UDC 577.35.4 doi: http://dx.doi.org/10.15407/ubj87.06.086

PROTECTIVE EFFECTS OF POTASSIUM TRANSPORT IN MITOCHONDRIA FROM RAT MYOMETRIUM UNDER ACTIVATION OF MITOCHONDRIAL PERMEABILITY TRANSITION PORE

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We demonstrated using PBFI K^+-sensitive fluorescent probe an enhancement of both components of K^+-cycle – the ATP-sensitive K^+-uptake and quinine-sensitive K^+/H^+-exchange – under the Ca^{2+} induced opening of mitochondrial permeability transition pore (MPTP) in rat myometrium mitochondria. Addition of $CaCl_2$ (100 μM) to K^+-free medium results in the enhancement of reactive oxygen species (ROS) production, which was eliminated by cyclosporine A. Addition of $CaCl_2$ to K^+-rich medium did not increase the rate of ROS production, but blocking of mitoK^+_{ATP}-channels with glybenclamide (10 mcM) increased production of ROS. We conclude that K^+-cycle exerts a protective influence in mitochondria from rat myometrium by regulation of matrix volume and rate of ROS production under the condition of Ca^{2+}-induced MPTP.

K e y w o r d s: Ca ions, MPTP, glybenclamide, K^+_{ATP}-channels, K^+/H^+-exchanger, mitochondria.

Mitochondria play an important role in regulation of cell life and death as they are involved in realization of the most important functions, such as providing of energy substrates and control over cell death [1]. The inner membrane of mitochondria is encrusted with respiratory chain complex and ATP-synthase, which produces ATP, using energy of $\Delta\mu_{H+}$ in the process. The inner membrane of mitochondria is thus impermeable to ions, and their matrix homeostasis is regulated via functioning of ion transporting systems of channels and exchangers [1, 2]. The mitochondrial ion homeostasis is a finely tuned process, imbalances in it may adversely affect mitochondrial functions and may provoke cell death. Particularly, matrix overload with Ca^{2+} is known to induce cyclosporine A (CsA) – sensitive mitochondrial permeability transition pore (MPTP), that exists in either high or low conductance state. The high-conductance state causes inner membrane rupture followed by cell death. Imbalanced Ca^{2+} homeostasis and MPTP induction caused by it are associated with a number of pathologies such as neurodegenerative diseases and disorders of heart, smooth and skeletal muscles [3, 4].

Mitochondria are capable of maintaining normal functioning under adverse conditions [5]. K^+ transport across the inner membrane may be one of the factors that help to sustain the mitochondrial function within physiological margins [6]. K ions homeostasis is maintained by potassium channels, which provide for accumulation of the ion in mitochondria, and by K^+/H^+-exchanger, which releases K^+ in cytosol in exchange for H^+. The latter's function servers to maintain stable matrix volume and mitochondrial membranes' integrity. Potassium channels, i.e. mitochondrial K^+_{ATP}-channels (mitoK^+_{ATP}-channels) regulate matrix volume, function of electron-transport chain, as well as indirectly affect F_0F_1-ATPase and generation of reactive oxygen species (ROS) [2, 7]. For instance, Costa et al. [8] demonstrated, that cooperative activity of mitoK^+_{ATP}-channel and K^+/H^+-exchanger in muscle mitochondria is responsible for creating and supporting a new equilibrium matrix volume. K^+ influx through the mitoK^+_{ATP}-channel causes alkalinization of matrix that mediates increased ROS production [9]. The last effect, in particular, is perceived as key to cytoprotective mitoK^+_{ATP}-channel effects under MPTP activation. For example, Costa et al. [8] showed, that mitoK^+_{ATP}-channel activation indirectly prevents MPTP opening through increased ROS production and consequent protein kinase C activation, which is one of the key enzymes protecting cells from death [10]. These effects are probably involved in the phenomenon of preconditioning – the protective influence of series of short ischemic periods prior to prolonged ischemia [7, 10]. Cell damage under adverse conditions, namely oxidative stress, has been proven

to include the disruption of Ca^{2+} homeostasis, which in turn causes necrotic or apoptotic cell death [11]. There is also data supporting activation and cytoprotective effects of mitoK^+_{ATP}-channels under increased cytosol Ca^{2+} concentration and induction of MPTP opening [12].

Nevertheless, the mechanism underlying cell protection by mitoK^+_{ATP}-channel has not been studied thoroughly. The mitoK^+_{ATP}-channels' priority role over K^+_{ATP}-channels of plasma membrane in preconditioning remains a speculative subject as well. The influence of activation of K^+ transport in smooth muscle mitochondria on ROS generation has not been investigated. Also, there is no data as to specifics of functioning of mitoK^+_{ATP}-channels from myometrium under stress conditions, such as Ca^{2+} overload. Therefore, we pursued the aim to investigate, using probes and selective blockers of K^+_{ATP}-channels and MPTP, the effect of Ca^{2+} on K^+ transport, dynamics of changes in matrix volume and ROS generation in isolated rat myometrium mitochondria.

Materials and Methods

Isolation of mitochondrial fraction from myometrium. Mature female white rats (150-200 g body mass) were anesthetized with ethyl ether and decapitated. Uterus tissue was cleaned from blood and fat, minced and homogenized on ice in 8 ml of isolation buffer solution of the following composition: 250 mM sucrose, 1 mM EDTA, 10 mM HEPES (pH 7.2 titred by 2 M Tris). The homogenate was centrifuged for 7 min at 1000 g and 4 °C. The supernatant was centrifuged for 7 min. at 12 000 g and 4 °C. The sediment of mitochondria was resuspended in isolation buffer without EDTA and stored on ice. Protein content was determined using Bradford assay.

Concentrated glibenclamide and CsA solutions in dimethyl sulfoxide were applied introduced into incubation medium in cuvette at 1 µl per 1.8 ml of working volume (final concentration of 10 µM). $CaCl_2$ solution was introduced in the cuvette to final concentration of 100 µM.

Measurement of K^+ influx in mitochondria loaded with PBFI (potassium-binding benzofuran isophthalate) probe. The mitochondria were loaded with PBFI probe as described [8]. The sediment after the second centrifugation was resuspended in 500 µl of buffer solution containing 250 mM sucrose, 10 mM HEPES (pH 7.2, 25 °C), 10 mM pyruvate. The suspension was incubated with 40 µM acetoxymethyl ether of PBFI (PBFI-AM) and 0.5 µl of 20% solution of F-127 non-ionic surfactant for 10 min at room temperature. The suspension was then mixed for 2 min with 500 µl of buffer for K ions substitution in mitochondrial matrix, consisting of 175 mM sucrose, 10 mM HEPES (pH 7.2 at 25 °C), 5 mM succinate, 5 mM Na_2HPO_4 1 mM $MgCl_2$, and 50 mM tetraethyl ammonium. 7 ml of mitochondria isolation buffer was then added to the mix, followed by centrifugation for 10 min at 12000 g and 4 °C. The sediment was resuspended in buffer with 250 mM sucrose and 10 mM HEPES (pH 7.2 at 4 °C and no EDTA added) and kept on ice. Probe fluorescence was registered at 340 nm and 380 nm wavelength of excitation and 480 nm wavelength of emission. Final protein concentration in a sample was 55-60 µg/ml.

The measurements were performed with PTI Quanta Master 40 spectrofluorometer (Canada) in a standard fluorometric cuvette in thermostatic sample holder with magnetic stirrer at 28 °C in standard incubation medium consisting of 10 mM HEPES (pH 7.2 at 28 °C titred by 2 M Tris), 125 mM KCl, 5 mM Na_2HPO_4, 1 mM $MgCl_2$, 5 mM succinate, 5 µM rotenone, 2 µg/ml oligomycin. KCl was replaced with NaCl on equimolar basis in certain samples. Final protein concentration in a sample was 55-60 µg/ml.

Mitochondria swelling was measured by registering side lateral light scattering at 520 nm in standard incubation medium (see above) as described [8]. The signal registration begun 1 s after the added mitochondria suspension had been introduced in the incubation medium.

ROS generation was assayed with ROS-sensitive 2′,7′-dichlorofluorescein diacetate probe (DCF-DA). Probe solution was introduced into incubation medium, and the readings were taken immediately. Final probe concentration was 4 µM. Fluorescence excitation wavelength was 540 nm, and emission was registered at 520 nm 1 s after mitochondria suspension had been introduced into the incubation medium.

The results were analyzed and plotted with Microcal Origin v. 5.0 Software (Microcal Software, USA). Data was evaluated with Student's *t*-test, the difference between groups was considered significant if $P < 0.05$.

We used the following reagents: Na_2HPO_4, $MgCl_2$ – of local origin, of chemically pure grade; ATP, diazoxide, sucrose, HEPES, KCl, rotenone, oli-

gomycin, succinate, CsA, ruthenium red, glibenclamide, DCF-DA – by Sigma–Aldrich (USA), $CaCl_2$ (1 M solution), Tris, EDTA, NaCl – by Fluka (Switzerland), PBFI-AM by Molecular Probes (USA).

Results and Discussion

Mitochondria isolation in sucrose-rich K^+-free medium leads to a markedly decreased content of these cations in matrix. Afterwards, as the mitochondria are introduced into incubation medium containing KCl, P_i anions, and respiratory chain substrates, they begin to uptake K^+ intensively, which has been demonstrated by various methods, including fluorescent spectroscopy with potassium-sensitive PBFI probe [8]. In order to ensure the adequacy of the probe's response to K^+ in isolated mitochondria from the rat myometrium, we studied intensity of fluorescence PBFI loaded in mitochondria depending on KCl concentration in incubation medium. Introduction of mitochondria loaded with PBFI into the incubation medium with various KCl concentrations (see Materials and Methods) led to dose-dependent increase in PBFI fluorescence, which testifies to the adequacy of response of the probe to K^+ accumulation in mitochondrial matrix (Fig. 1, *A*). The maximum probe's response was registered at KCl concentrations close to physiologic (125 mM), and consequently this concentration was used in the standard incubation medium. K^+ influx into mitochondrial matrix in these experimental conditions is by diffusion due to high membrane potential, and by K^+-transport channels (ATP-sensitive, in particular) on the inner mitochondrial membrane [13]. As has been demonstrated in numerous publications, the functioning of mitoK^+_{ATP}-channels is blocked by ATP [8]. ATP in concentration of 200 μM in the standard incubation medium inhibited K^+ accumulation in myometrium mitochondria as well (Fig. 1, *B*, *8*). The inhibiting effect of ATP was totally eliminated by mitoK^+_{ATP}-channels activator diazoxide (Fig. 1, *B*, *9*), which is also in accordance with data from experiments performed on mitochondria from other tissues [2, 8, 14]. Thus, our results corroborate the existence of K^+_{ATP}-channels in the rat myometrium mitochondria.

As has been mentioned by various authors, the pathological conditions inhibiting normal muscle tissue and cell functioning are mediated by disruptions in Ca^{2+} homeostasis, increase in its cytosol concentrations, sometimes by orders of magnitude above the physiological values, and may lead to negative consequences for the myocyte and the muscle in general [11]. Hence, the understanding of possible mechanisms underlying myocytes protection under damaging conditions with increased cytosol Ca^{2+} is an important research task. The possibility of regulation of K^+_{ATP}-channels by Ca^{2+} has been demonstrated [15]. Since the activation of these channels exhibits a cytoprotective effect in conditions associated with imbalanced Ca^{2+} homeostasis, it is interesting to investigate their functional properties in smooth muscle mitochondria under high Ca^{2+} concentration. K^+ accumulation in matrix was found to decrease in incubation medium with 100 μM of $CaCl_2$ (Fig. 1, *B, 2*). Ca^{2+}-induced decrease in K^+ accumulation was eliminated by 0.5 mM EGTA, a Ca^{2+}-chelator (Fig. 1, *B*, *4*). The data presented on Fig. 1, *B,* support the assumption that this influence of Ca^{2+} on K^+ accumulation in myometrium mitochondria is mediated via Ca^{2+}-uniporter. The inhibiting effect of Ca^{2+} on K^+ accumulation was eliminated by 10 μM of ruthenium red, an inhibitor of Ca^{2+}-uniporter (Fig. 1, *B*, 3).

The blockade of total accumulation of K^+ in mitochondria may be caused by either partial inhibition of K^+ uptake into the matrix or by activation of K^+ release. Diazoxide, an activator of K^+_{ATP}-channels, in concentration of 50 μM did not exert any effect on K^+ accumulation in the presence of 100 μM of $CaCl_2$ (Fig. 1, *B*, *6*). Lack of activation of K^+ accumulation by diazoxide in our conditions may have at least two explanations: a) K^+_{ATP}-channels are already in active state in the presence of Ca^{2+}, or b) K^+_{ATP}-channels are inhibited, and the activator cannot affect their functional state. Since introduction of 10 μM glibenclamide, an inhibitor of K^+_{ATP}-channels, in the presence of Ca^{2+} restores fluorescent signal to control levels, it evidences in favor of our first assumption (Fig. 1, *B*, *5*). We have demonstrated the specificity of inhibiting effect of glibenclamide on K^+ transport in myometrium mitochondria in particular in our previous work [16]. We have also demonstrated inhibition by glibenclamide of ATP-sensitive K^+ transport in myometrium mitochondria with PBFI probe (Fig. 1, *B*, *10*). This tendency of increase in PBFI signal in the presence of K^+_{ATP}-channels inhibitors allowed us to formulate a hypothesis of simultaneous activation of mitoK^+_{ATP}-channels and K^+/H^+-exchanger, which was confirmed in further experiments with quinine, an inhibitor of K^+/H^+-exchanger. Namely, quinine, when introduced in 0.5 mM concentration, restored K^+ to values higher than that of the control

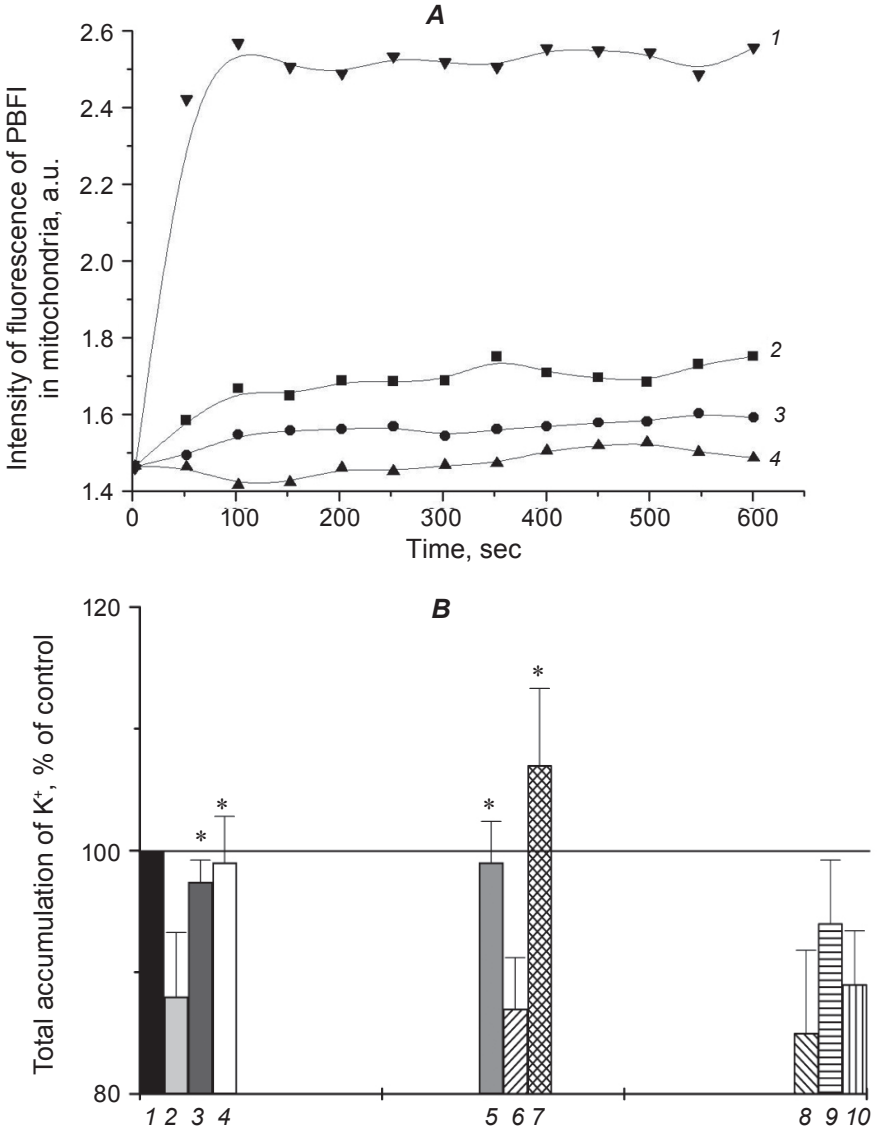

*Fig. 1. K ions accumulation in isolated mitochondria from myometrium under various conditions. A, fluorescence of PBFI probe loaded in isolated mitochondria depending on KCl concentration in incubation medium; 1 – 125 mM KCl, 2 – 50 mM KCl, 3 – 10 mM KCl, 4 – 50 mM choline chloride (in cases 2, 3 and 4 the incubation medium contained also sucrose, the final osmolarity was 250 mOsm/l). B, effect of Ca^{2+}, inhibitors of K^+ transport and Ca^{2+} uniporter on total accumulation of potassium ions in isolated mitochondria; 1 – K^+ accumulation in standard incubation medium, 2 – K^+ accumulation in the presence of 100 μM $CaCl_2$, 3 – 100 μM $CaCl_2$ and 10 μM ruthenium red, 4 – 100 μM $CaCl_2$ and 0.5 EGTA, 5 – 100 μM $CaCl_2$ and 10 μM glibenclamide, 6 – 100 μM $CaCl_2$ and 50 μM diazoxide, 7 – 100 μM $CaCl_2$ and 0.5 mM quinine, 8 – K^+ accumulation in standard incubation medium in the presence of ATP 200 μM, 9 – with addition of ATP 200 μM and diazoxide 50 μM, 10 – with addition of ATP 200 μM, diazoxide 50 μM and glibenclamide 10 μM. * Denotes significant differences from bar 2 ($P < 0.05$)*

(Fig. 1, *B*, *7*). We can thus assume that activation of mitoK^+_{ATP}-channels is coupled with activation of K^+/H^+-exchanger. Therefore, we can assume from these results, that in our conditions we observe potassium cycle activation in the presence of calcium in mitochondria of myometrium, particularly K^+ influx into matrix through the K^+_{ATP}-channels and release the K^+/H^+-exchanger, which is eliminated by the specific inhibitors – glibenclamide, ATP and quinine. Garlid et al. argue that the regulation of mitochondrial ma-

trix volume is the main function of potassium cycle under physiological conditions, which may also protect mitochondria under stress [2, 14]. It is known that increased mitochondrial volume is a condition for activation of the K^+/H^+-exchanger as a participant in potassium cycle. The activation of the potassium cycle helps to establish new matrix volume equilibrium, yet it is the K^+/H^+-exchanger that maintains integrity of mitochondrial membranes [2, 8]. Basing on our data we assume that potassium cycle activation, and the K^+/H^+-exchanger in particular, may be involved in maintenance of integrity of myometrium's mitochondrial membranes under Ca^{2+} overload and induction of MPTP.

It has been demonstrated that opening of Ca^{2+}-induced MPTP may result in uncontrolled increase in matrix volume followed by outer membrane rupture and induction of apoptosis or necrosis [11]. The change in mitochondrial volume resulting from MPTP opening may be observed by registering of side light scattering at 520 nm [17]. Introduction of mitochondrial suspension in the standard incubation medium with 100 μM $CaCl_2$ (see Material and Methods section) caused a noticeable drop in lateral light scattering in comparison to control (Fig. 2, *A*). According to our results, ruthenium red (10 μM), CsA (10 μM) and EGTA inhibited Ca^{2+}-induced swelling of mitochondria (Fig. 2, *A*). The Ca^{2+}-sensitivity of changes in lateral light scattering of mitochondria suspension leads to the following conclusions: *a*. Ca^{2+}-induced swelling of mitochondria is mediated by transport of Ca^{2+} into matrix through mitochondrial uniporter as it is inhibited by ruthenium red and EGTA, a Ca^{2+} chelator; *b*. this process is mediated by MPTP induction as it is inhibited by CsA, which is an MPTP inhibitor.

One of the functions of mitochondrial potassium cycle and the mitoK^+_{ATP}-channels has been presumed to be matrix volume regulation [2]. We hence investigated the possible involvement of the mitoK^+_{ATP}-channels in Ca^{2+}-induced swelling of mitochondria from myometrium. We found that glibenclamide, an inhibitor of mitoK^+_{ATP}-channels, in concentrations of 10 μM partially inhibited the changes in lateral light scattering of mitochondrial suspension induced by Ca^{2+} (Fig. 2, *B*). It must be noted that the inhibitor itself did not affect the process in any way in the absence of Ca ions (data not shown). Our data are in accordance with the results of Jaburek et al. [18], who propose the existence of various functional states for mitoK^+_{ATP}-channels with differing sensitivity to inhibitors. Also, glibenclamide did not affect the change of mitochondrial volume in K^+-free medium (replaced equimolarly with Na^+) (Fig. 2, *B*). These results may indicate that changes in light scattering of mitochondria suspension in the presence of 100 μM $CaCl_2$ are caused by two processes: the induction of CsA-sensitive MPTP and the swelling of mitochondria due to K^+ influx through potassium channels, i.e. glibenclamide-sensitive mitoK^+_{ATP}-channels. Costa et al. suppose that one of the main physiological effects of mitoK^+_{ATP}-channels activation on mitochondrial function is the increase in stable matrix volume, and the protective effect against damage to the cell that is ascribed to these channels results directly from regulation of mitochondrial volume [8].

ROS are by-products of cellular metabolism and are produced as a result of functioning of mitochondrial electron-transport chain, among other processes. They participate in intracellular signal pathways, regulating numerous processes under physiological conditions, including gene expression and muscle contraction. Nevertheless, under pathological conditions associated with imbalance in intracellular Ca^{2+} homeostasis the increased ROS generation leads to MPTP opening followed by necrosis or apoptosis [11]. Taking into account the cytoprotective effect of mitoK^+_{ATP}-channels activation under conditions arising from increased concentration of Ca^{2+} [3, 17], we also aimed to investigate the effect of K^+ transport in mitochondria of myometrium on the rate of ROS generation in the presence of Ca^{2+}. Under standard conditions (Ca^{2+}-free) ROS generation does not result in MPTP opening, as introduction of CsA, an inhibitor of MPTP, does not affect the process in any way (data not shown). It is worth mentioning that in the incubation medium containing K^+ the rate of ROS generation was higher than in the potassium-free medium (replaced by equimolar Na^+) (Fig. 3), which is in accordance with data by others [19] that activation of K^+ influx in mitochondria potentiates ROS generation in heart mitochondria. While addition of 100 μM $CaCl_2$ did not cause further increase in ROS generation in the medium containing K^+, it did result in higher ROS generation in the potassium-free medium, and these changes were eliminated by CsA (data not shown). Glibenclamide (10 μM) potentiates rate of ROS generation in the standard medium with KCl and did not affect the process in the potassium-free medium (Fig. 3), which proves yet again the specificity of its

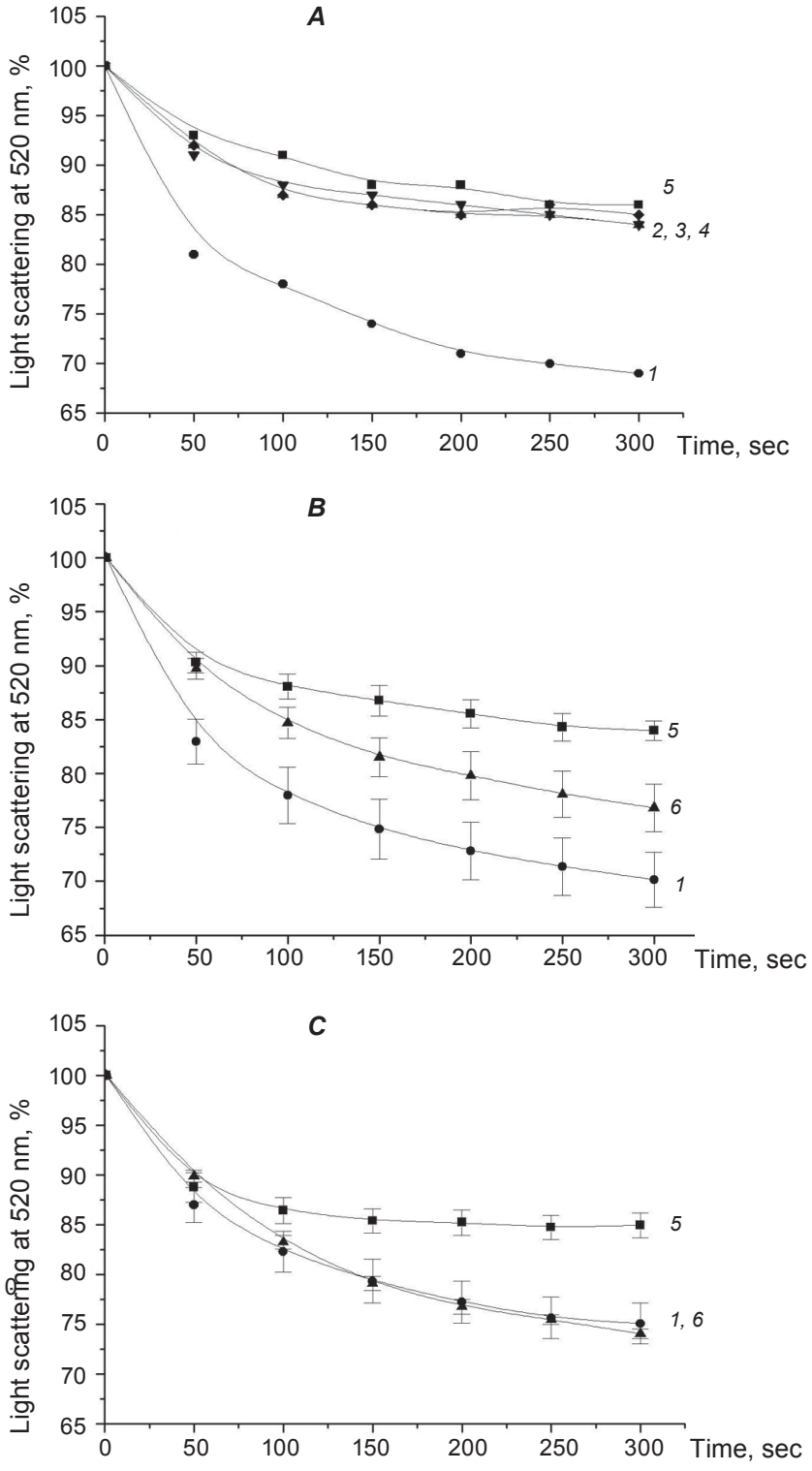

Fig. 2. Mitochondria swelling induced by $CaCl_2$. Dynamics of changes in light scattering of mitochondria suspension in the presence of: A, blockers of Ca^{2+} transport and MPTP (result of a typical experiment are shown); B, K^+_{ATP}-channels channels inhibitors in standard medium; C, K^+_{ATP}-channels channels inhibitors in K^+-free medium with KCl replaced by NaCl on equimolar basis. 1 – incubation with $CaCl_2$ (100 μM); 2 – incubation with $CaCl_2$ (100 μM) and ruthenium red (10 μM); 3 – incubation with $CaCl_2$ (100 μM) and CsA (10 μM); 4 – incubation with $CaCl_2$ (100 μM) and EGTA (0.5 mM); 5 – control (standard medium with no alterations); 6 – incubation with $CaCl_2$ (100 μM) and glibenclamide (10 μM)

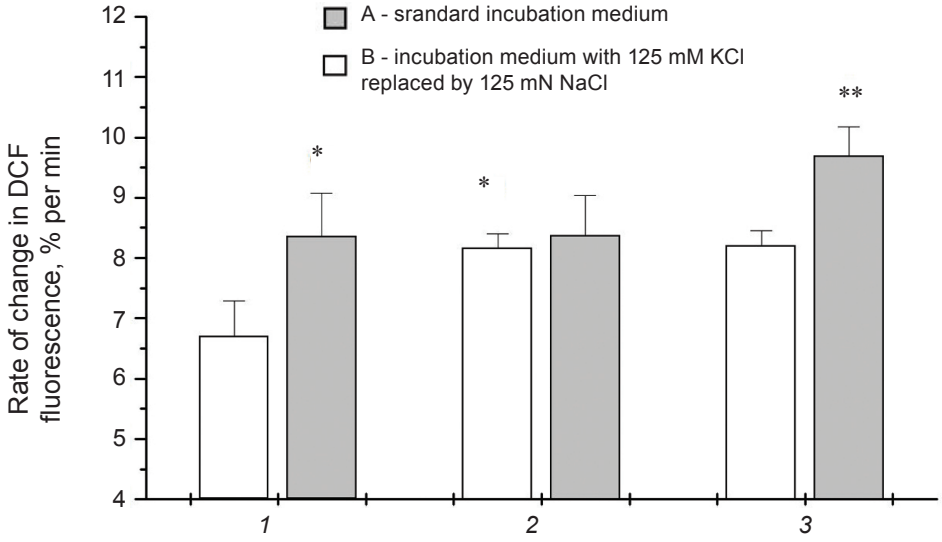

*Fig. 3. Effect of Ca^{2+} on rate of increase in fluorescence of DCF, a ROS-sensitive probe, in mitochondria isolated from the myometrium. 1 – control conditions; 2 – with addition of 100 μM $CaCl_2$; 3 – with addition of 100 μM $CaCl_2$ and 10 μM glibenclamide. * denotes changes that are significant in comparison to 1B, ** in comparison to 2A; $P < 0.05$*

effect on K^+ transport in mitochondria isolated from the myometrium. Thus, MPTP induction under inhibited K^+ transport results in the increased matrix ROS generation rate, while activation of K^+ transmembrane transport may protect the mitochondria to an extent from the damage caused by overproduction of ROS under opening of MPTP, which corroborates the data by Facundo et al., who demonstrated that activation of mitoK^+_{ATP}-channels of cardiomyocytes decreases ROS generation in the mitochondria in response to increased local oxidant levels [14].

Therefore, the obtained results lead to conclusion that Ca^{2+} induces opening of CsA-sensitive MPTP in mitochondria from the myometrium, followed by matrix swelling and increased rate of ROS generation. We suppose that both components of potassium cycle in the myometrium mitochondria – accumulation in matrix, including influx through mitoK^+_{ATP}-channels, and release through K^+/H^+-exchanger – are activated under the induction of MPTP. Activation of K^+ influx into the matrix intensifies ROS generation under normal conditions and inhibits it under induction of MPTP. While the induction of MPTP is associated with swelling of mitochondria and accumulation of cytotoxic ROS, the activation of potassium cycle, on the other hand, regulates the rate of ROS generation and matrix volume, thus preventing membrane rupture and overproduction of damaging ROS.

ПРОТЕКТОРНИЙ ЕФЕКТ ТРАНСПОРТУ K^+ В МІТОХОНДРІЯХ МІОМЕТРІЯ ЩУРІВ ЗА УМОВ ВІДКРИВАННЯ МІТОХОНДРІАЛЬНОЇ ПОРИ ПЕРЕХІДНОЇ ПРОНИКНОСТІ

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Методом флуоресцентної спектроскопії з використанням калійчутливого зонда було показано, що за індукції іонами Са циклоспоринчутливої мітохондріальної пори перехідної проникності відбувається активація обох компонент калієвого циклу в мітохондріях міометрія щурів – АТР- та глібенкламідчутливого входу K^+ в матрикс та хінінчутливого K^+/H^+-обміну. У присутності $CaCl_2$ (100 мкМ) в безкалієвому середовищі інкубації спостерігали зростання швидкості утворення активних форм кисню, яка пригнічувалась блокатором мітохондріальної пори циклоспорином А. У середовищі зі 125 мМ KCl внесення $CaCl_2$ (100 мкМ) не призводило до зростання швидкості утворення активних форм кисню. Але присутність блокатора АТР-чутливих калієвих каналів глібенкламіду (10 мкМ) в середовищі інкубації знімала протекторну дію іонів К на

підвищення продукції активних форм кисню в мітохондріях міометрія. Зроблено висновок, що в умовах відкривання Ca^{2+}-індукованої мітохондріальної пори калієвий цикл виявляє протекторний ефект у мітохондріях міометрія щурів, регулюючи об'єм матриксу та швидкість утворення АФК.

Ключові слова: іони Са, циклоспоринчутлива мітохондріальна пора, глібенкламід, $K^+_{АТР}$-канали, K^+/H^+-обмін, мітохондрії.

ПРОТЕКТОРНЫЙ ЭФФЕКТ ТРАНСПОРТА K^+ В МИТОХОНДРИЯХ МИОМЕТРИЯ КРЫС В УСЛОВИЯХ ОТКРЫВАНИЯ МИТОХОНДРИАЛЬНОЙ ПОРЫ

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Методом флуоресцентной спектроскопии с использованием K^+-чувствительного зонда было показано, что в условиях индукции ионами Са митохондриальной поры активируются обе компоненты К-цикла в митохондриях миометрия крыс – АТР- и глибенкламидчувствительный вход K^+ в матрикс и хининчувствительный K^+/H^+-обмен. В присутствии $CaCl_2$ (100 мкМ) в бескалиевой среде инкубации наблюдали увеличение скорости образования АФК, которое нивелировалось циклоспорином А. В KCl-содержащей среде внесение $CaCl_2$ (100 мкМ) не приводило к повышению скорости образования АФК. Но в присутствии блокатора $K^+_{АТР}$-каналов–глибенкламида (10 мкМ) – в среде инкубации протекторный эффект K^+ на генерацию АФК в митохондриях миометрия не наблюдался. Сделан вывод, что в условиях открывания Ca^{2+}-индуцированной циклоспоринчувствительной поры K^+-цикл оказывает протекторный эффект в митохондриях миометрия крыс, регулируя объем матрикса и скорость образования АФК.

Ключевые слова: ионы Са, циклоспоринчувствительная митохондриальная пора, глибенкламид, $K^+_{АТР}$-канали, K^+/H^+-обмен, митохондрии.

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Received 13.05.2015