Abstract

Skeletal muscle is the largest tissue in the body and plays a marked role in the homeostasis of the body metabolic state. Mitochondria have been proven to contribute to the pathophysiology of various metabolic diseases, either due to defects in their bioenergetic properties or the production of reactive oxygen species. In this work murine myoblasts C2C12 were used as a model of skeletal muscle in vitro, and rat muscle was used to prepare homogenate enriched in the mitochondrial fraction.

This work investigates the changes in respiratory parameters in models where mitochondrial oxidative phosphorylation is induced by changing the available consumable substrates in the culture media, such as replacing glucose by galactose, and the effect of treating the cells with high glucose concentration during the process of differentiation on mitochondrial performance. It also investigates the changes in bioenergetic profiles in samples treated with inactive derivatives of the widely used triphenylphosphonium (TPP^+) salts to target mitochondria by various probes and antioxidants.

The methods used in this study included evaluating mitochondrial parameters in intact and permeabilized cells by real time measurement of the oxygen consumption rate using the extracellular flux analyzer, measuring the enzymatic activity of Krebs cycle and the electron transport chain complexes spectrophotometrically, and measuring changes in mitochondrial membrane potential ($\Delta\psi_m$) fluorometrically.

The results confirmed that low glucose concentration is the main inducer of mitochondrial respiration and changes observed with galactose-treated models are due to glucose deprivation. The presence of glucose in the culture media is essential to induce differentiation and increasing the glucose level during the myogenic process decreases in the respiratory capacity due to the decrease in the enzymatic activity of complex I and III.

More hydrophobic long alkyl side chain of the TPP^+ derivatives induces mitochondrial uncoupling and proton leak respiration, while the least hydrophobic methytriphenylphosphonium (TPMP) causes a gradual decrease of mitochondrial respiration by interruption of the Krebs cycle and inhibition of oxoglutarate dehydrogenase complex.