

Ca⁽²⁺⁾n it be measured? High-resolution FluoRespirometric detection of extramitochondrial calcium movement

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Introduction: The mitochondrion plays an essential role in controlling calcium (Ca²⁺) homeostasis. However, physiological Ca²⁺ signalling can lead to deleterious processes in various disease states due to Ca²⁺ overload (e.g. ischaemia/reperfusion, sepsis); that can induce the opening of mitochondrial permeability transition pore (MPTP) and subsequent cell death. Our studies were aimed 1) to monitor simultaneously the extramitochondrial Ca²⁺ movement and oxygen flux (JO₂) through the electron transport system and 2) to assess whether the inhibition of MPTP affects mitochondrial Ca²⁺ influx/efflux using high-resolution FluoRespirometry (Oroboros O2k, Oroboros Instruments, Innsbruck, Austria).

Materials and Methods: Under ketamine and xylazine anaesthesia, SPRD rats (330-360 g; n=7) and SKH-1 mice (n=7; 20-30 g) were sacrificed then the left lateral liver lobe was immediately removed for homogenate preparation, or the whole liver was harvested for preparing isolated mitochondrial fraction. Samples were energized with complex I-specific substrates (10 mM glutamate and 2 mM malate) or a complex II substrate (10 mM succinate; after complex I blockade with 0.5 μM rotenone) in the presence or the absence of the MPTP inhibitor Cyclosporin A (1 μM). After reaching a stable respiration, Ca²⁺ movement was assessed by Calcium Green-5N fluorescent dye (2 μM) using Fluorescence-Sensor Blue (excitation 465 nm). Calcium influx was stimulated by CaCl₂ addition (50 μM). Finally, EGTA (1 mM) was used for the chelation of calcium.

Results: Exogenous CaCl₂ resulted in an abrupt elevation in Calcium Green-5N fluorescence intensity followed by a decrease (mitochondrial calcium uptake) with simultaneous elevation in oxygen consumption. This was followed by a rapid increase in the fluorescence signal (reflecting Ca²⁺ efflux), reaching a higher fluorescence (Ca²⁺-efflux) than the initial CaCl₂-induced elevation. Chelation of Ca²⁺ with EGTA completely abolished the fluorescence of the indicator. After the pre-incubation with Cyclosporin A marked delay (approx ~15 min) in mitochondrial Ca²⁺ movement was observed, not only in isolated liver mitochondria but also in tissue homogenate.

Conclusion: Our results indicate that Calcium Green-5N is an applicable method for monitoring the Ca²⁺ flux of biological samples, in isolated mitochondria and tissue homogenates as well. Further experiments are required for the better understanding of MPTP-independent way of Ca²⁺ efflux and how pharmacological/genetic inhibition of mitochondrial Ca²⁺ transport affects the Ca²⁺ movement.

Grant support: NKFIH K116689, GINOP-2.3.2-15-2016-00034, EFOP-3.6.2-16-2017-00006

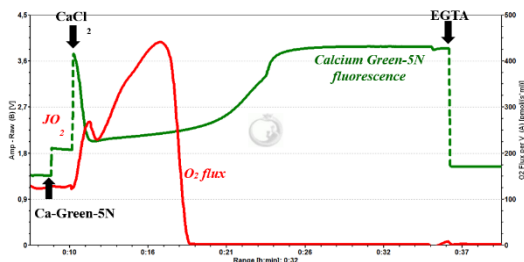


Figure 1. Simultaneous measurement of calcium- and O₂ flux in mouse liver homogenate