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ATP-SENSITIVE POTASSIUM TRANSPORT IN RAT BRAIN MITOCHONDRIA IS HIGHLY SENSITIVE TO mK_{ATP} CHANNELS OPENERS: A LIGHT SCATTERING STUDY

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The aspects of ATP-sensitive K^+ transport regulation by mitochondrial K^+,ATP-sensitive (mK_{ATP}) channels openers are important for understanding the properties of these channels. The effect of K_{ATP} channels openers (KCOs) diazoxide and pinacidil on ATP-sensitive K^+ transport in isolated brain mitochondria was studied in the absence and the presence of MgATP using light scattering technique. Without MgATP we observed high sensitivity of ATP-sensitive K^+ transport to both drugs with full activation at ≤ 0.5 μM. ATP-sensitive K^+ transport was specifically blocked by ATP in the presence of Mg^{2+}. Neither Mg^{2+} nor ATP affected V_{max} of ATP-sensitive K^+ transport activated by KCOs, but MgATP shifted the activation curve to micromolar scale. The blockage of ATP-sensitive K^+ transport by K_{ATP} channels blockers glibenclamide and 5-hydroxydecanoate in the absence and the presence of MgATP proved the sensitivity of ATP-sensitive K^+ transport to the blockers of mK_{ATP} channel. Full activation of mK_{ATP} channel by diazoxide and pinacidil on sub-micromolar scale in the absence of MgATP was shown. The sensitivity of ATP-sensitive K^+ transport to the known modulators of mK_{ATP} channel (diazoxide, pinacidil, glibenclamide, 5-HD and MgATP) proved the identity of ATP-sensitive K^+ transport with mK_{ATP} channel activity. Based on our studies, we hypothesized that mK_{ATP} channel might comprise high affinity sites for KCOs binding screened by MgATP. The results of this work reveal novel not described earlier aspects of the regulation of ATP-sensitive K^+ transport by mK_{ATP} channels openers, important for understanding of mK_{ATP} channel properties.

K e y w o r d s: mitochondrial K_{ATP} channel, brain, potassium transport, diazoxide, pinacidil.

Mitochondrial ATP-sensitive K^+ transport driven by ATP-sensitive potassium channels (mK_{ATP} channels) afford cytoprotection in different cell types under several pathophysiological and metabolic stress conditions [1, 2]. In central nervous system, mK_{ATP} channel was shown to be a promising target for the treatment of neurodegenerative diseases [3]. Under ischemic and hypoxic conditions pharmacological mK_{ATP} channels openers (KCOs) used for mK_{ATP} channel activation effectively prevented the development of apoptosis and necrosis in the neurons [4-6]. However, in spite of the well established beneficial therapeutic effects of pharmacological mK_{ATP} channels openers, the progress in the understanding the molecular background of their action was prevented by unknown molecular composition of mK_{ATP} channels, their different tissue-specific isoform distribution, the lack of knowledge about molecular mechanisms of the channel interaction with pharmacological mK_{ATP} channel openers and several side effects of these drugs [7].

ATP-sensitive potassium transport appears to be an all-round modulator of mitochondrial functions: the respiration, matrix volume, ATP synthe-

sis, Ca^{2+} transport and ROS production [1, 2, 4]. As it was shown in multiple studies, neuro- and cardioprotection conferred by mK_{ATP} channels openers was largely based on bioenergetic effects of ATP-sensitive K^+ transport, which under pathophysiological conditions improved mitochondrial functions, reduced mitochondrial calcium loading and prevented the damage of mitochondrial Ca^{2+} overload, such as the opening of permeability transition pore. The modulation of mitochondrial ROS production by ATP-sensitive K^+ transport and triggering of cytoprotective signaling in several studies suppressed the development of apoptosis and cell death in neurons and other cell types [2, 4, 8].

Diazoxide and pinacidil are most often used pharmacological mK_{ATP} channels openers. By analogy with plasmalemmal K_{ATP} channels, it is generally supposed that MgATP is required for the opening of mK_{ATP} channels and the activation of ATP-sensitive K^+ transport by these drugs [9, 10]. However, published data obtained on isolated mitochondria are controversial, and the activation of ATP-sensitive K^+ transport by KCOs was shown as well without MgATP [11-13]. Besides, it needs to be considered that Mg^{2+} severely suppresses potassium cycle (K^+ uptake and K^+/H^+-exchange) known to underlie bioenergetic effects of mK_{ATP} channels opening [14]. Thus the aim of this work was to study the effect of diazoxide and pinacidil on the ATP-sensitive K^+ transport in brain mitochondria in the absence of MgATP.

Materials and Methods

Mitochondrial preparations. The work has been carried out in accordance with "Guide for the Care and Use of Laboratory Animals" 8th ed. Washington, DC: National Research Council of the National Academies: The National Academic Press, 2011 approved by the Ethics Commission on Animal Experiments of A.A. Bogomoletz Institute of Physiology, NAS of Ukraine. Adult Wistar-Kyoto female rats with 180-200 g mean body weight were used. Brain was washed by cold 0.9% KCl solution (4°C), minced and homogenized in 1:5 volume of the isolation medium: 250 mM sucrose, 1 mM EDTA, 1 mg/ml BSA, 20 mM Tris-HCl buffer, 4°C (pH 7.2). Mitochondria were isolated by centrifugation for 7 min at 1000 g, 4°C and after the pellet have been discarded; supernatant was centrifuged again for 15 min at 12000 g, 4°C. Final pellet was resuspended in a small volume of isolation medium without EDTA and stored on ice. The protein content was determined by the Lowry method.

The study of potassium transport. Light scattering is known to decrease because of mitochondrial swelling due to obligatory water uptake during potassium transport into matrix [10]. Initial rates of potassium transport (V_0) were found by monitoring light scattering at 520 nm excitation/emission wavelengths in 1 cm^3 cell starting from the addition of mitochondria at 0.3 mg/ml in standard incubation medium: 120 mM KCl, 0.5 mM EDTA, 5 mM sodium glutamate, 1 mM KH_2PO_4, 5 mM Tris-HCl buffer (pH 7.4), oligomycin (1 μg/mg protein). When required, K^+ was isotonically replaced by Na^+. Dependent on the conditions, $MgCl_2$ (1 mM), ATP (0.3 mM), glibenclamide (5 μM), and 5-HD (100 μM), were added. Diazoxide and pinacidil were added to the standard incubation medium at concentrations required. In the presence of Mg^{2+} EDTA was replaced by EGTA.

Oxygen consumption assay. Oxygen consumption was studied polarographically in 1 cm^3 closed termostated cell at 26°C with platinum electrode at constant stirring in the same standard incubation medium. Mitochondria were added at 1.5-2.0 mg/ml protein.

Chemicals. All reagents were from Sigma-Aldrich, USA. Deionized water was used for medium preparations.

Statistical analysis. The data were expressed as mean ± S.E. of 4-6 independent experiments. Statistical analysis was performed using paired Student's *t*-test; $P < 0.05$ was taken as the level of significance.

Results and Discussion

Light scattering is considered as one of the most sensitive techniques to assess potassium transport in mitochondria because potassium entrance to the matrix, as well as potassium efflux from mitochondria is accompanied by water translocation in stoichiometric proportions [10, 14]. This enables reliable monitoring of both stages of potassium cycle, potassium uptake and K^+/H^+ exchange as we have shown earlier [15]. Thus in this work we used light scattering to study the effects of diazoxide and pinacidil on the potassium uptake in native isolated mitochondria, in the absence of Mg^{2+} and ATP. Representative curves reflecting the effect of diazoxide on the mitochondrial matrix volume and the effects of both drugs on mitochondrial respiration are shown on Fig. 1.

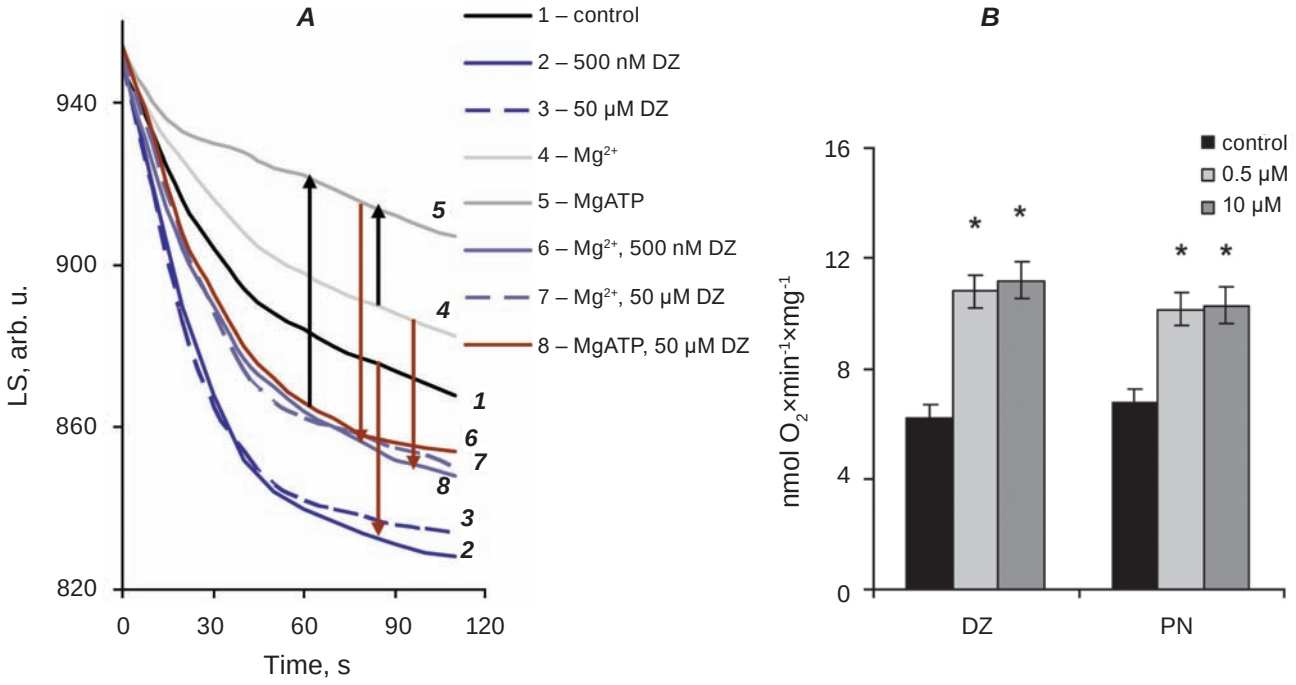

Fig. 1. The effect of diazoxide on the matrix volume and state 4 respiration of rat brain mitochondria. ***A*** *– representative curves reflecting the time courses of light scattering of mitochondria suspensions after the following additions to standard incubation medium: control, no additions (1), 500 nM DZ (2), 50 μM DZ (3), Mg^{2+} (4), MgATP (5), Mg^{2+}, 500 nM DZ (6), Mg^{2+}, 50 μM DZ (7), MgATP, 50 μM DZ (8);* ***B*** *– the effect of mKATP channels openers on state 4 respiration. M ± m, n = 4; *P < 0.05 as compared to control (incubation medium). The activation of K^+ transport by DZ and the blockage by ATP in the presence of Mg^{2+} are shown by the arrows*

The data of Fig. 1 gave evidence of high sensitivity of brain mitochondrial K^+ transport to sub-micromolar concentrations of mK_{ATP} channels openers. Thus, maximal swelling was reached in the presence of as low as 500 nM of diazoxide and was not enhanced further by the increase of diazoxide concentration up to high micromolar level (50 μM) (Fig. 1, *A*, 1-3). Mg^{2+} partially suppressed matrix swelling, which was further reduced to the minimum by the addition of ATP (Fig. 1, *A*, 4, 5). While the effect of Mg^{2+} on mitochondrial volume could be explained by its ability to block several types of K^+ channels [16], the ATP-sensitive difference in swelling rates and swelling amplitude was reported to reflect the activity of mK_{ATP} channel, which in the energized mitochondria is in its open state [9, 10]. In the presence of Mg^{2+} the stimulatory effect of diazoxide on K^+ uptake remained, and the addition of diazoxide at 0.5 μM increased matrix volume to the maximum possible in the presence of Mg^{2+} (Fig. 1, *A*, 5). This could not be exceeded either by further increasing concentration of diazoxide up to 50 μM (Fig. 1, *A*, 6), or by conventional block of K_{ATP} channel by MgATP with consequent reactivation by the high micromolar concentrations of diazoxide, about 30 μM (Fig. 1, *A*, 7, 8). The same effects were observed with other K_{ATP} channels opener pinacidil (not shown).

Our observations on the activation of potassium transport were supported by monitoring state 4 respiration under the same conditions. Both diazoxide and pinacidil stimulated mitochondrial respiration on glutamate at the same nanomolar concentrations of the drugs (Fig. 1, *B*). The effects were specific for K^+ based medium, and were not repeated in Na^+ based medium (not shown). In K^+ based medium, added at high micromolar concentrations (10 μM), diazoxide and pinacidil were unable of further respiration stimulation. This agreed with our earlier observations on liver mitochondria [15]. Considering the existence of stoichiometric relations between the rates of respiration and potassium transport [17], and reliable increase in matrix swelling shown by the light scattering, the experiments clearly testified increase in potassium uptake in brain mitochondria caused by sub-micromolar concentrations of K_{ATP} channels openers.

To assess the sensitivity of mitochondrial potassium transport towards K_{ATP} channels openers, the concentration dependence of the effects of these drugs on K^+ uptake was studied. For this purpose, the normalized initial rates of potassium transport $R_n = (V_0 - V_{0min})/(V_{0max} - V_{0min})$ were plotted against a logarithm of KCOs concentration (Fig. 2). As the experiments have shown, diazoxide and pinacidil were similarly effective in the stimulation of K^+ transport (Fig. 2, *A*). By our estimations based on the transformation of the concentration dependences to linearized Hill plots, the half-maximal activation of potassium transport in brain mitochondria by diazoxide (EC_{50}) was of the order of ~160 nM, and for pinacidil this value too was as low as ~130 nM. These data well coincided with EC_{50} ~140 nM for the activation of potassium transport in liver mitochondria by diazoxide in the absence of MgATP found in our earlier work [15]. Thus, the experiments gave convincing evidence that without MgATP K^+ transport in native isolated brain mitochondria, similar to native liver mitochondria, could be activated by mK_{ATP} channels openers with high affinity, showing full activation on sub-micromolar scale.

In the presence of an MgATP complex, a shift of the sensitivity of K^+ transport to much higher micromolar concentrations of mK_{ATP} channel openers was observed, and full activation was conventionally

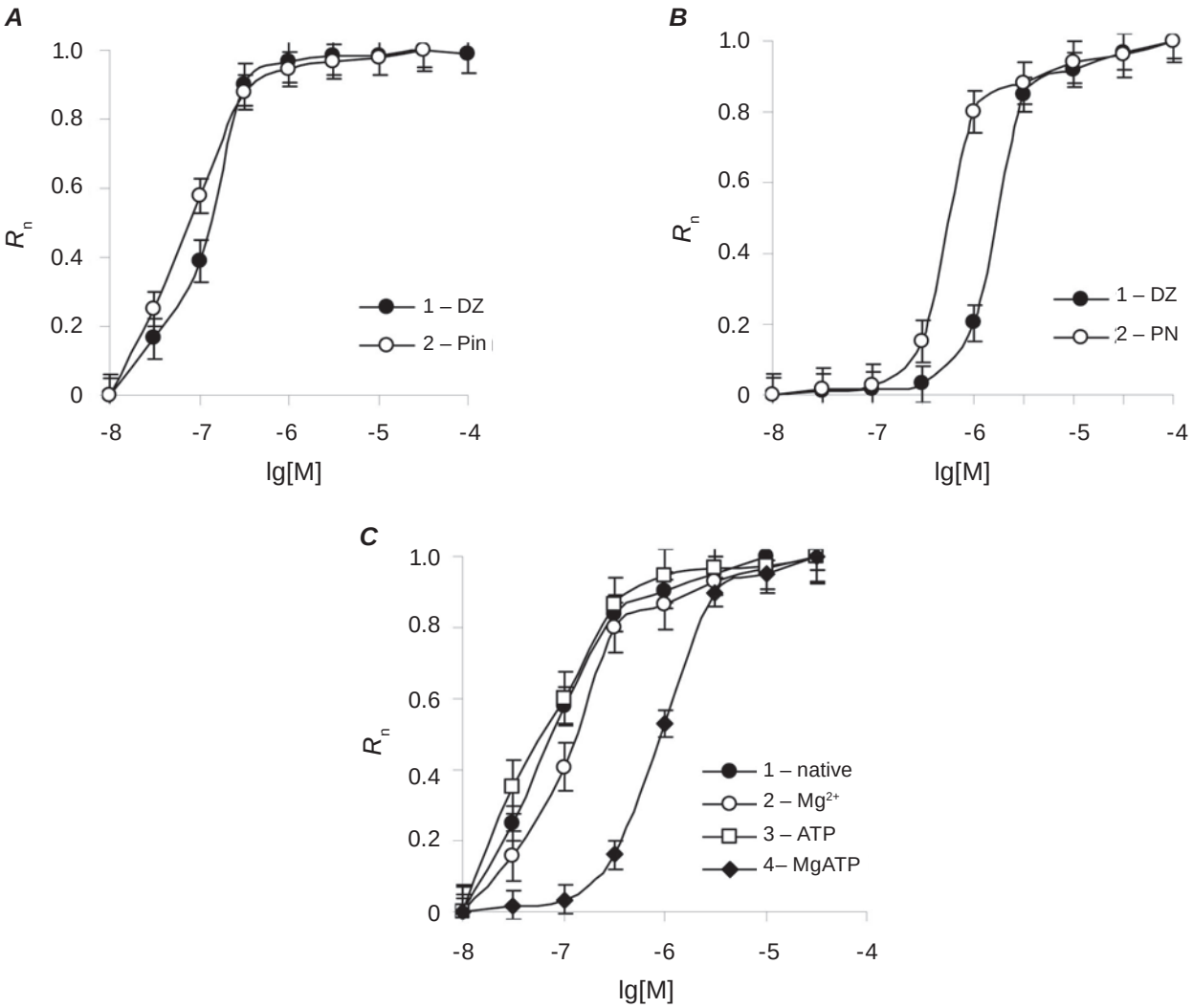

*Fig. 2. The effect of mKATP channels openers on the V_0 of potassium transport in the absence (**A**) and the presence (**B**) of MgATP;* **C** *– the effect of MgATP on the V_0 of K^+ transport stimulated by pinacidil (1 – control, no additions, 2 – Mg^{2+}, 3 – ATP, 4 – MgATP). M ± m, n = 9. On the abscissa axis – logarithm of the drugs concentration, [M], on the ordinate axis – the normalized rates of potassium transport*

reached only at ≥ 1 μM of the drugs (Fig. 2, *B*, 1, 2). As it is known, in the presence of MgATP only ATP-sensitive potassium transport could be specifically activated by diazoxide and pinacidil [10]. With MgATP, obtained concentration dependences were close to the literary data reporting the affinity of mK_{ATP} channel to these drugs [9]. So, it was of interest to find which of the individual components of an MgATP complex was responsible for the observed decrease in the sensitivity of potassium transport to mK_{ATP} channels openers. With this aim, we studied the effect of pinacidil on potassium transport in the presence of individual Mg^{2+} and ATP (Fig. 2, *C*).

As we have observed, ATP-sensitive K^+ transport was inhibited by ATP only in the presence of Mg^{2+} with IC_{50} ~ 15 μM ATP (Fig. 3); no inhibition by either Mg^{2+} (1-3 mM) or ATP alone (up to 0.3 mM ATP) was observed, which agreed with the properties of mK_{ATP} channel described in the literature [18]. Thus, under our experimental conditions Mg·ATP complex formed from 1 mM of added Mg^{2+} and 300 μM of added ATP (which correspond to 255 μM of MgATP complex) was in excess and fully sufficient to block mK_{ATP} channel. Full blockage of native mK_{ATP} channel by MgATP was confirmed by the additions of glibenclamide and 5-HD, which were unable of further blockage of K^+ transport in the presence of MgATP (not shown). Mg^{2+} at concentrations ≤3 mM partially reduced V_0 of total K^+ transport, but was of no effect on ATP-sensitive K^+ transport either native or activated by KCOs (Fig. 1, *A*, 1 vs. 4, 2-3 vs. 6-7). Without Mg^{2+}, ATP at ≤300 μM was of no effect on potassium uptake, and respectively mK_{ATP} channel activity in native mitochondria, which too agreed with the published data showing no effect of ATP on mK_{ATP} channel activity in the absence of Mg^{2+} [18]. Neither Mg^{2+}, nor ATP alone affected the sensitivity of K^+ transport to diazoxide (not shown) and pinacidil (Fig. 2, *C*, 1-3), and the shift to lower affinity was observed only in the presece of the complex MgATP (Fig. 2, *C*, 4). Thus, the experiments indicated that MgATP complex, but not individual components of it, were responsible for the observed decrease in the sensitivity of K^+ transport to diazoxide and pinacidil (Fig. 2, *A*-*C*).

So, to ascertain the activation of ATP-sensitive K^+ transport in the absence of MgATP more specifically, we blocked mK_{ATP} channel, native and activated by mK_{ATP} channel opener, sequentially by Mg^{2+} and MgATP monitoring the effects of mK_{ATP} channels opener on the ATP-sensitive and ATP-insensitive components of K^+ transport (Fig. 4, *A*). Also, the effects of KCOs on ATP-sensitive K^+ transport were assessed using pharmacological blockers

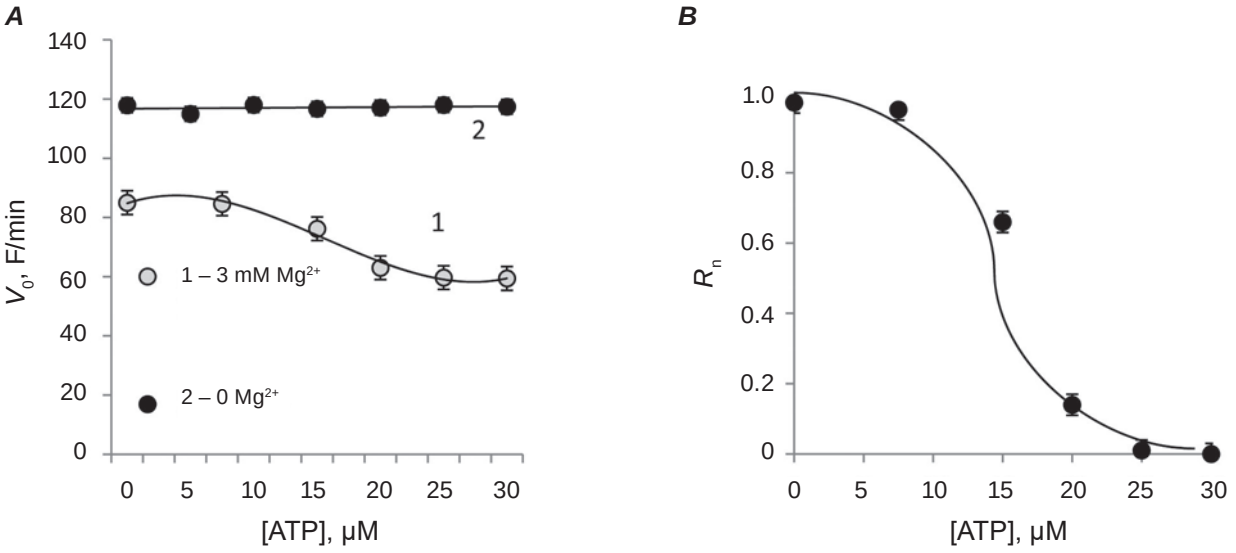

Fig. 3. The effect of ATP on the initial rate of K^+ transport: ***A*** *– in the presence of 3 mM Mg^{2+} (1) and the absence of Mg^{2+} (2);* ***B*** *– normalized rates of potassium transport in the presence of 3 mM Mg^{2+}. M ± m, n = 4. On the abscissa axis – ATP concentration, [μM], on the ordinate axis – the normalized rates of potassium transport*

of mK_{ATP} channel glibenclamide and 5-hydroxydecanoate (5-HD). In these experiments mK_{ATP} channel opener pinacidil was used. According to the literature [9, 10], the activation of the channel by micromolar concentrations of mK_{ATP} channels openers, such as diazoxide and pinacidil, in the presence of MgATP and consequent channel blockage by glibenclamide (or 5-HD) reflect full mK_{ATP} channel activity. Thus, the absolute differences in V_0 of K^+ transport after its blockage by ATP in the presence of Mg^{2+}, activation by micromolar concentrations of pinacidil in the presence of MgATP, and specific blockage of the activated mKATP channel by glibenclamide and 5-HD, were assumed to reflect the contribution of mK_{ATP} channel to the total K^+ transport [10].

From the experiments, we have found that in the native mitochondria ATP essentially blocked K^+ transport in the presence of Mg^{2+}, which could be ascribed to the blockage of native mK_{ATP} channel activity. This ATP-sensitive transport was reliably activated by pinacidil (shown by the arrow, Fig. 4, *A*), which agreed with similar effect of diazoxide (Fig. 1, *A*, 4-5 vs.4-6) and gave evidence for the activation of the native ATP-sensitive K^+ transport by mK_{ATP} channels openers. Selective mK_{ATP} channel activation by high micromolar concentrations of pinacidil up to 50 μM in the presence of MgATP gave the

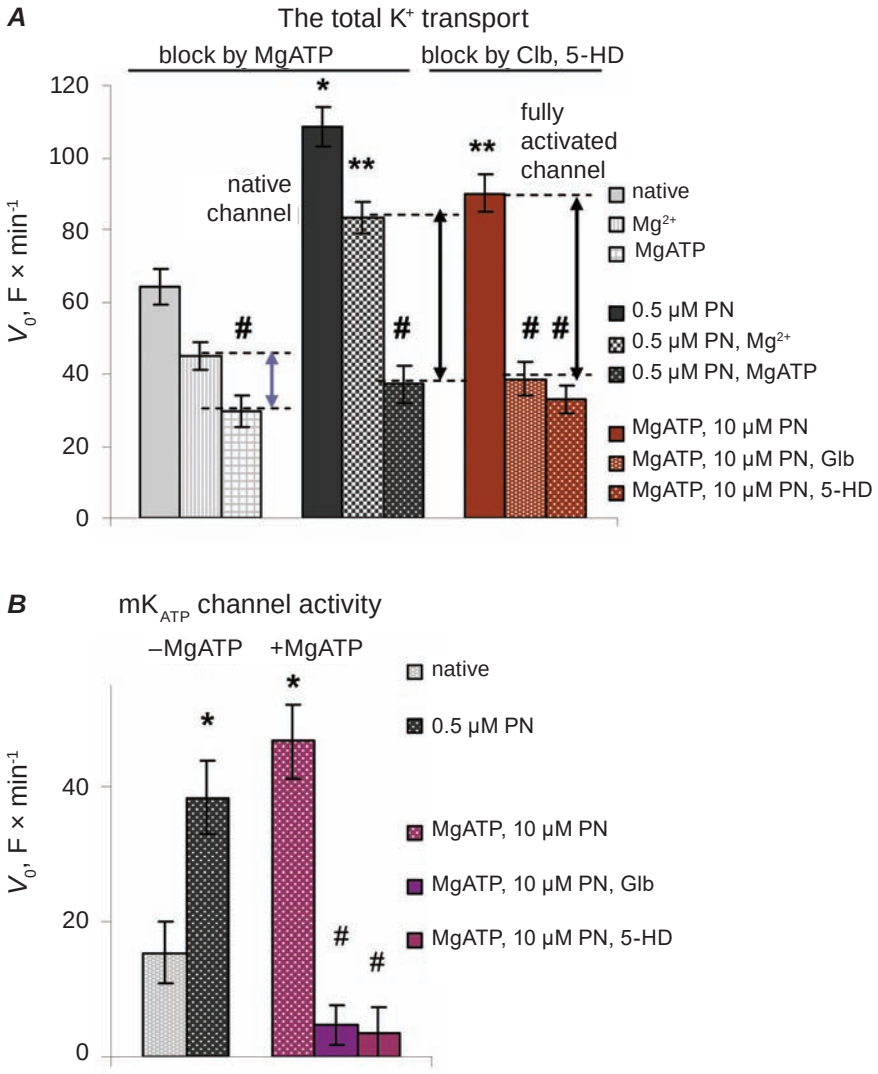

*Fig. 4. The effects of pinacidil and mK_{ATP} channels blockers (glibenclamide and 5-HD on the total (**A**) and ATP-sensitive K^+ transport (**B**). Control: incubation medium, no additions; other additions were as in the figure legend. $M \pm m$, $n = 6$; *$P < 0.05$ as compared to control; **$P < 0.05$ as compared to 0.5 μM pinacidil; #$P < 0.05$ as compared to MgATP + pinacidil*

estimate of full mK_{ATP} channel activity close to the estimate of fully activated ATP-sensitive K^+ transport (Fig. 4, *A*, shown by the arrows). Our observations were confirmed by consequent blockage of the activated channel by glibenclamide and 5-HD in the presence of MgATP (Fig. 4, *A*), which confirmed the above estimate of maximal mK_{ATP} channel activity. Meanwhile the component of K^+ transport blocked by Mg^{2+} only was not reliably affected by pinacidil (Fig. 4, *A*), which proved the ability of mK_{ATP} channels opener to specifically elicit full activation of ATP-sensitive K^+ transport on nanomolar concentration scale without MgATP.

As showed our experiments, diazoxide and pinacidil were potent activators of native ATP-sensitive K^+ transport in rat brain mitochondria in the absence of MgATP. However, main limitation of our approach is the lack of the means for molecular identification of ATP-sensitive K^+ transport, which is required to identify its relation to mK_{ATP} channel. Thus, an uncertainty remains, whether ATP-sensitive K^+ transport studied in our work belongs to mK_{ATP} channel activity. One of the first steps to resolve this issue is the study of the sensitivity of ATP-sensitive K^+ transport to pharmacological and physiological modulators of mK_{ATP} channel.

Diazoxide and pinacidil are most commonly used pharmacological mK_{ATP} channels openers, and most of the well known functional and bioenergetic effects of mK_{ATP} channels opening were established with the aid of these drugs [7]. Generally, mK_{ATP} channels openers were supposed to bind to the receptor SUR subunits of the channel, which possesses MgATPase activity. So, the presence of MgATP is considered to be indispensable for mK_{ATP} channel opening [9]. However, it worth notion that in the literature uncertainty still exists about the mechanism of mK_{ATP} channel interaction with mK_{ATP} channel openers [19, 20].

After the first discovery in 1991, molecular structure of mK_{ATP} channel for about three decades remained unknown. None of the hypotheses about K^+ conductant subunit of mK_{ATP} channel could satisfactory explain the mechanism of mK_{ATP} channels' response to pharmacological openers, such as diazoxide [19, 20]. However, quite recently, molecular composition of mK_{ATP} channel was disclosed, based on combined proteomics, biophysical and biochemical studies [21]. This work principally confirmed the knowledge on molecular architecture of mK_{ATP}channel, which is an octameric multiprotein complex composed of four K^+ conductant and four receptor subunits, named MITOK and MITOSUR respectively. Also, it was confirmed that K^+ conductant subunit of mK_{ATP} channel plays a number of vital functions, such as volume regulation, maintenance of mitochondrial membrane potential, regulation of ATP synthesis and Ca^{2+} transport [21]. Genetic deletion of MITOK caused instability of $\Delta\Psi_m$, suppression of phosphorylation, and loss of cardioprotective effect of diazoxide [21], which confirmed the results of earlier study, showing that K^+ conductant subunit of mK_{ATP} channel was indispensable for cardioprotective effect of diazoxide [19].

Nevertheless, there remains unexplained diversity of the properties of mK_{ATP} channel in different preparations [18], and uncertainty about the direct and off-target effects of pharmacological modulators of mK_{ATP} channel [7]. These issues still are to be answered and the disclosure of molecular nature of mK_{ATP} channel will help in filling numerous gaps in the knowledge on mK_{ATP} channels and their physiological functions. Also, it remains to be explained possible simultaneous presence of other type(s) of K^+ conductant subunit of ATP-sensitive channels in mitochondria, such as renal outer medulla K^+ channel, ROMK (Kir 1.x), recently supposed to represent mK_{ATP} channel, which isoforms were found in different tissues (brain, heart, liver [22, 23]). Thus, the properties of mK_{ATP} channel in isolated mitochondria and the mechanism of its interaction with pharmacological and physiological modulators still require extensive studies, and we suppose that now this is a time to reconsider some existing concepts about basic biochemical properties of mK_{ATP} channel.

While in several works no effects of diazoxide and pinacidil on mitochondrial matrix volume and respiration were observed without MgATP [9, 10, 18], a number of published data argued for the susceptibility of native ATP-sensitive K^+ transport to the activation by diazoxide and pinacidil in the absence of MgATP [11-13]. However, while in the works referred to high micromolar concentrations of the drugs were used, known to produce multiple off-target effects [7], in our work we established full stimulation of ATP-sensitive K^+ transport by diazoxide and pinacidil on sub-micromolar concentration scale.

As it was shown in our experiments on rat brain mitochondria, not only mitochondrial swelling, but the respiration was as well sensitive to the same

sub-micromolar concentrations of mK_{ATP} channels openers (Fig. 1), which was close to the results of our earlier study showing high sensitivity of rat liver mitochondria K^+ transport to diazoxide within the same concentration limits [15]. Taken together, these data testified susceptibility of mitochondrial ATP-sensitive K^+ transport to the activation by mK_{ATP} channels openers in the absence of MgATP.

From the study of the effects of mK_{ATP} channels openers on the ATP-sensitive and ATP-insensitive K^+ transport blocked by Mg^{2+} ions, we obtained convincing data showing high affinity of ATP-sensitive K^+ transport in brain mitochondria to diazoxide and pinacidil in the absence of MgATP. By our estimation of K^+ transport using light scattering technique, the increment of ATP-sensitive K^+ uptake under the action of mK_{ATP} channels openers in native mitochondria well matched the same effect obtained by conventional activation of mK_{ATP} channel in the presence of MgATP (Fig. 4, *A*, *B*). Also, the same differences in the rates of K^+ transport were obtained after the blockage of activated channel by mK_{ATP} channels blockers, glibenclamide and 5-HD in the presence of MgATP (Fig. 4, *A*, *B*). Both diazoxide and pinacidil were similarly effective in the activation of ATP-sensitive K^+ transport. Thus, specific blockage of the K_{ATP} channel, native and activated by KCOs, by MgATP, its reactivation by micromolar concentrations of KCOs, and consequent blockage by glibenclamide and 5-HD (Fig. 4, *A*) strongly indicates the ability of diazoxide and pinacidil of full activation of brain mK_{ATP} channel on nanomolar concentration scale in the absence of MgATP. This was confirmed by the observation that after the blockage of ATP-sensitive K^+ transport, activated by KCOs, by MgATP, no other blocking effect of glibenclamide or 5-HD on K^+ transport were observed. However, to explain the activation of ATP-sensitive K^+ transport in the absence of MgATP, we can hypothesize a difference in the mechanisms of mK_{ATP} channel activation dependent on the presence of MgATP.

As it was shown by the experiments on pharmacological modulation of mK_{ATP} channel activity (Fig. 4), only ATP-sensitive K^+ transport was affected by the openers; the component of K^+ uptake non-specifically blocked by Mg^{2+} was almost unaffected by these drugs (Fig. 1, *A*, 3-8 vs. 1-4; 3, *A*). Thus, the concentration dependences of the normalized rates of K^+ transport obtained in this work (Fig. 2, *A*) were related to the activation of ATP-sensitive K^+ transport, which, based on our study, reflected mK_{ATP} channel activity. The sensitivity of ATP-sensitive K^+ transport to the known modulators of mK_{ATP} channel (diazoxide, pinacidil, glibenclamide, 5-HD and MgATP) proves identity of ATP-sensitive K^+ transport with mK_{ATP} channel activity and shows the ability of diazoxide and pinacidil to elicit full activation of mK_{ATP} channel on sub-micromolar scale without MgATP.

Without MgATP, ATP-sensitive K^+ transport exhibited full activation at $\leq$ 500 nM of both drugs. Its affinity to KCOs in this work (EC_{50} ~130-160 nM) resembled that of native liver mitochondria, which we established earlier [15], and was decreased by the order in the presence of MgATP (EC_{50} ~1.6 μM). Neither Mg^{2+} nor ATP alone affected the affinity of ATP-sensitive K^+ transport to pharmacological openers, but MgATP complex conventionally shifted it to micromolar concentration level (Fig. 2, *C*), which was close to the literary data on mKATP channel [9, 10].

In this work we obtained strong evidence that the presence of MgATP complex is not a prerequisite for the activation of ATP-sensitive K^+ transport by diazoxide and pinacidil. Close results found in this work on rat brain mitochondria and in our earlier work on rat liver mitochondria, reveal novel common features of the mechanism of the activation of ATP-sensitive K^+ transport by mK_{ATP} channel openers.

Based on our experiments we came to the conclusions that 1) native ATP-sensitive K^+ transport in brain mitochondria is highly sensitive to sub-micromolar concentrations of K_{ATP} channels openers: diazoxide and pinacidil; both K_{ATP} channels openers were similarly effective in the activation of ATP-sensitive K^+ transport; 2) the regulation of native ATP-sensitive K^+ transport by the modulators of mK_{ATP} channel allows us ascribe ATP-sensitive K^+ transport in native mitochondria to mK_{ATP} channel activity; 3) neither Mg^{2+}, nor ATP alone affected the mK_{ATP} channel affinity to K_{ATP} channels openers, and only binding of MgATP complex shifted it to much higher micromolar concentrations of the drugs.

Based on the results of our study, we assume that mK_{ATP} channel might comprise high affinity sites for binding of pharmacological mK_{ATP} channels openers, which possible screening by MgATP decreases the channel affinity to these drugs. This assumption allows us hypothesize an existence of the alternative mechanism of mK_{ATP} channel activation by diazoxide and pinacidil, in which MgATP is dis-

pensable of mK_{ATP} channel activation by KCOs. The results of our study reveal novel not described earlier aspects of the regulation of ATP-sensitive K^+ transport by pharmacological openers of mK_{ATP} channel.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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АТР-ЗАЛЕЖНИЙ ТРАНСПОРТ КАЛІЮ В МІТОХОНДРІЯХ МОЗКУ ЩУРІВ Є ВИСОКОЧУТЛИВИМ ДО АКТИВАТОРІВ mK_{ATP}-КАНАЛУ ЗА ДАНИМИ СВІТЛОРОЗСІЮВАННЯ

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Методом світлорозсіювання вивчено вплив активаторів K_{ATP}-каналів (КСО), діазоксиду і пінацидилу, на АТР-залежний транспорт K^+ в ізольованих мітохондріях мозку щурів за відсутності і в присутності MgATP. За відсутності MgATP виявлено високу чутливість АТР-залежного транспорту K^+ до обох активаторів, із максимальним ефектом при ≤ 0,5 мкМ. У K^+-вмісному середовищі АТР-залежний транспорт K^+ блокувався АТР у присутності Mg^{2+}. Ні Mg^{2+}, ані АТР не впливали на V_{max} АТР-залежного транспорту K^+ за дії КСО, однак, згідно з даними літератури у присутності MgATP крива активації зсувалась в область мікромолярних концентрацій. Блокування АТР-залежного транспорту K^+ блокаторами K_{ATP} каналів, глібенкламідом і 5-гідроксидеканоатом за відсутності і в присутності MgATP показує чутливість АТР-залежного транспорту K^+ до блокаторів mK_{ATP}-каналу. Чутливість АТР-залежного транспорту K^+ до відомих модуляторів активності K_{ATP} каналів (діазоксиду, пінацидилу, глібенкламіду, 5-HD і MgATP) дозволяє віднести АТР-залежний транспорт K^+ до активності mK_{ATP}-каналу і свідчить, що його активація діазоксидом і пінацидилом за відсутності MgATP відбувається в області субмікромолярних концентрацій активаторів. За результатами експериментів, ми припускаємо, що нативний mK_{ATP}-канал може містити високоафінні сайти зв'язуваня КСО, що екрануються MgATP. Результати нашого дослідження виявляють нові, раніше невідомі аспекти регуляції АТР-залежного транспорту K^+ активаторами mK_{ATP}-каналу.

Ключові слова: мітохондріальний K_{ATP}-канал, мозок, транспорт калію, діазоксид, пінацидил.

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