- Organellar Transporters and Ion Channels -

How to access their electrophysiology by using the SURFE²R technology and Planar Patch Clamp

Maria Barthmes¹, Tom A. Goetz², Stephan Holzhaus³, Melanie Glider³, Christian Pätzig³, Raphael Bonde³, Timo Stegeli³, Marius Vogel³, Johannes Stiehler³, Andrea Brüggemann³, Michael George¹, Niels Fertig¹

Abstract

Electrophysiological characterization of ion channels from organellar membranes is vastly restricted due to limited accessibility. Even more so the direct measurement of transporter activity in these membranes is extremely challenging, because noise usually exceeds signal size due to comparably low transport rates. Here we present electrophysiological data of the ADP:ATP exchanger (ANT) purified from mitochondrial membranes (IMM) acquired with the SURFE²R technology which is based on a solid supported membrane approach. By measuring the total current of a large number of transporter molecules triggered simultaneously, the signal becomes available for pharmacological studies. Also we show data from mitochondrial BK⁹⁶ channel to demonstrate how planar patch clamp technology facilitates the study of organellar mitochondrial ion channels.

The SURFE²R Technology

Surface Electrogenic Event Reader (formerly ionGATE)

Solid Supported Membrane (SSM) based biosensors A gold electrode of 3 mm diameter is functionalized by a triad and a lipid layer. This allows to immobilize membrane fragments or liposomes containing the target transporter on the sensor.

Sensor preparation
- 3 mm gold surface
- 30 min 0.5 M 1 octadecane-thiol
- 15 µl 1µM DPPPC
- loading buffer added
- 6 µl sonicated liposome
- centrifugation 1 h, 2000 g

Accumulated transport current

The protein on the sensor is synchronously activated by a fast perfusion system. The transport of charged substances over the membrane generates an electrical current (like capacitor plate). It’s measured as a sum signal of all the protein on one sensor, thereby enabling a high amplification.

ANT and Respiratory Chain Complexes from IMM preparations

Adenine Nucleotide Translocator (ANT)

Two modes, ADP:ATP exchange and transport separately. Preparations from pig heart mitochondria inner membrane. Example: traces activation of ANT in the presence of ADP on IMM.

Respiratory chain complexes (I, IIIiti, IV, V)

Oxidation of NADH leads to transport of four protons across the membrane. The electrons are transferred to ubiquinone. The signal is generated by addition of NADH.

Oxidation of succinate to fumarate. The electrons are transferred to cytochrome c via complex II while four protons are transported to the intermembrane space. Signal is evoked by oxidized cytochrome c. Succinate must be provided.

Reduction of oxygen by consumption of electrons from cytochrome c and transport of two protons to the intermembrane space. Signal is evoked by reduced cytochrome c.

Complex V generates ATP from ADP plus phosphate. Here it works reverse as ATPase. Therefore signal is generated by addition of ATP. Membranes were treated with 50 µM BFA (Brefeldin acid = specific ANP inhibitor).

Planar Patch Clamp - from Port-A-Patch to SyncroPatch 384PE -

preparation of agar salt bridge (3x KC1)

The Search of the Putative Mitochondrial BK⁹⁶ channel

Does KCNMA1 gene not only code for KCa1.1 (plasma membrane) or also mitochondrial BK⁹⁶?

- Preparation of mitoplasts (2-5 µm) and mitochondria from HEK293
- mito-tracker red stained, CMAC as "cap" structure (Kirschik et al. 2004)
- control for GMI, electrophysiology of liposomes + intact mitochondria
- liposome measurements, large activities, VDAC

A-C macroscopic current + single channel events largely attributed to CI-channel/MAC-100µs [Baroyley et al.]
- Propranolol: 5µM iCl-C1 replaced by MSA: methane sulfonic acid
- NPC-chip: 1 µm aperture, R: 10-15SMOhm

Statistical analysis

A-K channel appearances in wt BK⁹⁶ and mutant BK⁹⁶: mitochondrial
- Conductance of 190ps in different conditions, NS41021, increased appearance by 10%, P<0.05 reduced by >20%

Analysis of single channel characteristics
- A current histograms of ROMY
- B scatter IV of positive voltages, conductance of 190ps
- C dwell time of closed and open state

References


¹ Nanion Technologies GmbH, Gabrieleinstraße 9, Munich Germany
info@nanion.de