HIGH-RESOLUTION RESPIROMETRY WITH MULTIPLE SUBSTRATES TITRATION IN PERMEABILIZED MYOCARDIAL FIBERS

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HIGH-RESOLUTION RESPIROMETRY WITH MULTIPLE SUBSTRATES TITRATION IN PERMEABILIZED MYOCARDIAL FIBERS (Abstract): The present study was purposed to standardize the high-resolution respirometry technique for the analysis of oxidative phosphorylation (OXPHOS) in saponin-skinned cardiac fibers. Preparation of permeabilized myocardial fibers allows the assessment of respiratory function of the entire population of mitochondria in their normal intracellular position. Adult male rat ventricular bundles were permeabilized with saponin (50 μg/ml) and samples (1-3 mg wet weight) were transferred into the Oxygraph-2k (OROBOROS Instr., Austria) chambers containing air saturated incubation buffer in order to measure Complex I (CI) and II (CII) dependent respiration. The following values (expressed in pmol O₂·s⁻¹·mg⁻¹) were obtained: CI_LEAK, 67.18±5.12 (CI dependent basal respiration, after glutamate and malate addition); CI_P, 247.37±49.90 (CI_OXPHOS state after ADP addition); CI_Pc, 252.036±53.13 (the intactness of the outer mitochondrial membrane assessed after cytochrome c addition); CI+II_P, 342.90±62.48 (maximum OXPHOS state obtained after succinate addition by activating convergent electron flow from CI+II into the Q junction of the electron transport system (ETS)); CII_P, 302.26±50.16 (CII dependent OXPHOS state obtained after the addition of a CI inhibitor - rotenone, with the subsequent entry of electrons from CII only into the Q junction); ETS capacity, 331.11±62.39 (uncoupled respiration). The standardized technique will allow the systematic characterization of mitochondrial respiratory dysfunction associated with several cardiac pathologies in both animals and humans. **Key words:** HIGH-RESOLUTION RESPIROMETRY, PERMEABILIZED MYOCARDIAL FIBERS.

Cardiovascular diseases and coronary heart disease, in particularly, remain the leading cause of mortality due to myocardial infarction and morbidity due to heart failure worldwide. Mitochondria are recognized to be directly involved in pathophysiology of ischemia-reperfusion injury (1-3) and also in cardioprotection (4). In the past decades, analysis of mitochondrial respiratory (dys)function has become a common research tool in basic cardiology, and strategies able to reversibly modulate respiration in order to protect mitochondria during the postischemic reperfusion are widely investigated (5).

The present study was aimed to standardize the technique for preparation of permeabilized fibers from rat hearts view subsequent studies of high-resolution respirometry (HRR). Standard procedures of