16P.6 Large conductance potassium channel opener NS1619 regulates endothelial function

Agnieszka Łukasiak[1], Antoni Wrzosek[2], Stefan Chłopicki[3], Adam Szewczyk[2], Krzysztof Dołowy[1]
[1]*Warsaw University of Life Sciences SGGW, Department of Biophysics, Warsaw, Poland*
[2]*Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland*
[3]*Jagiellonian University Medical College, Department of Experimental Pharmacology, Cracow, Poland*
E-mail: a.lojek@nencki.gov.pl

Mitochondria play crucial role both in energetic and regulatory pathways within the cell. Inner mitochondrial membrane contains various ion channels, among which potassium channels are well described due to protective activities. Large conductance calcium activated potassium channel (BK_{Ca}) can be activated by channel openers such as NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2*H*-benzimidazole-2-one). NS1619 can regulate functioning of endothelial cells EA.hy 926 in many aspects. In our study it was shown that NS1619 changes mitochondrial function both by decreasing mitochondrial potential and by increasing oxygen consumption probably due to activating BK_{Ca} channels present in the inner mitochondrial membrane and thus promoting K^+ flux. Additionally NS1619 caused increase in calcium concentration within the endothelial cells. Calcium is well known regulator of many signaling pathways within the cells. Ionophore A23187 (1 μM) causes increase in calcium concentration, which subsequently increased nitric oxide (NO) production in EA.hy 926 cells *via* activation of nitric oxide synthase. Similar activity is proposed for NS1619. Along with these results it was observed that NS1619 increased coronary flow in isolated guinea pig hearts in NO dependent manner (100 μM L-NAME, inhibitor of nitric oxide synthase, partially reversed the effect of NS1619). It seems that NS1619 can have beneficial effect on endothelium *via* vasodilating activity, however, the exact mechanism which seems to involve both BK_{Ca} channel activation and other places of action, needs further investigation.

This study was supported by a Nencki Institute of Experimental Biology and Polish Mitochondrial Network MitoNet.pl.

doi:10.1016/j.bbabio.2010.04.401

16P.7 Cytoprotective action of the potassium channel opener NS1619 under conditions of disrupted calcium homeostasis

Dominika Malińska, Ludwika Chmielewska, Adam Szewczyk
Nancki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland
E-mail: d.malinska@nencki.gov.pl

Cytoprotective properties of potassium channel openers (KCOs) have already been shown in several models of cell injury, mainly in ischemia–reperfusion-induced damage of cardiac muscle. The mechanism responsible for the observed cytoprotection as well as the relative contribution of potassium channels located in the plasma membrane and in the inner mitochondrial membrane to the beneficial effects exerted by KCOs remains unclear. This work demonstrates the cytoprotective properties of NS1619, an opener of large-conductance calcium-activated potassium channels (BK_{Ca} channels), in C2C12 myoblasts injured by calcium ionophore A23187 treatment. Application of two BK_{Ca} channel inhibitors, paxilline and iberiotoxin, abolished this cytoprotective effect. At the applied concentrations (10–100 μM), NS1619 increased the respiration rate of C2C12 cells in a dose-dependent manner. However 0.2 μM paxilline, which effectively abolished the protective effect of NS1619, failed to counteract the opener-induced increase in cellular respiration. This result indicates that the NS1619-mediated increase in the survival rate of A23187-treated C2C12 cells is distinct from its effect on mitochondrial functioning and suggests that activation of BK_{Ca} channels in the plasma membrane is responsible for cytoprotection by NS1619.

The study was supported by the Ministry of Science and Higher Education grant no. P-N/031/2006 and no. N N301 304 337.

doi:10.1016/j.bbabio.2010.04.402

16P.8 Influence of ATP-sensitive potassium channel activities on respiration and membrane potential in plant mitochondria

Karolina Matkovic[1], Izabela Koszela-Piotrowska[2], Adam Szewczyk[2], Wiesława Jarmuszkiewicz[1]
[1]*Laboratory of Bioenergetics, Adam Mickiewicz University, Poznan, Poland*
[2]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*
E-mail: matkovic@amu.edu.pl

We describe the existence of a potassium ion transport mechanism in the mitochondrial inner membrane of plants. We found that substances known to modulate ATP-sensitive potassium channel ($mitoK_{ATP}$) activity influenced the bioenergetics of potato (*Solanum tuberosum)* tuber mitochondria, i.e. the rate of resting respiration and membrane potential. In isolated mitochondria, diazoxide (a potassium channel opener) was found to depolarize the mitochondrial membrane potential (measured with a TPP^+-specific electrode) and to stimulate resting respiration. These effects were blocked by glibenclamide and ATP, potassium channel blockers, dependently on the presence of potassium ions in the incubation medium. We investigated monovalent cation (chloride salts) selectivity of the diazoxide-induced ATP-sensitive mitochondrial membrane depolarization. Pharmacological profile and immunoreactivity with specific antibodies indicate that the plant $mitoK_{ATP}$ channel belong to inward rectifier K^+ channel family — Kir.6.x. Our results suggest that an ATP-sensitive potassium channel similar to that of mammalian mitochondria is present in plant mitochondria.

This work was supported by the Polish Mitochondrial Network.

doi:10.1016/j.bbabio.2010.04.403

16P.9 Kidney cortex mitochondria are non-functional in a potassium-based media whereas heart mitochondria improve with increasing potassium concentration

Fredrik Palm*[1,2], Malou Friederich*[1], Christopher S. Wilcox[2]
[1]*Uppsala University, Department of Medical Cell Biology, Uppsala, Sweden*
[2]*Georgetown University Medical Center, Department of Medicine, Division of Hypertension and Nephrology, Washington DC, USA*
E-mail: Fredrik.Palm@mcb.uu.se

A medium of containing high levels of potassium chloride (KCl) is commonly used when assessing respiratory function of isolated mitochondria from various tissues. However, the measured intracellular [K^+] in kidney proximal tubular cells is about 60 mM and in cardiac myocytes approximately 130 mM. Therefore, the use of a similar media [K^+] for all tissues seems unsupported. Here we investigated the effect of different [K^+] on respiratory function in mitochondria isolated from kidney cortex and heart of healthy male Sprague–Dawley rats. Oxygen consumptions and the respiratory

control ratios (RCR) were measured using respiratory medias containing [K^+] of 15, 37, 81, 111 and 146 mM. In all measurements, the media contained (in mM): 1 EGTA, 20 HEPES, 5 $MgCl_2$, 5 KPO_4^- and 1 g/l bovine serum albumin. pH was adjusted to 7.4 and the osmolarity to 330 mOsm/kg H_2O using a 1:3 ratio of sucrose and mannitol. The RCR of kidney cortex mitochondria decreased when the [K^+] was elevated compared to the media containing 15 mM K^+ (5.2 ± 0.2 vs. 2.5 ± 0.2, 3.7 ± 0.2, 3.9 ± 0.2, and 3.0 ± 0.1, respectively). However, RCR of heart mitochondria was lowest at 37 mM (3.9 ± 0.3) and was highest at 146 mM K^+ (10.1 ± 0.45). A two-way ANOVA showed that kidney cortex mitochondria have a different sensitivity towards K^+ compared to heart mitochondria (interaction $p < 0.05$, treatment $p < 0.05$, and group $p < 0.05$). Glibenclamide (100 μM), an inhibitor of the ATP-sensitive K^+ channel, increased RCR in kidney cortex mitochondria at 15 mM K^+ (+32%), but significantly more at 146 mM K^+ (+47%). Blockade of the voltage-gated K^+ channel by 4-aminopyridine (4-AP, 1 mM) together with glibenclamide improved RCR by +73% at 146 mM K^+. Neither of the applied K^+-channel blockers had any effect on the RCR of heart mitochondria. Mitochondria swelling at increasing [K^+] were observed in kidney cortex mitochondria, measured as loss of absorbance at 540 nm. Kidney cortex mitochondria in K^+-based media are non-functional in [K^+] ranging from 37 to 146 mM. Heart mitochondria do not display K^+-sensitivity to the same degree, but rather increase respiratory function with increasing [K^+]. Furthermore, we demonstrated that a tissue specific difference in mitochondria K^+-channels may explain these differences. The present study therefore demonstrates the importance of choosing a correct *in vitro* media to ensure a high quality of mitochondria research.

*These authors contributed equally to this work.

doi:10.1016/j.bbabio.2010.04.404

16P.10 Novel assay and regulation of the mitochondrial K_{ATP} channel

Andrew P. Wojtovich[1], Keith W. Nehrke[2], Paul S. Brookes[3]
[1]*University of Rochester Medical Center, Pharmacology and Physiology, USA*
[2]*University of Rochester Medical Center, Medicine, USA*
[3]*University of Rochester Medical Center, Anesthesiology, USA*
E-mail: Andrew_wojtovich@urmc.rochester.edu

The mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$) protects the heart from damage induced by ischemia–reperfusion injury. Despite its central role in cardioprotection, the **mo**lecular identity of mitoK$_{ATP}$ remains controversial, and the validity of current methods to assay mitoK$_{ATP}$ activity is disputed. We sought to apply new and updated methods to investigate properties of the K^+ transporting component of mitoK$_{ATP}$ and its role in IPC. Using a thallium (Tl^+) sensitive fluorophore, a novel assay was developed to measure Tl^+ flux through mitoK$_{ATP}$. With both this assay and the classical mitoK$_{ATP}$ osmotic swelling assay, four key observations were made: (i) The IC_{50} for ATP-dependent channel inhibition was 4.5 μM. (ii) The EC_{50} for UDP-dependent channel activation was 20 μM. (iii) In isolated mitochondria, mitoK$_{ATP}$ activity rapidly degraded with time, and this channel "run-down" was reversed by phosphatidylinositol-4,5-bisphosphate (PIP_2). (iv) The antidepressant fluoxetine (Prozac™) both inhibited mitoK$_{ATP}$ (IC_{50} 2.4 μM) and blocked mitoK$_{ATP}$-dependent cardioprotection, while the related drug zimelidine was without effect. These findings are consistent with the hypothesis that the pore-forming subunit of mitoK$_{ATP}$ is an inward rectifying K^+ channel (Kir), likely Kir6.2. The ability of PIP_2 to reverse channel run-down is the first demonstration of modulation of a mitochondrial ion channel by PIP_2, and provides a mechanism to extend the assayable lifetime of mitoK$_{ATP}$ activity. The effect of fluoxetine on mitoK$_{ATP}$-dependent cardioprotection has implications for the widespread use of antidepressants in cardiovascular disease patients.

doi:10.1016/j.bbabio.2010.04.405

16P.11 Potassium channel opener CGS7184 modulates activity of mitochondria by Ca^{2+} release through ryanodine receptor

A. Wrzosek[1], Z. Tomaskova[2], K. Ondrias[2], A. Łukasiak[3], A. Szewczyk[1]
[1]*Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Pasteura 3, PL 02-093 Warszawa, Poland*
[2]*Institute of Molecular Physiology and Genetics, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Vlarska 5, 833 34 Bratislava, Slovakia*
[3]*University Warsaw of Life Sciences-SGGW, Department of Biophysics, Nowoursynowska 159, 02-776 Warszawa, Poland*
E-mail: A.Wrzosek@nencki.gov.pl

Large-conductance Ca^{2+}-activated potassium channel (BK_{Ca} channel) opener ethyl-1-{[(4-chlorophenyl)amino]oxo}-2-hydroxy-6-trifluoromethyl-1H-indole-3-carboxylate (CGS7184) acts on endothelium in the aorta and coronary circulation, NO production, calcium homeostasis, and mitochondrial function, especially on mitochondrial membrane potential, and mitochondrial respiration in cultured endothelial cells. All effects may be triggered by the CGS7184-induced modulation of intracellular Ca^{2+} homeostasis. To find the source of Ca^{2+} we studied the calcium homeostasis in H9C2 and C2C12 cell lines, and isolated sarcoplasmic reticulum (SR). The effects of CGS7184 on calcium homeostasis in C2C12 and H9C2 cell lines were measured with fura-2 fluorescence. Calcium uptake and Ca^{2+}-ATPase activity in isolated SR vesicles from rat skeletal muscles were applied. Single channel properties of calcium RYR channel were studied in bilayer lipid membrane (BLM). The BK_{Ca} channel opener CGS7184 has a profound effect on release of calcium from internal stores in the concentration-dependent manner. Data demonstrate that potassium channel opener CGS7184 modulates cytosolic Ca^{2+} concentration in H9C2, C1C12, and EA.hy 926 cell lines due to SR modulation. CGS7184 (5 μM) activated the cardiac RYR channel from both cytosolic and luminal sides, when the open probability of the control channel was >0.01. The large conductance potassium channel opener CGS7184 affects intracellular calcium homeostasis by interaction with RYR receptor.

This study was supported by the Nencki Institute of Experimental Biology and grant from the Ministry of Sciences and Higher Education (P-N/31/2006) and by financial support from the Polish Mitochondrial Network MitoNet.pl.

doi:10.1016/j.bbabio.2010.04.406