Application Note

Cells: Mitoplasts
Tools: Port-a-Patch®

Mitoplasts from HEK cells on Nanion's Port-a-Patch®

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Summary

Mitochondria play an important role in metabolism by providing the cell with ATP due to oxidative phosphorylation. But they are also supposed to be involved in apoptosis and cytoprotection.
The mitochondrial membranes contain a large number of ion channels, transporters and pores. However the physiological role of mitochondrial channels is largely unknown. The main channel of the outer membrane is VDAC, a well described anion conductance. In the inner membrane most prominent are the permeability transition pore (PTP) and the inner membrane anion channel IMAC. Different potassium channels are described as well (mitoBK, mitoKATP and Kv1.3) and a number of calcium conducting channels and receptors (for example MCU).
To study the channels of the inner mitochondrial membrane the outer membrane can be stripped of by osmotic swelling. This method was used for the examples of electrophysiological measurement with the Port-a-Patch shown below.

Preparation of mitoplasts

Use two 10 cm dishes or a T75 flask confluent with HEK 293. Harvest with 2 ml trypsin for 3 min. Add 2 ml of serum containing media, and spin down cells at 1200 g for 5 min. From this point every step has to be done at 4°C. Resuspend pellet in 800 µl of IBc buffer. Potter by hand 40-50 times. Wash the potter with 800 µl. Spin down the cells at 700 g for 10 minutes at 4 °C in 2 ml Eppendorf tubes. Put the supernatant in two fresh 1.5 ml tubes. Centrifuge at 3000 g for 15 min. Resuspend the pellet in 200 µl and centrifuge again at 3000 g for 15 min. Remove the supernatant as good as possible. Mitochondria can now be stored for a day on ice. For the preparation of mitoplasts resuspend pellet in 100 µl of swelling buffer (mix 160 µl of hypotone buffer with 40 µl of hypertone buffer). Incubate for 7 min at room temperature. Stop process with 20 µl of hypertone buffer. Mitoplasts can now be used for measurements. Keep on ice.

Electrophysiology

Mitoplasts can be measured with high success rates by using NPC1 chips with a resistance of 10-15 MOhm and Nanion's standard electrophysiological solutions. A mix of different conductances was observed under these conditions. Some examples are displayed in fig.1 and fig. 2

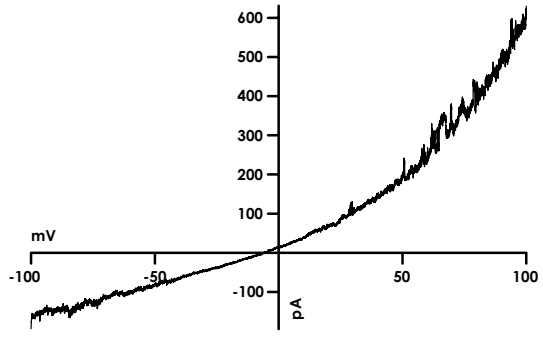

Figure 1:
Voltage ramp from -100 mV to 100 mV in standard solutions. Resulting current is a mix of macroscopic and single channel currents. Different conductances can be identified.

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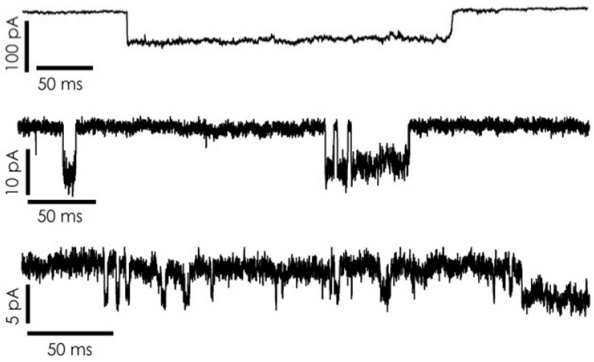

Figure 2:
Individual traces of mitoplast recordings at continuous voltage (-20 mV) in standard solutions are shown in this figure. Ion channels with different single channels properties can be found.

A chloride conductance was identified by replacing external chloride by MSA, resulting in a reduced outward current (see Figure 3).

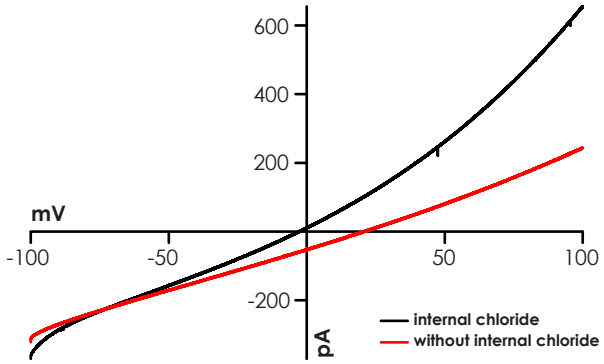

Figure 3:
Chloride conductance. Black trace shows current with external chloride, red without.

The Port-a-Patch allows exchanging the external solution as well as the internal solution. Such an experiment is shown in Figure 4.

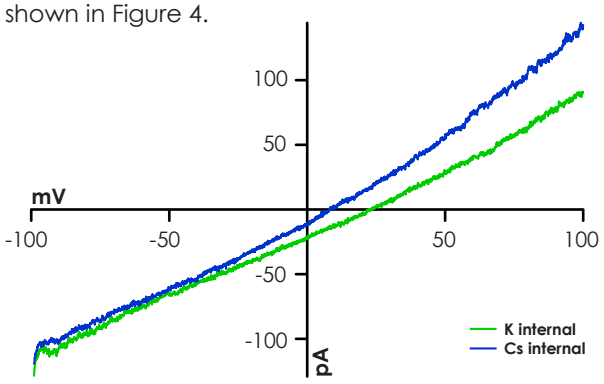

Figure 4:
Mitoplast current with different internal solutions. Blue trace is with internal cesium, green with potassium.

To identify potassium channels, a chloride free external solution was used in figure 5. The single channel on top of the macroscopic current has a conductance of of about 300 pS, and therefore could be the mitoBK.

Figure 5:
Step to +60 mV with internal potassium and chloride free external solution.

Methods

Solutions and buffer

Buffer for cell mitochondria isolation (IBc): Prepare 100 ml of IB by adding 10 ml of 0.1 MTris–MOPS (ph7.4 with MOPS) and 1 ml of 0.1 EGTA/Tris (pH7.4 with Tris) to 20 ml of 1 M sucrose. Bring the volume to 100 ml with distilled water. Adjust pH to 7.4. Add proteinase inhibitor before use 1:20-25.
Hypothone buffer: 5 K-HEPES and 1 CaCl2 (pH 7.2).
Hypertone buffer: 750 KCl, 80 K-HEPES, 1 CaCl2 (pH 7.2).

Nanion Standard Solutions:
Internal K Solution : 50 mM KCl, 10 mM NaCl, 60 mM KF, 20 mM EGTA, 10 mM Hepes /KOH, pH 7.2

Internal Cs Solution : 50 mM CsCl, 10 mM NaCl, 60 mM CsF, 20 mM EGTA, 10 mM Hepes /CsOH, pH 7.2

External Solution : 140 mM NaCl 4 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 5 mM D-Glucose monohydrate, 10 mM Hepes /NaOH pH 7.4

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