Novel approach to the expression of the mitoBKCa channel.

P-02.5-05

S. Galecka[I], P. Bednarczyk[II], A. Szewczyk[I], B. Kulawiak[I]

[I]Nencki Institute of Experimental Biology, Warsaw, Poland, [II]Warsaw University of Life Sciences, Warsaw, Poland

Ischemia/reperfusion (I/R) injury of brain and heart tissue is one of the most common causes of death in most Western countries. Activation of mitochondrial potassium channels is a promising direction for development of therapeutic strategy reducing tissue damage after I/R induced insults. The mitochondrial large conductance calcium activated potassium (mitoBK$_{Ca}$) channel is present in both heart and brain. Experimental data show that activation of the channel with potassium channel openers results in cardioprotection.

Expression of the mitoBK$_{Ca}$ channel in HEK293T cells as a model system.

The pore forming subunit of the channel is encoded by KCNMA1 gene and BK-DEC splice variant has been demonstrated to localize to mitochondria. However, it was not known whether this isoform forms an active channel in mitochondria. After transient transfection of HEK293T cells with plasmid encoding this splice variant we were able to record electrophysiological activity of this channel in isolated mitoplasts. The recorded channel had all properties typical for mitoBK$_{Ca}$. Interestingly, the activity of this channel was not detected in wild type cells. Additionally, in untransfected cells we detected expression of the regulatory ß4 subunit of the channel. The presence of this regulatory subunit was verified using both Western Blot and quantitative PCR reaction. Our data also suggests that ß4 might localize to mitochondria.

Results of our experiments show that BK-DEC splice variant forms a fully functional channel in the inner mitochondrial membrane. Moreover, the wild type cells might express ß4 subunit of the channel. The presence of the ß4 in wild type cells raises the question about the role of this protein in mitochondria. Additionally, our data shows that the HEK293T cells can be used as a model for mitochondrial potassium channel research.

This project was supported by Polish National Science Centre grant No.2015/18/E/NZ1/00737 and the Nencki Institute of Experimental Biology.