Application Note

Organelles: Mitochondria
Tools: Port-a-Patch

Recordings from mitochondria and mitoplasts on Nanion's Port-a-Patch

The electrophysiology team at Nanion Technologies GmbH, Munich.
Mitoplasts courtesy of Dr. Shin-Young Ryu and Professor Shey-Shing Sheu at the University of Rochester, USA.
Mitochondria courtesy of Dr. Valentin Gribkoff, Dr. Armando Signore and Dr. Steven Dworetzky, Knopp Neurosciences Inc., Pittsburgh, PA, USA.

Summary

Mitochondria are often referred to as the "power house" of the cell since they are responsible for making most of the cell's energy supply in the form of adenosine triphosphate (ATP). In addition to providing the cell with energy, mitochondria are thought to have roles in cell signalling, cellular differentiation and apoptosis[1]. They have also been implicated in the pathophysiology of neurodegenerative disorders such as Parkinson's Disease[2], and may also play a role in diabetes[3] and in the ageing process[4].

Mitochondria are usually rod shaped and range in size from approximately 1 - 10 µm. They have an outer membrane and a highly folded inner membrane. The outer membrane is highly permeable and contains one of the most well studied mitochondrial proteins, the voltage-dependent anion channel (VDAC). The inner membrane contains many ion channels, including the Ca^{2+} uniporter, a K_{ATP} channel, the Ca^{2+}-activated K^+ channel (K_{Ca}) and the inner membrane anion channel (IMAC)(for brief review see ref. 5). To study the mitochondrial inner membrane using the patch clamp technique, mitoplasts were formed. This is a process whereby the mitochondria are swelled, thus rupturing the outer membrane and exposing the inner membrane.

Results

Mitochondria play a central role within eukaryotic cells providing the cell with energy. They are generally rod-shaped and have an outer and inner membrane. Figure 1 shows a schematic representation of a mitochondrion.

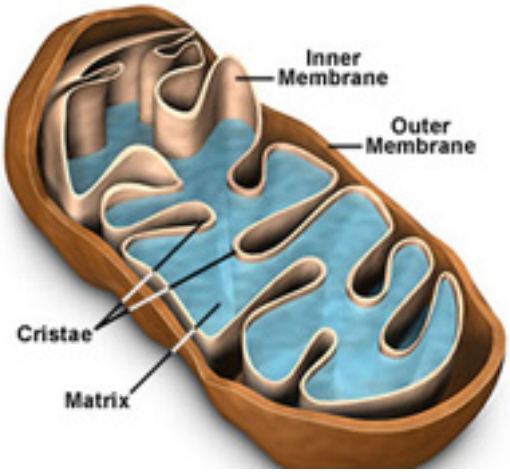

Figure 1: Schematic representation of a mitochondrion showing outer and inner membranes. (Adapted from http://micro.magnet.fsu.edu/cells/mitochondria/mitochondria.html)

Application Note

We were able to successfully capture and record from intact mitochondria using the Port-a-Patch. Figure 2 shows the current response to a voltage ramp from -120 mV to +100 mV over 500 ms from a mitochondrion.

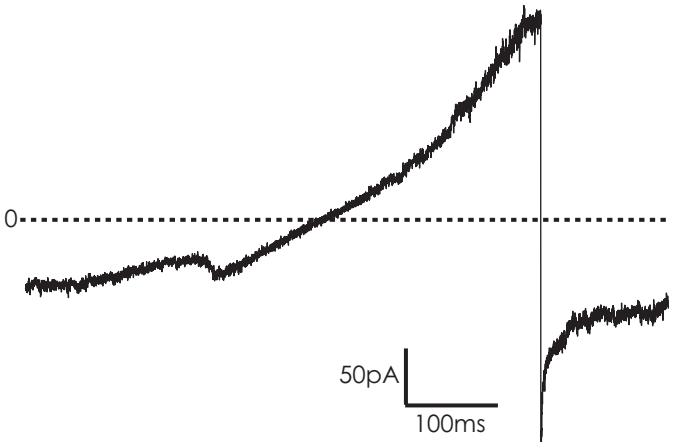

Figure 2: The current response of a mitochondrion to a voltage ramp from -120 mV to +100 mV over 500 ms approximately 30 secs after application of 100 µM Ca^{2+} on the external surface. There is a small "bump" that appeared on the ramp at approximately -55 mV. It is not known whether the mitochondrion, however, was in the "on-cell" or "whole-cell" configuration. It was not determined whether this is an inward Na^{+} or Ca^{2+} current at negative potentials, or an outward K^{+} (or other positively charged ion) at positive potentials.

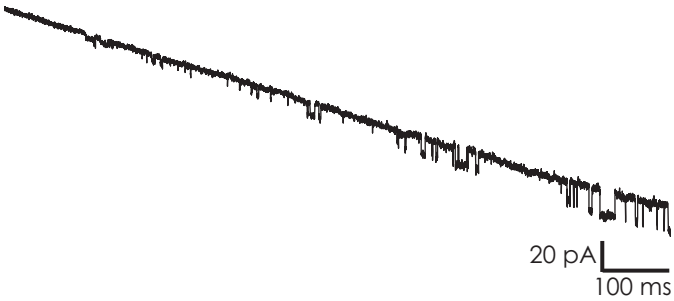

Figure 3: Single channel events recorded from mitoplasts on the Port-a-Patch. Currents were elicited using a voltage ramp protocol, the portion here shows from 0 to -80 mV over 1 s. Single channel events are seen as small downward deflections at voltages more negative than -20 mV

Besides intact mitochondria we were also able to record from mitoplasts. The recording in Figure 3 shows single channel events from a mitoplast. A voltage ramp protocol was used in the cell attached configuration from 0 mV to -80 mV over 1 s. Inward currents can be seen at voltages more negative than approximately -20 mV.

In conclusion, we successfully captured and recorded from mitochondria and mitoplasts using the Port-a-Patch. Both macroscopic and single channel events could be elicited using a voltage ramp.

References

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Methods

Organelles

Intact mitochondria were acutely isolated from liver at Knopp Neurosciences. Mitoplasts prepared from mitochondria isolated from rat heart were prepared by Dr. Shin-Young Ryu at the University of Rochester, USA.

Electrophysiology

Patch clamp recordings were conducted on the Port-a-Patch using NPC-1 chips with high resistance either using an Axon Axopatch 200B or a Multiclamp 700B amplifier.

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