Characterization of the Inhibitory Effect of Oligomycin on Protonophore-Induced Maximal Oxygen Consumption in Tumor Glioma Cells

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Introduction: The maximal capacity of the mitochondrial electron transport system (ETS) in intact cells is frequently estimated by promoting protonophore-induced maximal oxygen consumption preceded by inhibition of oxidative phosphorylation by oligomycin. **Objectives:** The present study aimed to characterize the inhibitory effect of oligomycin on maximal oxygen consumption in cultured glioma cells. **Material and Methods:** Human glioma cells (T98G and U-87MG) were titrated with different concentrations of the protonophore CCCP to induce maximal oxygen consumption rate (OCR) within respirometers in a conventional growth medium. **Results and Discussion:** The results demonstrate that the presence of oligomycin leads to underestimation of maximal ETS capacity. In the presence of oligomycin, the spare respiratory capacity (SRC), i.e. the difference between the maximal and basal cellular OCR, was underestimated by 25 to 45%. The inhibitory effect of oligomycin on SRC was more pronounced in T98G cells and was observed in both suspended and attached cells. Further experiments indicated that the inhibitory effect of oligomycin on CCCP-induced maximal OCR did not occur when glucose was replaced by glutamine as metabolic substrate. We replaced CCCP by FCCP, another potent protonophore and similar results were observed. Lower maximal OCR and SRC values were obtained with the weaker protonophore 2,4-dinitophenol, and these parameters were not affected by the presence of oligomycin. In addition, exogenous ATP, but not ADP, inhibited CCCP-induced maximal OCR in permeabilized cells. **Conclusions:** We conclude that unless a previously validated protocol is employed, maximal ETS capacity in intact cells should be estimated without oligomycin. The inhibitory effect of oligomycin on potent protonophore-induced maximal OCR may be associated with inhibition of ATP hydrolysis by reverse ATPase activity, maintenance of intracellular ATP levels and consequently ATP inhibition of cytochrome *c* oxidase (respiratory complex IV) activity.

Keywords: ATP synthase; Mitochondrial electron transport system; Oxidative Phosphorylation; Spare respiratory capacity; Tumor cells.

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