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Spontaneous Na^+ Concentration Transients in Individual Mitochondria of Intact Astrocytes

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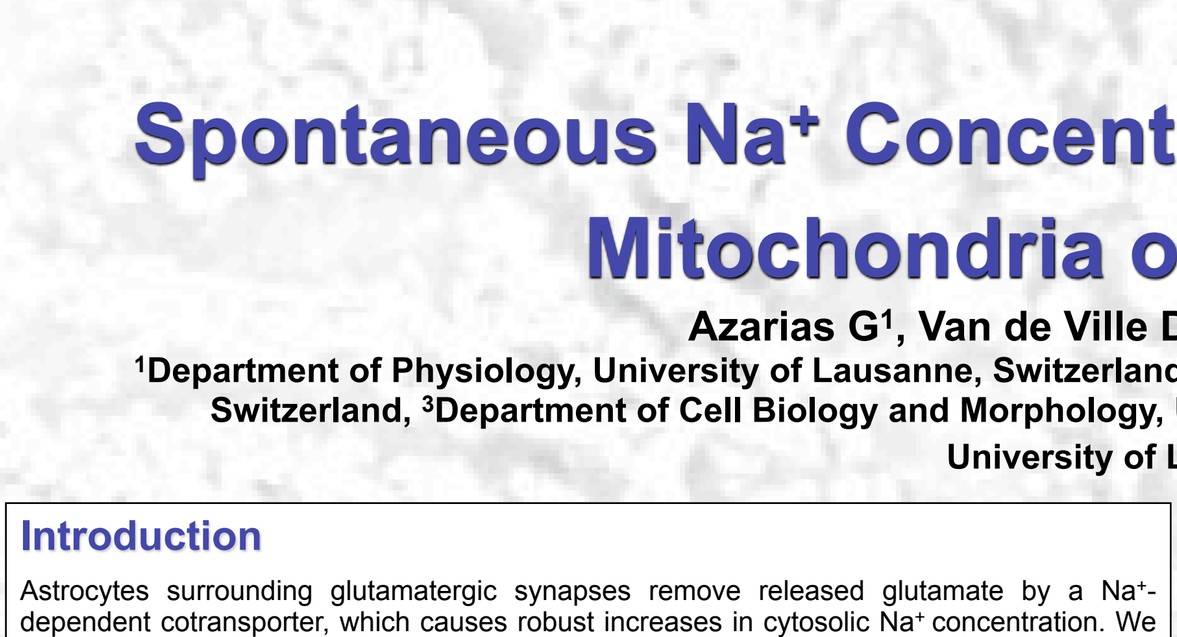
Introduction

Astrocytes surrounding glutamatergic synapses remove released glutamate by a Na^+-dependent cotransporter, which causes robust increases in cytosolic Na^+ concentration. We recently showed using a fluorescent probe specific for mitochondrial Na^+ (CoroNa Red), that Na^+ increases occur also in the mitochondrial population where it is dynamically regulated (see Bernardinelli et al, GLIA 54:460-470, 2006). Using the same fluorescent probe, we performed a study of individual mitochondria at the resting state and observed spontaneous transient increases in mitochondrial sodium (TIMS). We have developed an algorithm based on the wavelet transform to analyze and quantify TIMS in several pharmacological conditions. This study shows that individual mitochondria can exhibit dynamic regulation of their sodium content.

Characterization

Fig. 1



Transient increase in mitochondrial sodium (TIMS) occur spontaneously in intact astrocytes (*a*, 5 sec between images). Up to 1000 counts could be detected in a field of 30 confluent astrocytes with a mean duration of 12.2 sec +/- 0.9 (*b*) and homogeneous time course (*e*). Amplitudes were more heterogeneous (*c*). A mitochondrion exhibited one or several TIMS in the recording period (*e*).

Mitochondrial transmembrane potential seems required but not involved during TIMS. Partial uncoupling of mitochondria led to significant decreases in the frequency of TIMS (*d*) and complete uncoupling abolished them (*not shown*). However, TIMS occur without detectable change in mitochondrial potential simultaneously measured with Rhodamine 123 (*e*).

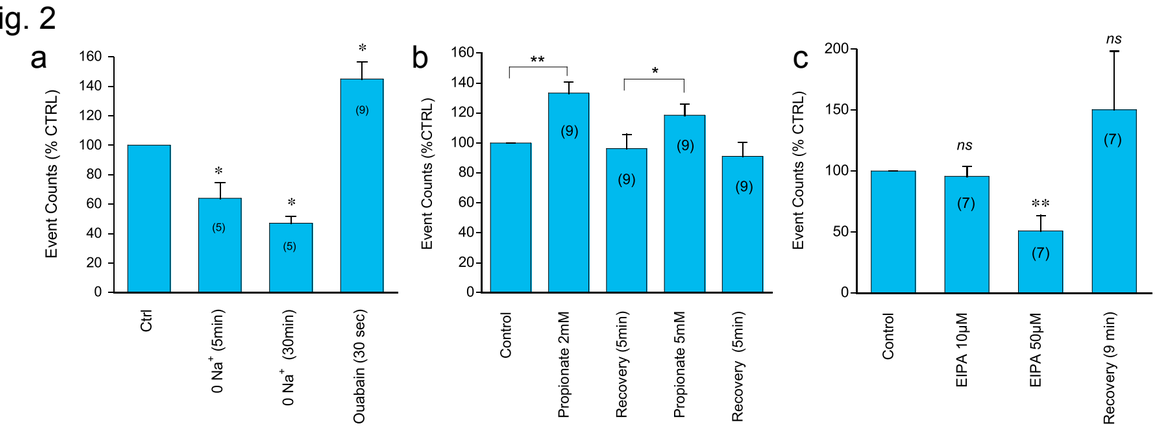
Methods

Mitochondrial Na^+ imaging : Mitochondrial Na^+ was measured using the fluorescent probe CoroNa Red (CR), and could be calibrated as shown in Bernardinelli et al. CR fluorescence was excited at 560 nm and detected at >580 nm. Cells were observed at 37 °C in a physiological buffer using a low-light level widefield fluorescence microscope. Data in figures 1a and 1e have been obtained on a Zeiss LSM 510 Meta confocal microscope.

Images analysis : We developed an algorithm based on the wavelet transform to quantify the frequency and cumulative intensity of TIMS. The operation was applied in the spatio-temporal domain, i.e. XY planes along time. First, 3D discrete wavelet transforms were computed for the stacks of images. Wavelet coefficients were then treated to retain only subbands mainly responsible for TIMS. This corresponds to separating the signal due to fluctuations from the slowly varying background and the highly uncorrelated noise. In practice, TIMS were identified as spatially clustered signal changes over 3-16 planes. The thresholding parameter was selected by estimating the noise variance from the highpass subband.

Proton and sodium homeostasis are linked during TIMS

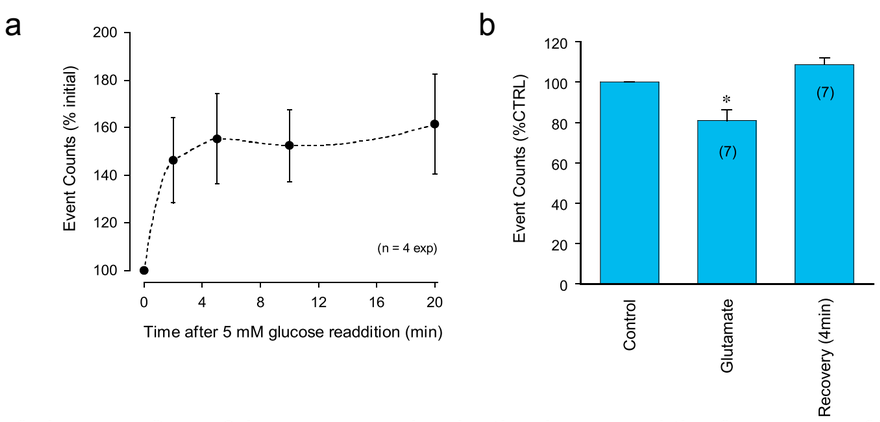
Fig. 2



Extracellular Na^+ removal (*a*) and cytosol acidification (*b*) altered TIMS frequency. Moreover mitochondrial Na^+/H^+ exchanger blockade strongly impaired TIMS (*c*).

Link between energy metabolism and TIMS

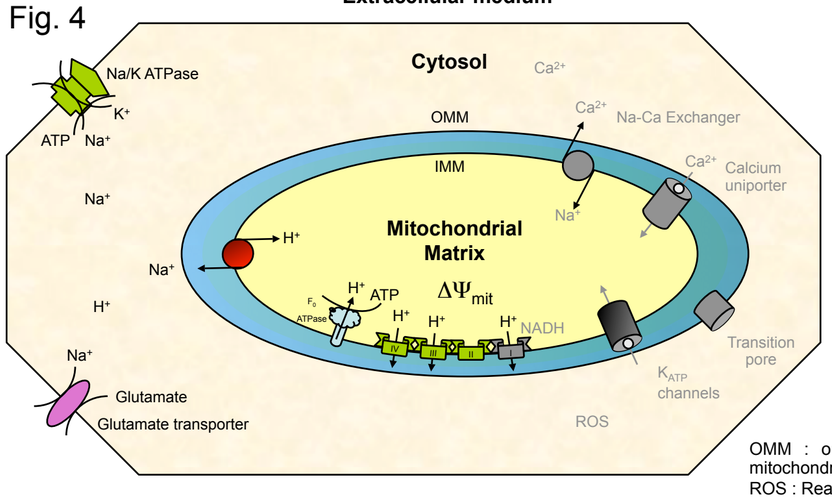
Fig. 3



Readdition of glucose after a 3-hour energy deprivation increased the frequency of TIMS (*a*). A 200 µM glutamate application, known to decrease ATP level in astrocytes, decreased weakly the frequency of TIMS (b).

Further investigations: identify the sodium entry pathway

Fig. 4



To address the question of the sodium entry pathway, calcium pathways (Na-Ca exchanger, Ca^{2+} uniporter), transition pore, K_{ATP} channels and ROS pathway have been tested and did not alter frequency or intensity of TIMS. (*not shown*)

OMM : outer-mitochondrial membrane ; IMM : inner-mitochondrial membrane ; ΔΨmit : mitochondrial potential ; ROS : Reactive oxygen species

Summary

- **Individual mitochondria exhibit spontaneous transient increases in their sodium concentration.**
- **Proton and sodium homeostasis are linked to each other by the mitochondrial Na^+/H^+ exchanger during TIMS.**
- **The physiological significance of TIMS might be related to a subcellular regulation of energy metabolism.**

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