenously (i.v.) by bolus (b) injection 30 min before onset of 40-min LDCA occlusion. Preliminary experiments have shown that this metilin administration regimen caused the most pronounced reduction in the MI size. (The dose-dependent effects of different routes of metilin administration on the MI size are given in Supplementary Materials, Table S1.)

4) Combined administration of 5-hydroxydecanoate (5HD, Merck) and metilin (HD+M). 5HD (5 mg/kg, i.v., b) was administered 10 min before metilin (5 mg/kg, i.v., b). The dose and administration route of 5HD were taken from the literature [14]. Preliminary experiments showed that administration of 1.0 mg/kg or 5.0 mg/kg 5HD alone did not affect the MI size or plasma CK-MB activity (Supplementary Materials, Table S2).

After reperfusion, plasma CK-MB activity was determined in five animals from each of the four groups, and the size of MI and AAR were assessed histochemically. The remaining five rats from these groups were used for isolation of their hearts, which were frozen in liquid nitrogen by means of Wollenberger clamps for subsequent analysis of energy metabolism metabolites.

The Model of Rat Heart Regional Ischemia and Reperfusion

Rats were anesthetized with 20% urethane (1200 mg/kg body weight, i.p.) and artificially ventilated with room air using a KTR-5 apparatus (Hugo Sacks Electronik, Germany) under thoracotomy. Mean arterial pressure, heart rate, and ECG were monitored and recorded on a computer using a USB 6210 analog-to-digital converter (National Instruments, USA) and LabView 7 software (National Instruments) [15]. After the manipulations, a period of hemodynamic stabilization followed. The animals were then subjected to 40-min LDCA occlusion followed by 60-min reperfusion. At the end of the experiment, in order to identify the MI and the intact myocardial area, the LDCA was reoccluded, and 2 ml of 2% Evans Blue (Merck) was injected into the jugular vein. The heart was then excised, the left ventricle (LV) was isolated to determine the MI size, and the sample was frozen at -20°C.

Assessment of Cardiomyocyte Membrane Damage

Cardiomyocyte membrane damage was assessed by the increase in plasma CK-MB activity at the end of reperfusion. Blood samples (0.5 ml) were collected in heparinized tubes from a venous catheter at BL (before LDCA occlusion) and after 1 h reperfusion. Plasma CK-MB activity was determined using a BioSystems kit (Spain) on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) at $\lambda = 340$ nm.

Myocardial Infarction Size Assessment

The frozen left ventricle (LV) was cut perpendicular to the long axis of the heart into 4–5 slices approximately 1.5-2.0 mm thick; they were then incubated for 10 min in 1% solution 2,3,5-triphenyltetrazolium chloride (TFT, Merck) in 0.1 M potassium phosphate buffer (pH 7.4 at 37°C). Histochemical assessment of the MI size using TFT is based on the formation of an insoluble red pigment, formazan, upon reduction of TFT by NAD^+- and $NADP^+$-dependent dehydrogenases. As a result, the intact LV region was stained dark blue, AAR was stained red, and the MI zone remained unstained (flesh-colored). The obtained samples were scanned from both sides using an Epson Perfection 2480 scanner (Japan), and zones of the MI and AAR were determined by computer planimetry using ImageJ software (NIH, USA). The sections were then weighed to determine the LV mass. In each group the AAR/LV and MI/AAR ratios were calculated in % [15].

Determination of Metabolite Content in the Cardiac Area at Risk

AAR cardiac tissue frozen in liquid nitrogen was homogenized in cold 6% $HClO_4$ (10 ml/g tissue) using an Ultra-Turrax T-25 homogenizer (IKA-Labortechnik, Germany). Proteins were precipitated by centrifugation (Sorvall RT1 centrifuge, Thermo Fisher Scientific, USA) at 2800 *g* for 10 min at 4°C. Supernatants were neutralized with 5 M K_2CO_3 to pH 7.4. The $KClO_4$ precipitate was separated by centrifugation under the same conditions. Protein-free extracts were stored at -70°C until metabolite analysis. The dry weight of homogenized tissue was determined after drying the samples for 24 h at 100°C. The content of ATP, ADP, AMP, phosphocreatine (PCr), and creatine (Cr) in tissue extracts was determined by means of modified enzymatic methods [16] using a Shimadzu UV-1800 spectrophotometer.

Statistical Data Analysis

Statistical data analysis was performed using the SigmaPlot 11.2 software package (SysStat, USA). Data are presented as mean ± standard error of the mean (M±SEM). Differences between groups were statistically confirmed using analysis of variance (ANOVA). Student's *t*-test with Bonferroni correction was used for comparison of multiple groups with the control. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Reduction of Cardiac IRI by Metilin Preconditioning

Histochemical analysis of LV sections after reperfusion revealed insignificant differences in AAR between the control and IP, M, and HD+M groups (Fig. 2A). This indicates that IRI was modeled similarly in all animals. In the control group, the magnitude of MI, expressed as the MI/AAR ratio in %, was 43.8±1.2% (Fig. 2B). After IP or metilin administration (5 mg/kg i.v. b, 30 min before regional ischemia), the extent of necrotic LV damage at the end of reperfusion was 26.9±2.0% and 29.3±2.6%, respectively ($p < 0.01$ versus the control group). Intravenous administration of 5HD (5 mg/kg) 10 min before metilin abolished the preconditioning effect of the peptide and increased the MI size to a value insignificantly differed from the control (39.4±2.8%).

The development of MI in animals of the control group was accompanied by a 5-fold increase in plasma CK-MB activity by the end of reperfusion compared to the animals of the IS group (Fig. 2B). IP and metilin, reduced plasma CK-MB activity at the end of reperfusion by 48.4% ($p < 0.01$) and 39.8% ($p < 0.05$), respectively, compared to the control. Administration of 5HD before the bolus injection of metilin increased plasma CK-MB activity to the value insignificantly differed from that in the control group (2258.2±179.1 IU/ml of plasma). Figure 2D shows the AAR localization in LV sections after staining with TFT. Increased formation of the red pigment formazan as a result of TFT reduction by NAD^+- and $NADP^+$-dependent dehydrogenases in animals of the IP and M groups indicated a reduction in the MI size due to the influence of IP and PP.

The Effect of Metilin on the Energy State of the Myocardial Area at Risk

Table 1 shows energy parameters of the LV AAR at the end of reperfusion in the studied groups in comparison with the IS group. IRI modeling had a significant impact on the content of adenine nucleotides and the PCr-Cr system in AAR of control group animals in the end of reperfusion. ATP and total adenine nucleotide (ΣAN) levels were reduced by 2- and 1.5-fold, respectively. PCr levels were reduced by 40%; since free Cr levels remained basically unchanged, this caused a 25% decrease in total intracellular creatine (ΣCr) compared to the baseline level. IP significantly increased AAR ΣAN

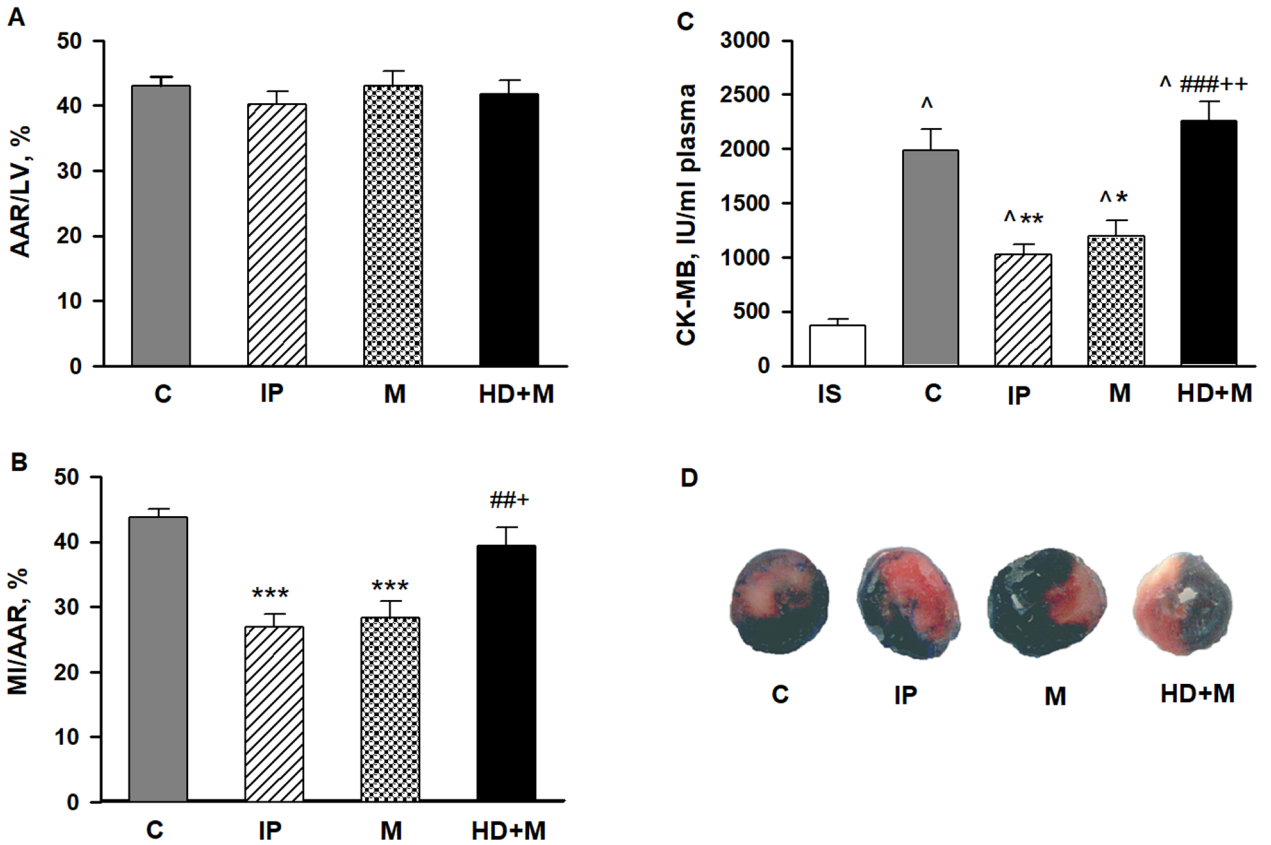

Figure 2. Effect of ischemic preconditioning, metilin, and 5-hydroxydecanoate on myocardial injury during regional cardiac ischemia and reperfusion in rats. **A** – AAR; **B** – MI size; **C** – plasma creatine kinase-MB activity; **D** – 2,3,5-triphenyltetrazolium chloride staining of LV sections at the end of reperfusion. IS – initial state baseline, C – control, IP – ischemic preconditioning, M – metilin administration before ischemia, HD+M – 5-hydroxydecanoate and metilin administration before ischemia. Statistically significant difference from: IS $^p < 0.001$; C $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; IP $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$; M $^{+}p < 0.05$, $^{++}p < 0.01$.

Table 1. Energy metabolism parameters of rat myocardium

	IS	K	IP	M	HD+M
ATP	20.58±1.09	10.74±0.76^^^	15.02±0.62^^,***	13.87±0.58^^^,*	11.98±0.72^^^,#,+
ADP	5.42±0.38	5.05±0.33	5.43±0.10	5.47±0.27	4.81±0.13##
AMP	0.98±0.10	1.43±0.24	1.26±0.15	1.45±0.11^	1.07±0.04+
ΣAN	26.98±1.25	17.11±0.81^^^	21.71±0.77^^,***	20.80±0.65^^,**	17.86±0.82^^^,##,+
PCr	31.68±1.73	19.29±1.19^^^	27.35±1.42**	24.72±1.31^,*	21.38±1.14^^^,#
Cr	26.15±1.17	24.81±1.25	21.49±1.80	23.91±1.12	23.43±0.87
ΣCr	57.83±1.85	43.90±1.37^^^	48.84±2.23^	48.64±1.46^^,*	44.81±1.29^^^

Results of metabolite content determination are expressed as μmol/g dry weight. IS – initial state; C – control; IP – ischemic preconditioning; M – metilin preconditioning (5 mg/kg); HD+M – administration of 5-hydroxydecanoate (5 mg/kg) and metilin (5 mg/kg) before regional ischemia. ΣAN = ATP+ADP+AMP; ΣCr = PCr+Cr. Significantly differed from IS: ^$p < 0.05$; ^^$p < 0.01$; ^^^$p < 0.001$; from K *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; from IP: #$p < 0.05$; ##$p < 0.01$; from M +$p < 0.05$.

compared to the control group due to an increase in the ATP content. In addition, an increase in the PCr levels was noted (by 30% compared to the control group, $p < 0.01$). Similarly to IP, metilin administration before LDCA occlusion influenced the adenine nucleotide system of AAR: the treatment increased the content of ATP and ΣAN ($p < 0.05$ and $p < 0.01$, respectively) and did not affect ADP and AMP levels. Metilin preconditioning increased the PCr content in the AAR thus causing an increase in ΣCr compared to the control ($p < 0.05$). In general, metilin improved the energy parameters of AAR to the same extent as IP. Administration of 5HD before metilin decreased the content of high-energy phosphates and ΣCr to control values. In animals of the HD+M group, the content of ATP, ADP, ΣAN, and PCr was significantly lower than after IP, and also lower than the content of ATP, AMP, and ΣAN compared to these parameters in animals of the metilin preconditioning group. These data indicate that 5HD blocks the effect of metilin on the energetics of damaged myocardium.

DISCUSSION

The use of pharmacological agents of various chemical structures for initiation of preconditioning mechanisms can significantly improve the prevention and treatment of coronary heart disease [17]. The ability of natural and modified C-terminal fragments of apelin to increase cardiac contractility and myocardial resistance to IRI has been confirmed in various experimental models, including isolated cardiomyocytes and H9c2 cells [9, 18–20], isolated perfused rat hearts [21–24], and *in vivo* myocardial infarction model in laboratory animals [25, 26]. In these experiments, apelins were administered before prolonged ischemia/hypoxia, suggesting the possibility of their use as preconditioning agents. Apelin-12, a structural analogue of metilin, was selected for this study. Its advantage over natural analogs of C-terminal fragments of apelin is associated with its higher stability with respect to proteolytic enzymes with simultaneous maintaining vasodilatory activity and antioxidant properties [8, 10]. In this study, we used a generally accepted protocol for modeling IP (three cycles of 5-min occlusion / 5-min reperfusion of the coronary artery before prolonged ischemia [12]) and assessed the effect of metilin under these conditions.

This study demonstrates for the first time the effects of metilin PP in rat hearts subjected to regional ischemia and reperfusion *in vivo*. These effects included a decrease in the MI size compared to the control, which was insignificantly differed from that after IP; a decrease in plasma CK-MB activity by the end of reperfusion; and an improvement in the energy state of the reperfused myocardium. Preliminary administration of 5HD, a mitoK_{ATP} inhibitor, abolished/reduced the effect of metilin on these parameters. This suggests that mitoK_{ATP} channels, which are the main signaling element of conditioning [27], are involved in the mechanisms of metilin PP. It is important that mitoK_{ATP} channels are involved in the effect of metilin on the energy state of the reperfused AAR. It is believed that mitoK_{ATP} opening may lead to mitochondrial membrane depolarization, causing dissipation of mitochondrial potential and a reduction in the driving force for Ca^{2+} uptake, as well as preserving mitochondrial function during ischemia and reperfusion [28]. Furthermore, a reduction in mitochondrial Ca^{2+} loading prevents mPTP opening, which inhibits oxidative phosphorylation and promotes the release of proapoptotic proteins [3]. Metilin stimulation of mitoK_{ATP} may be a promising therapeutic strategy to reduce IRI, particularly in hearts with damaged K_{ATP} channels.

Recent studies have shown that C-terminal fragments of apelin bind to the G-protein-coupled APJ receptor on the surface of cardiomyocytes and activate intracellular signaling cascades that include PI3K-Akt, Erk1/2, and transcription factors such as hypoxia-inducible factor (HIF1). Activation

of these mediators leads to the mitoK$_{ATP}$ opening [10]. Previously, in a model of isolated rat hearts subjected to global ischemia and reperfusion, we found that administration of specific inhibitors of mitogen-activated protein kinase 1/2 (MEK1/2) (UO126), phosphoinositide 3-kinase (PI3K) (LY294002), NO synthase (L-NAME), or mitoK$_{ATP}$ (5HD) reduced the cardioprotective efficacy of metilin administered into the perfusate immediately before ischemia [29]. This was indicated by a decrease in the product of LV pressure and heart rate, cardiac output, and an increase in LV diastolic pressure during reperfusion. The impaired recovery of isolated rat heart function was accompanied by a decrease in myocardial energy state and an increase in LDH release into the perfusate during early reperfusion. It was found that the enhancement of functional and metabolic recovery of the reperfused rat heart as a result of metilin administration before ischemia was suppressed by inhibitors of phospholipase C (PLC) (U-73122), protein kinase C (PKC) (chelerythrine), or inhibitors of sarcolemmal Na^+/H^+ and Na^+/Ca^{2+} exchange (amiloride or KB-R7943, respectively). Similar actions of these inhibitors of signal transduction and terminal effectors have been shown for apelin-12, apelin-13, [Pyr1] apelin-13, apelin-17, and apelin-36 in various models of cardiac IRI [18, 24, 25, 30–32]. This convincingly suggests that the synthetic APJ receptor agonist metilin triggers the same signaling cascades initiated by natural C-terminal fragments of apelin. Thus, it is most likely that PP by metilin is mediated by signaling through PLC and survival kinases PI3K and MEK1/2 with activation of downstream targets, NO synthase and mitoK$_{ATP}$ (Fig. 3). At the same time, the existence of alternative protective mechanisms of PP by metilin associated with its antioxidant properties cannot be ruled out [10].

Some limitations of our study may be related to the fact that 5HD is not a specific mitoK$_{ATP}$ inhibitor. For example, Hanley et al. showed that 5HD could be activated within mitochondria and further metabolized via β-oxidation [33]. 5HDCoA is known to bind to adenine nucleotide translocase and acetyl-CoA carboxylase, thereby inhibiting their activity [34]. This effect of 5HD on the metabolism of the postischemic heart may result in exacerbation of IRI [35]. However, administration of 5HD alone did not affect the MI size or CK-MB activity in our experiments (Supplementary Materials, Table S2). These results allow us to ignore possible nonspecific metabolic effects of 5HD, thus indicating that its action is mainly associated with mitoK$_{ATP}$ inhibition.

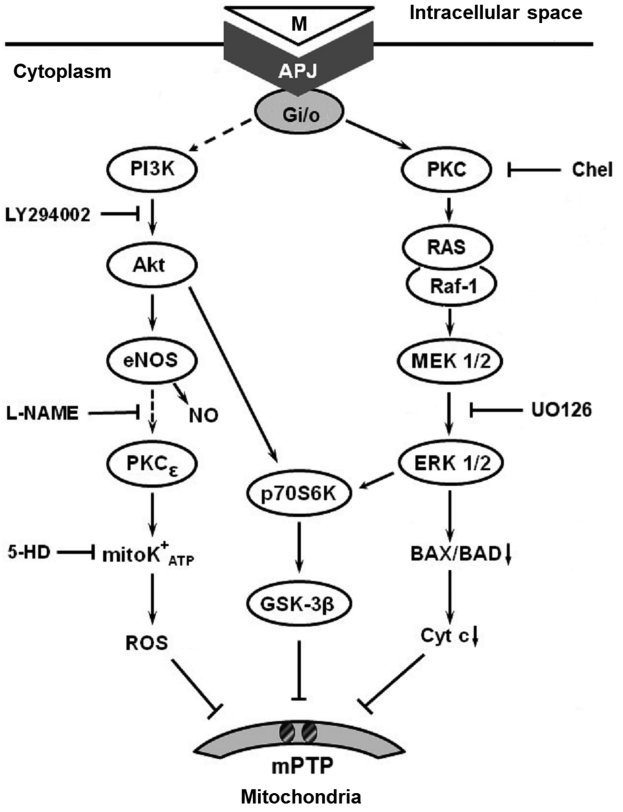

Figure 3. Activation of intracellular signaling by metilin preconditioning prevents mitochondrial mPTP pore opening in an ischemic cardiomyocyte. Ras – small G protein family; MEK1/2 – mitogen-activated protein kinase kinase (MAP2K); ERK1/2 – extracellular signal-regulated kinase; BAX/BAD – proapoptotic proteins; Cyt *c* – cytochrome *c*; PI3K – phosphatidylinositol 3-kinase; Akt – protein kinase B; eNOS – endothelial NO synthase; PKCε – ε-isoform of protein kinase C; mitoK$^+_{ATP}$ – mitochondrial ATP-dependent K^+ channels; GSK-3β – glycogen synthase kinase 3β; p70S6K – ribosomal protein kinase S6 β1; Chel – chelerythrine; ROS – reactive oxygen species; LY294002 – selective PI3K inhibitor; Raf-1 – serine/threonine protein kinase.

CONCLUSIONS

The apelinergic system is a promising therapeutic target for treatment of cardiovascular diseases. The short half-life of natural C-terminal fragments of apelin [36] suggests a clear need to create modified apelin analogs that are less susceptible to degradation in biological systems and retain their efficacy upon binding to the APJ receptor. Our results demonstrate that proteolytically stable metilin is capable of reproducing the preconditioning effects of apelin-12 in the *in vivo* model of MI in rats. In addition to reducing the MI size, this peptide increased the content of high-energy phosphates in the damaged region of the heart during reperfusion and reduced damage to cardiomyocyte membranes. Using 5HD, the involvement of mitoK$_{ATP}$ in signal transduction, which mediates the cardioprotective effects of metilin, has been confirmed. Metilin may be a tool for treating IRI not only in the heart but also in other vital organs. This view is consistent with the ability of apelin-13 to exert a protective effect in renal IRI [37]. In cerebral IRI, apelin-13 and apelin-36 protect neurons and inhibit inflammation-induced damage

by activating the PI3K/Akt pathway [38, 39]. Clearly, studies of the mechanisms of pharmacological pre- and post-conditioning with modified apelin peptides may be an important task for future research.

FUNDING

This study was supported by the Russian Ministry of Public Health (State Assignment no. 125022803024-3).

COMPLIANCE WITH ETHICAL STANDARDS

The study was performed in accordance with the fundamental principles governing experiments on laboratory animals and humane animal care (Declaration of Helsinki, 2000; Rules for Conducting Qualitative Clinical Trials in the Russian Federation (approved by the Ministry of Public Health of the Russian Federation on December 29, 1998) OST 42-511-99; and Rules for Laboratory Practice in the Russian Federation (approved by Order of the Ministry of Public Health of the Russian Federation dated June 19, 2003, no. 267).

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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Received: 07.10.2025.
Revised: 18.10.2025.
Accepted: 20.10.2025.

ПРЕКОНДИЦИОНИРУЮЩЕЕ ДЕЙСТВИЕ СТРУКТУРНОГО АНАЛОГА АПЕЛИНА-12 В МОДЕЛИ ОСТРОГО ИНФАРКТА МИОКАРДА У КРЫС

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Использование фармакологических агентов для запуска механизмов прекондиционирования может улучшать профилактику и лечение ишемической болезни сердца. Целью данной работы было оценить способность структурного аналога апелина-12 ((N$^{\alpha}$Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH, метилина) воспроизводить действие ишемического прекондиционирования (ИП) сердца крысы *in vivo*. В контроле использовали 40-мин окклюзию левой нисходящей коронарной артерии (ЛНКА) с последующим 60-мин восстановлением коронарного кровотока (реперфузией). ИП моделировали тремя циклами 5-мин окклюзии / 5-мин реперфузии ЛНКА перед длительной региональной ишемией миокарда и реперфузией. Метилин (5 мг/кг) вводили крысам внутривенно болюсом за 30 мин до окклюзии ЛНКА. Под действием ИП или метилина размер некротического повреждения левого желудочка, выраженный процентным отношением инфаркт миокарда/зона риска (ИМ/ЗР, %), составил в конце реперфузии 26,9±2,0% и 29,3±2,6% соответственно по сравнению с 43,8±1,2% в контроле ($p < 0,01$), активность креатинкиназы-МВ (КК-МВ) в плазме крови снижалась до 1026,1±93,9 МЕ/мл и 1195,2±142,0 МЕ/мл соответственно по сравнению с 1986,3±193,7 МЕ/мл в контроле ($p < 0,02$). Введение метилина, также как и ИП, способствовало увеличению сниженного содержания АТР, общего фонда адениннуклеотидов (ΣAN) и фосфокреатина (PCr) в ЗР в конце реперфузии по сравнению с контролем ($p < 0,05$–$0,01$). В группе метилина содержание общего креатина (ΣCr) в ЗР было более высоким, чем в контроле ($p < 0,05$). Внутривенное введение 5-гидроксидеканоата (5ГД) — ингибитора митохондриальных АТР-зависимых K^+-каналов (митоК$_{АТР}$) в дозе 5 мг/кг отменяло прекондиционирующее действие метилина, увеличивая отношение ИМ/ЗР, % и активность КК-МВ в плазме до значений, достоверно не отличающихся от контроля (39,4±2,8% и 2258,2±179,1 МЕ/мл соответственно). Одновременно 5ГД достоверно снижал содержание АТР и ΣAN в ЗР по сравнению с этими показателями в группе метилина и содержание АТР, ΣAN и PCr по сравнению с группой ИП. Результаты указывают на снижение ишемического/реперфузионного повреждения сердца с помощью фармакологического прекондиционирования метилином и участия митоК$_{АТР}$ в механизме действия метилина.

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

Ключевые слова: метилин; сердце крысы; прекондиционирование; инфаркт миокарда; креатинкиназа-МВ; энергетическое состояние зоны риска

Финансирование. Исследование выполнено при финансовой поддержке Минздрава России (Госзадание № 125022803024-3).

Поступила в редакцию: 07.10.2025; после доработки: 18.10.2025; принята к печати: 20.10.2025.