UDC 612.1+577

Mitochondria-derived H_2S mediates myocardial oxygen-sparing and mitochondria membrane integrity in ischemic preconditioning

Y.V. Goshovska, Y.P. Korkach, N.A. Strutynska, V.F. Sagach

Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv;
e-mail: goshovska@biph.kiev.ua

Ischemic preconditioning (IPC), brief episodes of ischemia and reperfusion, is known to activate endogenous protective mechanisms against ischemia-reperfusion (IR) injury. Despite extensive studies, the role of hydrogen sulfide (H_2S) produced in mitochondria by 3-mercaptopyruvate sulfurtransferase (3MPST) in IPC remains unknown. The aim of this study was to investigate the effect of o-carboxymethylhydroxylamine (OCA), a mitochondrial H_2S synthesis inhibitor, on recovery of cardiac function and mitochondrial membrane integrity after ex vivo IPC and IR. The experiment was performed on 44 male Wistar rats divided into four groups: control; IR; IPC + IR; and OCA (50 mg/kg, i.p.) + IPC + IR. After recording cardiac function using the Langendorff apparatus, cardiac mitochondria were isolated and subjected to a swelling test. Our results demonstrated that OCA reduced left ventricular developed pressure, dP/dt_{min}, and coronary flow, and simultaneously increased the oxygen cost of myocardial work. Moreover, OCA abolished the cardioprotective effect of IPC, impairing the recovery of contractile activity and enhancing inefficient oxygen utilization in the ischemic myocardium. IR mitochondria showed a 1.54-fold increase in swelling amplitude in Ca^{2+}-free medium, which was reduced by 15% in the IPC group. OCA increased the swelling amplitude 1.6-fold compared to IPC (even in Ca^{2+}-free medium) and 1.36-fold compared to IR. Notably, mitochondria from the OCA-treated group were insensitive to Ca^{2+} at all tested concentrations (10^{-4}-10^{-6} mol/l). These findings suggest that inhibition of mitochondrial H_2S production impairs IPC mechanisms responsible for preservation of energy production and proper Ca^{2+} handling during reperfusion. Thus, H_2S generated by 3MPST appears to play a crucial role in maintaining mitochondrial stability and mediating IPC-induced cardioprotection against IR injury. These results may have potential implications for pharmacological targeting of H_2S signaling pathways.

Keywords: ischemic preconditioning; heart; ischemia; mitochondria; hydrogen sulfide; 3-mercaptopyruvatesulfurtransferase; o-carboxy-methyl-hyrdoxylamine; mitochondrial permeability transition pore; oxygen consumption.

INTRODUCTION

In 2026, it marks the 40th anniversary since Murry, Jennings, and Reimer first reported the phenomenon of ischemic conditioning of the heart [1]. Paradoxically, a series of brief ischemic episodes, termed ischemic preconditioning (IPC), increases myocardial resistance to subsequent prolonged ischemia. IPC is a powerful cardioprotective strategy with potential clinical applications in organ transplantation, cardiac surgery, and other surgical interventions aimed at limiting ischemia–reperfusion (IR) injury caused by the restoration of oxygenated blood flow to previously ischemic tissues [2].

An enormous number of studies have been conducted to reveal the molecular mechanisms of IPC with the goal of their pharmacological recruitment. It is now well established that IPC involves multiple signaling pathways, including the Reperfusion Injury Salvage Kinase (RISK) pathway, the Survivor Activating Factor Enhancement (SAFE) pathway, AMP-activated protein kinase, protein kinase Cε signaling, HIF-1 signaling, and others [3, 4], most of which converge on the mitochondria.

It has been widely demonstrated that IPC provides its protective effects by preventing structural disintegration of mitochondria associated with massive opening of the nonspecific, cyclosporine A-sensitive mitochondrial permeability transition pore (MPTP) during reperfusion [5-8]. In IR, prolonged and extensive opening of the MPTP is triggered by high intracellular concentration of Ca^{2+} ions and reactive oxygen species (ROS) [8, 9]. Irreversible opening of the MPTP results in mitochondrial swelling, proton (H^+) leakage, dissipation of the proton gradient, and a sharp decline in mitochondrial membrane potential. Consequently, mitochondria shift to an uncoupled state, characterized by a dramatic reduction in ATP production and wasting of O_2 as ROS.

Energy deficiency impairs ATP-dependent channels and pumps, such as K^+-ATP channels, Na^+/K^+-ATPase, and SERCA, which are essential for maintaining intracellular ionic homeostasis. ATP depletion and alterations in ion concentration in different compartments of cardiomyocytes affect contractility and relaxation of IR myocardium. Antioxidants (such as coenzyme Q precursors, ferrostatin-1, and MitoQ), direct MPTP inhibitors (e.g., cyclosporin A), as well as IPC itself, prevent excessive mitochondrial swelling and energetic collapse at reperfusion [7, 9, 10]. In addition, inhibition of MPTP opening is essential for preventing cytochrome c release and apoptotic cell death in IR [11, 12].

Hydrogen sulfide (H_2S) is an endogenous gaseous transmitter that exerts multiple effects on the cardiovascular system. Under physiological conditions, H_2S participates in vasorelaxation by activating soluble guanylyl cyclase, which synthesizes cGMP from GTP and facilitates its activation by nitric oxide (NO) [13]. In mitochondria, H_2S acts as a modulator of mitochondrial function, stimulating bioenergetics through an increase in NAD^+ levels, contributing to O_2 sensing and mitochondria biogenesis [14, 15].

H_2S is produced by three enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MPST) along with cysteine aminotransferase (CAT). While CBS predominates in the brain and nervous system, CSE and 3MPST/CAT are localized in the heart and vascular endothelium [16, 17]. It has been demonstrated that H_2S produced by 3MPST/CAT supports basal physiological cellular bioenergetic functions [18, 19]. However, little is known about the physiological role of H_2S of mitochondrial origin. We hypothesized that H_2S produced by 3MPST/CAT within mitochondria plays an important role in the cardioprotective effect of IPC through modulation of the MPTP.

The aim of this study was to investigate the effect of o-carboxy-methyl-hydroxylamine (OCA), a mitochondrial H_2S synthesis inhibitor, on recovery of cardiac function and mitochondrial membrane integrity following ex vivo IPC and IR. Here, we demonstrate that inhibition of 3MPST/CAT with OCA attenuates the cardioprotective effect of IPC, impairing the recovery of Langendorff-perfused rat heart function after IR, and is associated with dysregulation of the MPTP and the ability of mitochondria to maintain Ca^{2+} homeostasis.

METHODS

All experiments were performed in accordance with the International Principles of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986), the Animal Protection Act No. 3447-IV (2006), and Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (22 September 2010). The study protocol was approved by the Bioethics Committee of Bogomoletz National Medical University (Protocol No. 1/26, dated 11 February 2026).

A total of 44 male Wistar rats, aged 6 months, with a mean body weight of 355 g were included in the study. Animals were kept on a standard vivarium diet at Bogomoletz Institute of Physiology (Kyiv, Ukraine), housed 4-5 per cage with free access to food and water. The animals were randomly assigned to four groups: group 1: IR (n = 14); group 2: IPC and IR (n = 12); group 3: o-carboxy-methyl-hydroxylamine hemihydrochloride (OCA), IPC and IR (n = 6); group 4: control (hearts were perfused on the Langendorff apparatus without any interventions, n = 12).

OCA (synonym: aminooxyacetic acid; "Sigma-Aldrich", USA, #C1340; 50 mg/kg), an inhibitor of mitochondrial H_2S production, was dissolved in 0.4 ml of 0.9% NaCl and administered intraperitoneally 30 min prior to sacrifice. The other groups received an equal volume of 0.9% NaCl. The study protocol is summarized in Fig. 1.

The Langendorff technique was performed as described previously [7]. Briefly, after decapitation and thoracotomy, the hearts were rapidly excised, the aorta was cannulated, and the coronary vessels were perfused under constant pressure with non-recirculating (37°C) Krebs–Henseleit buffer (mmol/l): NaCl – 118; KCl – 4.7; $MgSO_4$ – 1.2; $NaHCO_3$ – 24; KH_2PO_4 – 1.2; glucose – 10; $CaCl_2$ – 2.5; pH 7.4, aerated with a gas mixture (95% O_2, 5% CO_2).

Continuous monitoring of cardiac contractility was performed using a water-filled latex balloon inserted into the left ventricle and inflated to maintain the end-diastolic pressure (EDP) at 5-10 mmHg. The balloon was connected to a tensosensor ("Elema", Sweden), an analog-to-digital converter, and a PC equipped with Global Lab software. Left ventricular developed pressure (LVDP), EDP, the maximum (dP/dt_{max}) and minimum (dP/dt_{min}) values of the first derivative, and heart rate were calculated. Coronary flow was determined as the volume of perfusate passing through the heart per minute.

Oxygen tension in inflow ('arterial') and outflow ('venous') perfusate samples was measured using a gas analyzer (Radiometer BMS3Mk-2, Copenhagen, Denmark). Oxygen consumption was calculated according to the Neely formula [20]. The oxygen cost of myocardial work (OCMW) was expressed as the ratio of oxygen consumption to cardiac work (LVDP × heart rate).

After 20 min of equilibration, baseline parameters were recorded, and the hearts were subjected to the IR protocol with or without IPC (three cycles of 5 min ischemia followed by 5 min reperfusion) (Fig. 1). After 40 min of reperfusion, hearts were removed from the Langendorff apparatus, washed in cold (4°C) 0.9% KCl, and homogenized in isolation buffer (mmol/l): sucrose, 250; EDTA, 1; Tris-HCl, 25; pH 7.4. Mitochondria were isolated by differential centrifugation: first, at 700g for 8 min at 4°C to pellet nuclei and cell debris, followed by centrifugation of the supernatant at 11,000g for 16 min at 4°C to obtain the mitochondrial fraction. The mitochondrial pellet was resuspended in medium (mmol/l): sucrose – 250; Tris-HCl – 25; pH 7.2. Protein concentration in the mitochondrial suspension was measured by the Lowry method. After 30 min, the isolated mitochondria were used for a swelling test. Aliquots containing 0.4-0.5 mg protein per 1 ml were placed in an isotonic incubation medium (mmol/l): KCl – 120; Tris-HCl – 25;

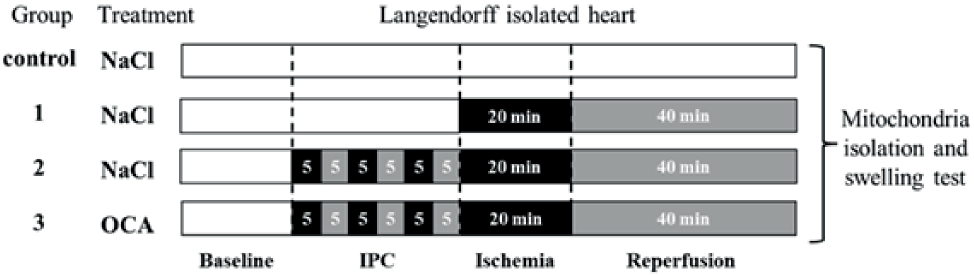

Fig. 1. Protocol of the study aimed to estimate the role of H_2S in ischemic preconditioning (IPC). OCA: o-carboxy-methyl-hyrdoxylamin, an inhibitor of mitochondrial synthesis of H_2S. NaCl: 0.9 % saline solution

KH_2PO_4 – 3; sodium succinate – 5; pH 7.4 (final volume, 3 ml). Changes in optical density (OD) were continuously recorded for 15 min at λ = 520 nm using a UV-1900 spectrophotometer (“Shimadzu”, Japan). Both Ca^{2+}-free and Ca^{2+}-induced mitochondrial swelling were assessed in each group. To evaluate mitochondrial sensitivity to Ca^{2+}, three concentrations of Ca^{2+} (as $CaCl_2$) were tested: 10^{-6}, 10^{-5}, and 10^{-4} mol/l. The change in OD amplitude (ΔOD) of swelling was calculated for each trace and then averaged.

Statistical analysis was performed using MS Excel and GraphPad Prism for Windows (“GraphPad Software”, USA). The Shapiro–Wilk test was used to assess the normality of data distribution. Comparisons were made using one-way ANOVA followed by Tukey’s HSD post hoc test or, for non-normally distributed data, the Kruskal–Wallis test with Mann–Whitney post hoc analysis. Differences were considered statistically significant at $P < 0.05$. Data are presented as mean ± SEM or as median with interquartile range.

RESULTS AND DISCUSSION

A total of nine cardiodynamic indices of Langendorff-isolated hearts were simultaneously recorded and analyzed. Figure 2 summarizes the baseline values. Statistical analysis revealed no significant differences between the baseline data of groups 1 and 2, confirming the homogeneity of the animals selected for the study.

Previously, we demonstrated that OCA administration reduced H_2S levels in blood plasma and cardiac mitochondria [21]. Therefore, OCA was used as a reliable tool to inhibit H_2S production. Pretreatment with OCA (group 3) suppressed the contractile activity of isolated hearts, as evidenced by a 31% decrease in LVDP (Fig. 2A) and a 15% reduction in dP/dt_{max} (data not shown). A pronounced decrease was observed in dP/dt_{min} (42%, Fig. 2B) and coronary flow (15.7%, Fig. 2C), this indicates impaired relaxation of the coronary vessels’ smooth muscles and the cardiomyocytes. Simultaneously, oxygen consumption by myocardium was lower by 19.8% than in group 1 (Fig. 2E). Although cardiac work was significantly reduced (Fig. 2D), the oxygen cost of myocardial work increased by 31%, indicating inefficient oxygen utilization in the myocardium of OCA-pretreated rats (Fig. 2F). These results indicate that H_2S inhibition by OCA depresses cardiac function and impairs myocardial oxygen utilization.

The dynamics of cardiac indices recovery is shown in Fig. 3. Analysis demonstrated that IPC significantly reduced post-ischemic disturbances. LVDP recovered to 79.6% of the pre-ischemic value by the 5th minute of reperfusion and remained stable throughout the observation period (85.3% at the 40th minute, Fig. 3A). A similar pattern was observed for dP/dt_{max}, which reached 85.3% at the 5th minute and 91% at the 40th minute of reperfusion (Fig. 3C), as well as for cardiac work (Fig. 3E).

IPC prevented post-ischemic myocardial stiffness, as indicated by nearly unchanged EDP; whereas in group 1, EDP sharply increased to 48.4 mmHg at the 5th minute and gradually declined to 28.3 mmHg at the 40th minute (Fig. 3B). IPC also substantially improved dP/dt_{min} (Fig. 3C) and coronary flow recovery (Fig. 3D), suggesting preserved energy supply, muscle relaxation, and Ca^{2+} handling in IPC hearts. Notably, IPC provided an oxygen-sparing effect: OCMW slightly changed during reperfusion, whereas in the IR group, OCMW increased dramatically, by 5.64-fold at the 5th minute, and remained at 192% of the pre-ischemic value at the 40th minute (Fig. 3F). Overall, IPC effectively limited IR-induced disturbances in isolated rat heart function and preserved energetic supply of myocardium contractility.

Pretreatment with OCA had a deleterious effect on the recovery of cardiac function. Notably, the frequency of post-ischemic myocardial fibrillation was 50% in Group 3, limiting the number of hearts available for analysis. In contrast, fibrillation occurred in 7% of group 1 and was absent in group 2 (0%).

OCA also impaired the reproducibility of IPC, as evidenced by reduced recovery of LVDP, dP/dt, and cardiac work, along with increased EDP during reperfusion (Fig. 3). Although these differences were not statistically significant compared with group 2, probably due to the small sample size, they also did not differ significantly from group 1, lying between the two datasets. The most pronounced effect of OCA was observed in coronary flow, which was 3-fold lower at the 5th minute of reperfusion compared with group 2. By the end of reperfusion, coronary flow recovered to levels similar to group 1 (Fig. 3D). These results indicate that the cardioprotective effect of IPC was substantially diminished by OCA pretreatment.

To evaluate mitochondrial integrity, membrane stability, and sensitivity to Ca^{2+}, mitochondria were isolated from hearts after completion of the Langendorff protocol and subjected to a swelling test. Fig. 4 demonstrates typical changes in the optical density (OD) of mitochondrial suspensions isolated from

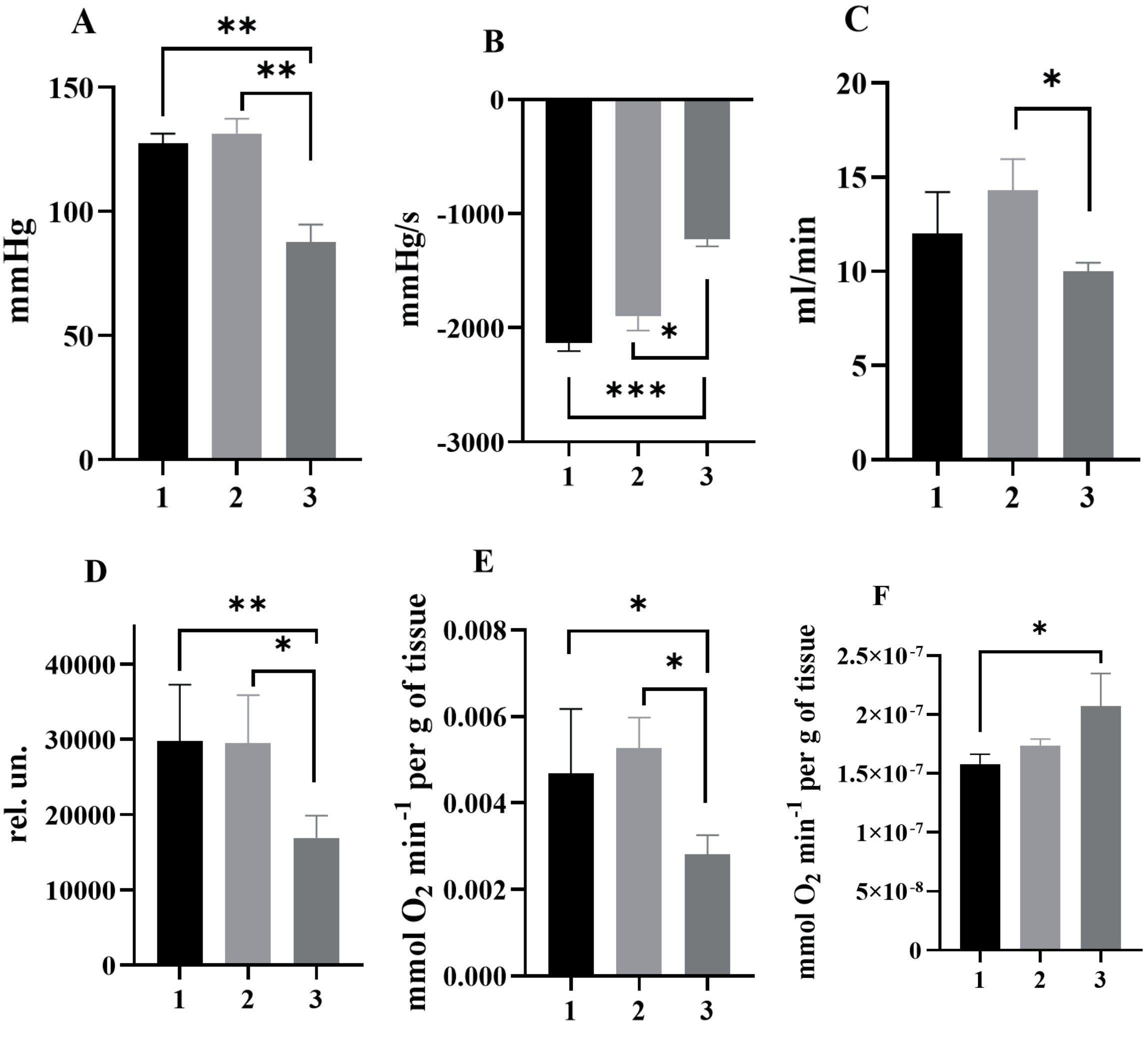

Fig. 2. Baseline cardiodynamic parameters of Langendorff-isolated rat hearts. A – left ventricular developed pressure (LVDP), B – dP/dt_{min}, C – coronary flow, D – LVDP×heart rate, E – oxygen consumption, F – oxygen cost of myocardial work. Groups: 1 – ischemia-reperfusion, 2 – ischemic preconditioning and ischemia-reperfusion, 3 – OCA, ischemic preconditioning and ischemia-reperfusion. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

control hearts in response to different Ca^{2+} concentrations.

Under normal conditions, mitochondria are capable of accumulating a certain amount of

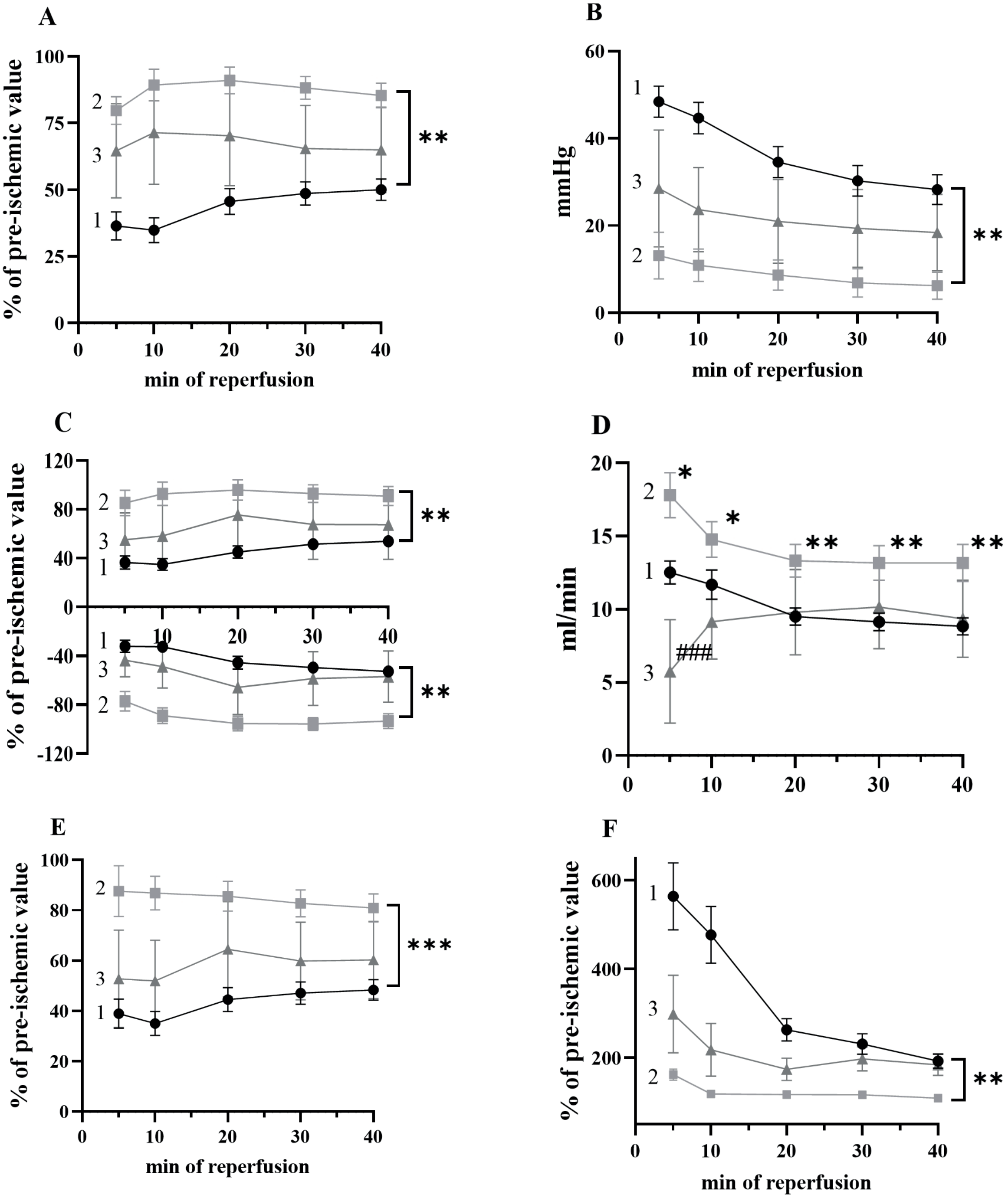

Fig. 3. Dynamics of post-ischemic recovery in Langendorff-isolated rat hearts. A – left ventricular developed pressure (LVDP), B – end-diastolic pressure, C – dP/dt, D – coronary flow, E – LVDP·Heart Rate, F – oxygen cost of myocardial work. Groups: 1 – ischemia-reperfusion, 2 – ischemic preconditioning and ischemia-reperfusion, 3 – OCA, ischemic preconditioning and ischemia-reperfusion. *$P < 0.05$ vs 1, **$P < 0.01$ vs 1, ***$P < 0.001$ vs 1, $^{\#\#\#}P < 0.001$ vs 2

Ca^{2+}; therefore, the OD changes in response to 10^{-6} and 10^{-5} mol/l Ca^{2+} were not dramatic. In contrast, a sharp decrease in OD observed in the presence of 10^{-4} mol/l Ca^{2+} indicates induction of MPTP opening and pronounced mitochondrial swelling (Fig. 4, curve 4). A swelling test was performed for each group; ΔOD values were calculated, averaged, and are presented in Fig. 5.

Interestingly, a significant difference in mitochondrial structural integrity was observed even in the absence of Ca^{2+} load, i.e., in Ca^{2+}-free medium. Mitochondria isolated from IR hearts demonstrated a 1.54-fold increase in swelling under Ca^{2+}-free conditions ($P < 0.05$), indicating altered permeability of the mitochondrial membranes.

One possible explanation is that Ca^{2+} from the cytosol had already been transported into mitochondria via the mitochondrial calcium uniporter (MCU), the principal pathway of pathological Ca^{2+} uptake in this model [22]. In addition, Ca^{2+} has been shown to inhibit H_2S synthesis by 3MST/CAT in a concentration-dependent manner, whereas in the absence of Ca^{2+}, H_2S production is maximal [23]. ROS also inhibits mitochondrial H_2S synthesis by oxidizing 3MST/CAT enzymes and downregulating their gene expression, thereby exacerbating H_2S deficiency in mitochondria [24].

These findings suggest negative feedback regulation of mitochondrial H_2S production by elevated Ca^{2+} and ROS during IR. Thus, IR induced deregulation of mitochondrial membrane integrity and irreversible MPTP opening, which was observed immediately in Ca^{2+}-free medium.

IPC reduced ΔOD of mitochondrial swelling in Ca^{2+}-free medium by 15% (n/s), as well as in Ca^{2+}-containing media (by 25, 12, and 11% at 10^{-6}, 10^{-5}, and 10^{-4} mol/l Ca^{2+}, respectively) (Fig. 5), indicating a higher Ca^{2+} threshold required for MPTP induction. These data confirm the protective effect of IPC against premature MPTP opening in response to elevated Ca^{2+} during IR.

Pharmacological inhibition of H_2S with OCA abolished the protective effect of IPC. The amplitude of mitochondrial swelling in group 3 was 1.6-fold higher than in group 2 ($P<0.01$) and 1.36-fold higher than in group 1 (n/s) under Ca^{2+}-free conditions. This finding indicates initial mitochondrial Ca^{2+} overload and engagement of H_2S produced via the 3MST/CAT

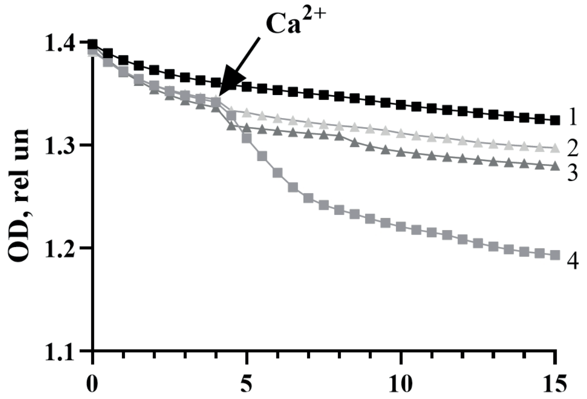

Fig. 4. Representative curves of optical density (OD) changes during the mitochondrial swelling tests. OD remained nearly unchanged in Ca^{2+}-free medium (curve 1), showed a slight decrease in response to 10^{-6} mol/l Ca^{2+} (curve 2) and 10^{-5} mol/l Ca^{2+} (curve 3), and decreased sharply in response to 10^{-4} mol/l Ca^{2+} due to MPTP opening and mitochondrial swelling (curve 4). The curves presented were obtained from mitochondria isolated from control rat hearts

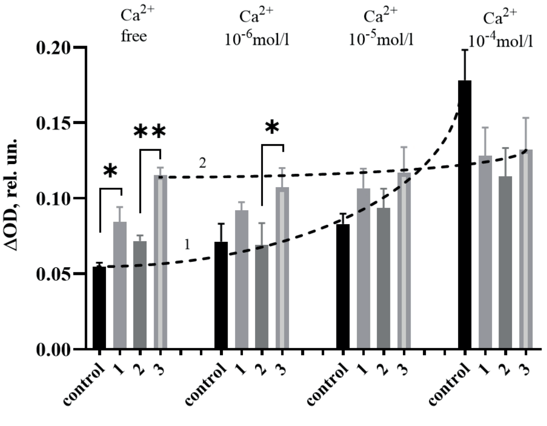

Fig. 5. Amplitude of mitochondrial swelling (ΔOD) in Ca^{2+}-free medium and in response to increasing Ca^{2+} concentrations. $^{*}P < 0.05$, $^{**}P < 0.01$. Groups: 1 – ischemia–reperfusion (IR); 2 – ischemic preconditioning (IPC) and IR; 3 – OCA, IPC and IR. IR induced ionic imbalance in mitochondria, resulting in increased ΔOD, whereas IPC reduced ΔOD. Control mitochondria exhibited a dose-dependent increase in ΔOD (dotted line 1), while group 3 demonstrated a Ca^{2+}-insensitive pattern (dotted line 2)

pathway in the protective mechanism of IPC.

H_2S, as an inorganic substrate, can serve as an electron donor for the mitochondrial respiratory chain and support ATP production under conditions of respiratory substrate deficiency [19]. Supporting this concept, exogenous H_2S donors have been shown to exert cardioprotective effects against IR injury by alleviating myocardial damage and providing antioxidant and anti-inflammatory actions [25, 26]. In our study, inhibition of H_2S synthesis by OCA likely reduced this emergency source of electrons, thus affecting energy production and diminishing the reproducibility of IPC-induced cardioprotection.

Fig. 5 clearly illustrates two distinct patterns of mitochondrial behavior in the swelling test, indicated by the dotted lines. In control hearts, OD changes show a dose-dependent increase, reflecting the classical Ca^{2+}-induced mitochondrial response. This indicates normal MCU activity and an intact capacity of mitochondria to store Ca^{2+}, with swelling occurring only at the highest Ca^{2+} concentration when the MPTP opens.

In group 3, depicted by the nearly horizontal dotted line, mitochondria from initially altered hearts exhibit loss of integrity and insensitivity to Ca^{2+} at all tested concentrations (Fig. 5). This likely results from the irreversibly opened MPTP, allowing free movement of Ca^{2+} across the mitochondrial membranes. This process was not accompanied by osmotic swelling due to increased membrane permeability and reduced barrier function, which may explain the absence of a sharp ΔOD drop in group 3 mitochondria in response to 10^{-4} mol/l Ca^{2+}.

Since recovery from ischemia requires enormous amounts of energy, preservation of mitochondrial coupling and ATP-producing capacity is a key factor ensuring cell survival and maintenance of myocardial function in the post-ischemic period [27]. IR induces structural and functional damage to cardiac mitochondria through excessive ROS production at complexes I and III. This, in turn, causes oxidative injury to these complexes and other components of the respiratory chain [22]. Elevated ROS levels together with increased cytosolic Ca^{2+} trigger irreversible MPTP opening, leading to deleterious consequences for cellular metabolism and energetics.

The protective effect of IPC is known to involve activation of multiple signaling cascades that converge on mitochondria. Several mitochondrial effectors of IPC have been identified as mediators of cardiac conditioning, including the mitoK_ATP channels [27, 28], uncoupling proteins (UCPs) [29–31], and connexin 43 [4].

In the present study, we propose that mitochondria-derived H_2S is involved in the protective cascades of IPC. We found that OCA, an inhibitor of 3MPST/CAT, affected the reproducibility of IPC, worsened relaxation of coronary vessels and myocardium, and attenuated the oxygen-sparing effect of IPC in the isolated heart. Furthermore, OCA abolished the membrane-stabilizing effect of IPC, increased sensitivity to Ca^{2+} overload, and disrupted mitochondrial Ca^{2+} handling.

With our work, we suggest that H_2S of mitochondrial origin, produced via the CAT/3MPST mechanisms, provides specific regulation of mitochondrial stability and at least partially contributes to the protective effect of IPC by inhibiting MPTP opening during reperfusion. This finding may be promising for future pharmacological application.

This work was supported and funded by the National Research Foundation of Ukraine [grant number 2021.01/0210], “The role of mitochondrial mechanisms in the development and prevention of cardiovascular dysfunctions in aging”.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

Ю.В. Гошовська, Ю.П. Коркач,
Н.А. Струтинська, В.Ф. Сагач

РОЛЬ МІТОХОНДРІАЛЬНОГО СІРКОВОДНЮ В ЕФЕКТИВНОСТІ ВИКОРИСТАННЯ КИСНЮ МІОКАРДОМ І ЗБЕРЕЖЕННІ ЦІЛІСНОСТІ МІТОХОНДРІАЛЬНИХ МЕМБРАН ПРИ ІШЕМІЧНОМУ ПРЕКОНДИЦІОНУВАННІ

Інститут фізіології ім. Богомольця НАН України, Київ;
e-mail: goshovska@biph.kiev.ua

Ішемічне прекондиціонування (ІП), чергування коротких епізодів ішемії та реперфузії, активує ендогенні механізми захисту від довготривалої ішемії-реперфузії (ІР). Незважаючи на безліч досліджень ІП, роль сірководню (H_2S), що утворюється в мітохондріях ферментом 3-меркаптопіруватсульфуртрансферазою (3MPST) при ІП, залишається невідомою. Метою нашої роботи було з’ясувати вплив інгібітора синтезу мітохондріального H_2S о-карбоксиметилгідроксиламіну (ОСА) на відновлення функції серця та цілісність мітохондріальної мембрани після ex vivo ІП та ІР. Експеримент було проведено на 44 самцях щурів лінії Вістар, розділених на 4 групи: контрольна і три дослідні. До 1-ї дослідної групи увійшли тварини з ІР; до 2-ї – з ІП та ІР; до 3-ї – з ІП та ІР, яким вводили ОСА (50 мг/кг, внутрішньоочеревинно). Після реєстрації показників кардіодинаміки, серця знімали з апарата Ландендорфа, гомогенізували, виділяли мітохондрії і вивчали їх набухання за наявності та без додавання Ca^{2+}. Показано, що ОСА чинив депресорний вплив на тиск у лівому шлуночку, dP/dt_{min}, коронарний потік, і при цьому достовірно збільшував кисневу вартість роботи міокарда. Крім того, зменшувався кардіопротекторний ефект ІП: погіршувалося відновлення скоротливої активності та збільшувалося неефективне використання кисню ішемізованим міокардом. У середовищі без Ca^{2+} амплітуда набухання мітохондрії після ІР була більшою у 1,54 раза, за умов ІП та ІР – на 15% нижча. Амплітуда набухання мітохондрій у групі з ОСА була у 1,6 раза більшою, ніж при ІП, та в 1,36 раза, ніж при ІР, навіть у середовищі без Ca^{2+}. Примітно, що ці мітохондрії не були чутливими до Ca^{2+} у жодній з досліджуваних концентрації (10^{-6} – 10^{-4} моль/л). Це свідчить про те, що пригнічення H_2S мітохондріального походження порушує механізми ІП, відповідальні за збереження продукції енергії та адекватну регуляцію кальцієвих транзієнтів мітохондріями. Таким чином, ми припускаємо, що продукція H_2S за допомогою 3MPST забезпечує унікальну регуляцію стабільності мітохондріальних мембран та є важливим фактором захисту функції серця від ІР-пошкодження за допомогою ІП. Це відкриття може бути перспективним для розробки фармакологічних антиішемічних засобів.

Ключові слова: ішемічне прекондиціонування; серце; ішемія; мітохондрії; сірководень; 3-меркаптопіруватсульфуртрансфераза; о-карбоксиметилгідроксиламін; мітохондріальна пора; споживання кисню.

REFERENCES

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74(5):1124-36.
2. Ji B, Liu M, Liu J, Wang G, Feng W, Lu F, Shengshou H. Evaluation by cardiac troponin I: the effect of ischemic preconditioning as an adjunct to intermittent blood cardioplegia on coronary artery bypass grafting. J Card Surg. 2007; 22 (5):394-400.
3. Sanada S, Komuro I, Kitakaze M. Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures. Am J Physiol Heart Circ Physiol. 2011 Nov;301(5):H1723-41. doi: 10.1152/ajpheart.00553.2011.
4. Jovanović A. Cardioprotective signaling: Outline and future directions. Biomedicines. 2025 Dec 3;13(12):2973. doi: 10.3390/biomedicines13122973.
5. Hausenloy DJ, Maddock HL, Baxter GF, Ye DM. Ingibiting mithochondrial permeability transition pore opening: a new paradigm for myocardial preconditiong? Cardiovasc Res. 2002; 55: 534-43.
6. Javadov SA, Clarke S, Das M, Griffiths EJ, Lim KHH, Halestrap AP. Ischaemic preconditioning inhibits opening of mithochondrial permeability transition pores in the reperfused rat heart. J Physiol. 2003; 549: 513-24.
7. Hoshovs’ka IuV, Shymans’ka TV, Rudyk OV, Korkach IuP, Sahach VF. Mitochondria permeability transition as a target for ischemic preconditioning. Fiziol Zh. 2011;57(4):34-45. doi: 10.15407/fz57.04.034. [Ukrainian].
8. Bauer TM, Murphy E. Role of mitochondrial calcium and the permeability transition pore in regulating cell death. Circ Res. 2020 Jan 17;126(2):280-93. doi: 10.1161/CIRCRESAHA.119.316306.
9. Mendoza A, Patel P, Robichaux D, Ramirez D, Karch J. Inhibition of the mPTP and lipid peroxidation is additively protective against I/R injury. Circ Res. 2024 May 10; 134(10): 1292-305. doi: 10.1161/CIRCRESAHA.123.323882.
10. Timoshchuk SV, Vavilova GL, Strutynska NA, Kuchmenko EB, Petukhov DN, Donchenko GV, Sagach VF. In vivo effect of precursors and modulator of coenzyme Q biosynthesis on sensitivity of mitochondrial permeability transition pore to inductors of its opening in old rats’ heart. Fiziol Zh. 2008; 54(3): 3-9. [Ukrainian].
11. Borutaite V, Jekabsone A, Morkuniene R, Brown GC. Inhibition of mitochondrial permeability transition prevents mitochondrial dysfunction, cytochrome c release and apoptosis induced by heart ischemia. J Mol Cell Cardiol. 2003 Apr;35(4):357-66. doi: 10.1016/s0022-2828(03)00005-1.
12. Chen Z, Chen J, Chen Y, Fang X. Opening of the mitochondrial permeability transition pore mediated myocardial damage after perinatal asphyxia in neonatal rats. Fetal Pediatr Pathol. 2025 May-Jun;44(3):177-95. doi: 10.1080/15513815.2025.2466804.

13. Zhou Z, Martin E, Sharina I, Esposito I, Szabo C, Bucci M, Cirino G, Papapetropoulos A. Regulation of soluble guanylyl cyclase redox state by hydrogen sulfide. Pharmacol Res. 2016 Sep; 111: 556-62. doi: 10.1016/j.phrs.2016.06.029.
14. Guo W, Kan JT, Cheng ZY, Chen JF, Shen YQ, Xu J, Wu D, Zhu YZ. Hydrogen sulfide as an endogenous modulator in mitochondria and mitochondria dysfunction. Oxid Med Cell Long. 2012;2012:878052. doi: 10.1155/2012/878052.
15. Paul BD, Snyder SH, Kashfi K. Effects of hydrogen sulfide on mitochondrial function and cellular bioenergetics. Redox Biol. 2021 Jan;38:101772. doi: 10.1016/j.redox.2020.101772.
16. Shibuya N, Mikami Y, Kimura Y, Nagahara N, Kimura H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. J Biochem. 2009 Nov;146(5):623-6. doi: 10.1093/jb/mvp111.
17. Szlęzak D, Hutsch T, Ufnal M, Wróbel M. Heart and kidney H2S production is reduced in hypertensive and older rats. Biochimie. 2022 Aug;199:130-8. doi: 10.1016/j.biochi.2022.04.013.
18. Módis K, Coletta C, Erdélyi K, Papapetropoulos A, Szabo C. Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. FASEB J. 2013 Feb; 27(2): 601-11. doi: 10.1096/fj.12-216507.
19. Szabo C, Ransy C, Módis K, Andriamihaja M, Murghes B, Coletta C, Olah G, Yanagi K, Bouillaud F. Regulation of mitochondrial bioenergetic function by hydrogen sulfide. Part I. Biochemical and physiological mechanisms. Br J Pharmacol. 2014 Apr;171(8):2099-122. doi: 10.1111/bph.12369.
20. Neely JR, Liebermeister H, Battersby EJ, Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol. 1967. 212: 804-14, doi:10.1152/ajplegacy.1967.212.4.804.
21. Luchkova AYu, Strutynska NA, Korkach YuP, Sagach VF. Inhibition of mitochondrial H2S synthesis increases oxidative stress and impairs the electron transport chain function. Fiziol Zh. 2018; 64(6): 9-16. doi: 10.15407/fz64.06.009. [Ukrainian].
22. Chen CL, Zhang L, Jin Z, Kasumov T, Chen YR. Mitochondrial redox regulation and myocardial ischemia-reperfusion injury. Am J Physiol Cell Physiol. 2022 Jan 1;322(1):C12-C23. doi: 10.1152/ajpcell.00131.2021.
23. Mikami Y, Shibuya N, Kimura Y, Nagahara N, Yamada M, and Kimura H. Hydrogen sulfide protects the retina from light-induced degeneration by the modulation of Ca2+ influx. J Biol Chem 286: 39379-86, 2011.
24. Kimura H. Production and physiological functions of hydrogen sulfide. Antioxid Redox Signal. 2014;20(5):783-804. doi: 10.1089/ars.2013.5309.
25. Shymans'ka TV, Hoshovs'ka IuV, Semenikhina OM, Sahach VF. Effect of hydrogen sulfide on isolated rat heart reaction under volume load and ischemia-reperfusion. Fiziol Zh. 2012;58(6):57-66. doi: 10.15407/fz58.06.057. [Ukrainian].
26. Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, Kimura H, Chow CW, Lefer DJ. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. Proc Natl Acad Sci USA. 2007 Sep 25;104(39):15560-5. doi: 10.1073/pnas.0705891104.
27. Strutynskyi RB. Protective properties of opening ATP-sensitive potassium channels. Fiziol Zh. 2019;65(3):73-85. doi: 10.15407/fz65.03.073. [Ukrainian].
28. Testai L, Rapposelli S, Calderone V. Cardiac ATP-sensitive potassium channels: a potential target for an anti-ischaemic pharmacological strategy. Cardiovasc Hematol Agents Med Chem. 2007 Jan;5(1):79-90. doi: 10.2174/187152507779315831.
29. Liu Y, Chen L, Xu X, Vicaut E, Sercombe R. Both ischemic preconditioning and ghrelin administration protect hippocampus from ischemia/reperfusion and upregulate uncoupling protein-2. BMC Physiol. 2009 Sep 22;9:17. doi: 10.1186/1472-6793-9-17.
30. Mehta SL, Li PA. Neuroprotective role of mitochondrial uncoupling protein 2 in cerebral stroke. J Cerebr Blood Flow Metab. 2009 Jun;29(6):1069-78. doi: 10.1038/jcbfm.2009.4.
31. Hoshovs'ka IuV, Shymans'ka TV, Sahach VF. Genipin – uncoupling protein inhibitor – reduces the protective effect of ischemic preconditioning. Fiziol Zh. 2011;57(6):38-45. PMID: 22420157. doi: 10.15407/fz57.06.038. [Ukrainian].

Received 14.01.2026