Inhibition of Apoptosis Partially Restores Mitochondrial Function

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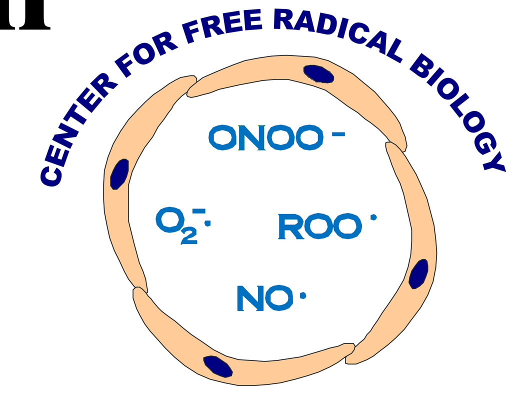
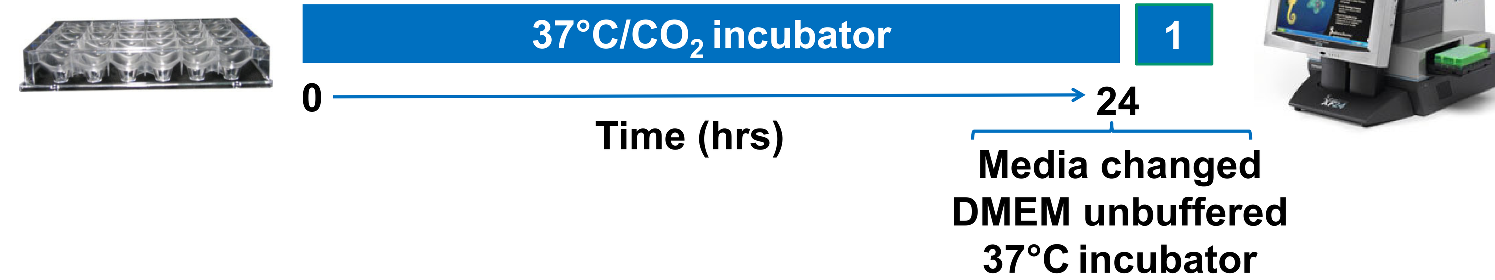


Introduction

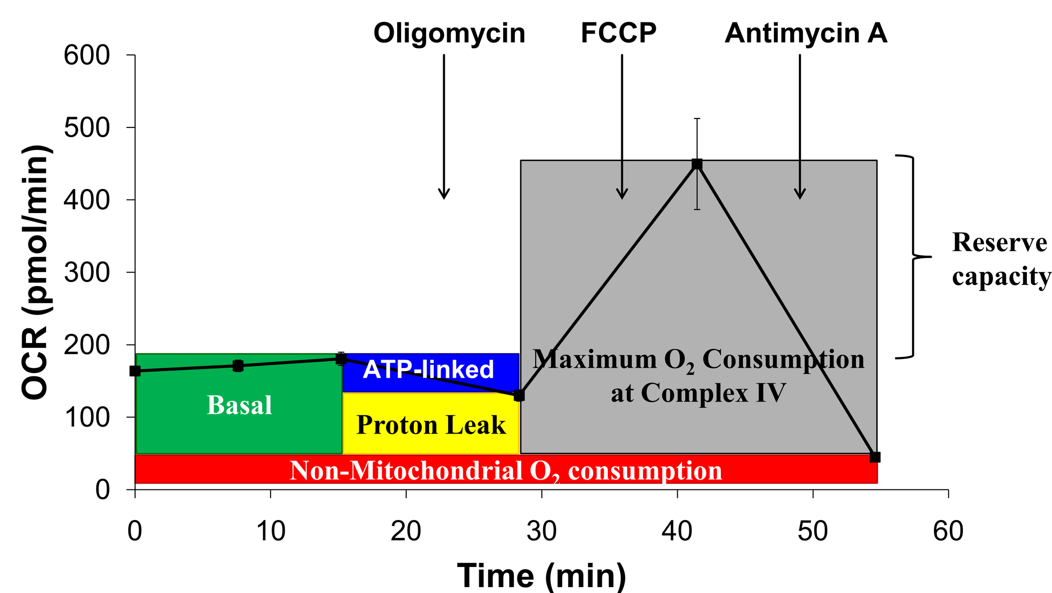
- It has been shown that mitochondrial dysfunction and reactive oxygen species (ROS) play a critical role in the pathogenesis of many neurodegenerative and neurovascular conditions, such as Alzheimer's disease, Parkinson disease, Huntington's disease, amyotrophic lateral sclerosis and stroke.
- It is generally accepted that once mitochondria are damaged, function cannot be restored and apoptosis and cell death occurs in neurons and endothelial cells; however, the mechanism of this process remains unclear.
- In the present study, we determine the effects of bolus doses of hydrogen peroxide (H_2O_2) and of intracellular H_2O_2 production by 2,3-dimethoxy-1,4-naphtoquinone (DMNQ) on mitochondrial respiration of endothelial and SHSY5Y cells using a Seahorse Bioscience XF24 Extracellular Flux Analyzer. To further investigate the relationship between mitochondrial function and apoptosis, we used a specific pharmacological inhibitor of caspase-3 and caspase-9 activation.

Methods

Standard Protocol



Measurement of Mitochondrial Function by the extracellular flux analyzer



- Protein concentration was determined using the Lowry assay.
- SDS/PAGE and Western blot. Lysates were obtained by adding 25 µL of Laemli Buffer into each well. Two replicate wells were combined to run samples on SDS-PAGE.

Results

Bioenergetic profile of BAEC treated with H_2O_2 (Dose-response)

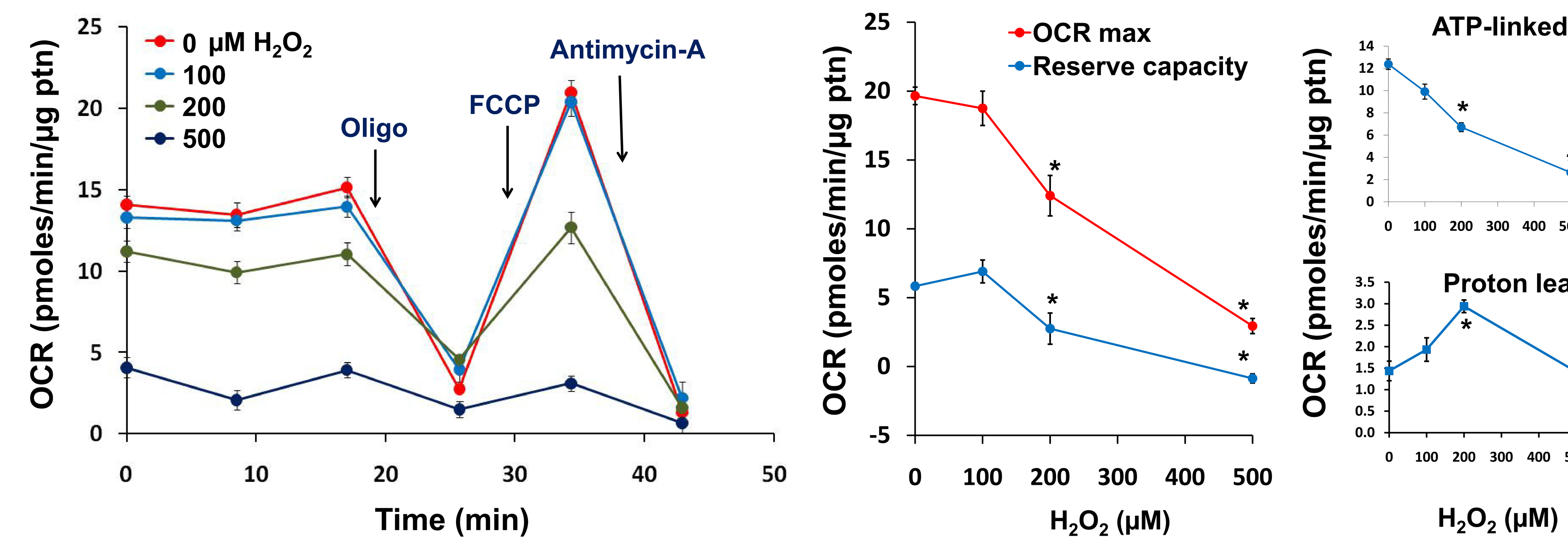


Fig. 1. Oxygen consumption rate (OCR) of Bovine Aortic Endothelial Cells (BAEC) treated with inhibitors of mitochondrial respiration. BAEC were pretreated with H_2O_2 (0 to 500 µM) for 1 hour previous to XF24 Analyzer. Shown is a representative oxygen consumption rate (OCR) trace of basal mitochondrial function followed by the sequential addition of oligomycin (1 µM), FCCP (1 µM) and antimycin–A (10 µM). Results are mean SEM, n≥3 per group *p≤0.05 vs. Control.

Mitochondrial Function was Partially Restored by Caspase-3 Inhibitor

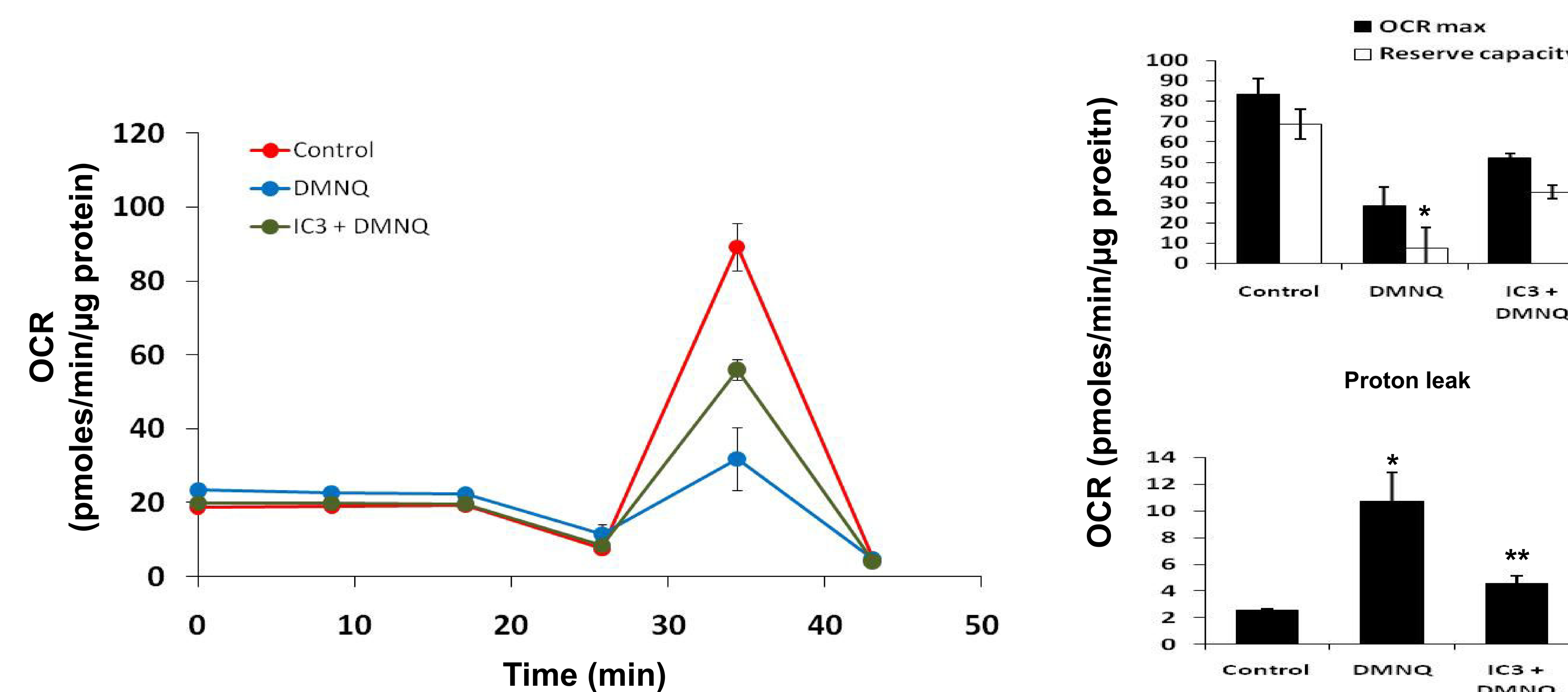


Fig. 2 Mitochondrial function was partially restored by caspase-3 inhibition. Bovine Aortic Endothelial Cells (BAEC) were treated with 20 µM caspase-3 inhibitor (IC3) for 1 hour and 15 µM DMNQ for an additional of 3 hours prior to bioenergetics measurement. Shown is a representative oxygen consumption rate (OCR) trace of basal mitochondrial function followed by the sequential addition of oligomycin (1 µM), FCCP (1 µM) and antimycin–A (10 µM). Data shown are the mean sem, n=3-5 per group, *p≤0.05 vs. Control, **p ≤0.05 vs. DMNQ treated cells.

Caspase-3 Inhibition

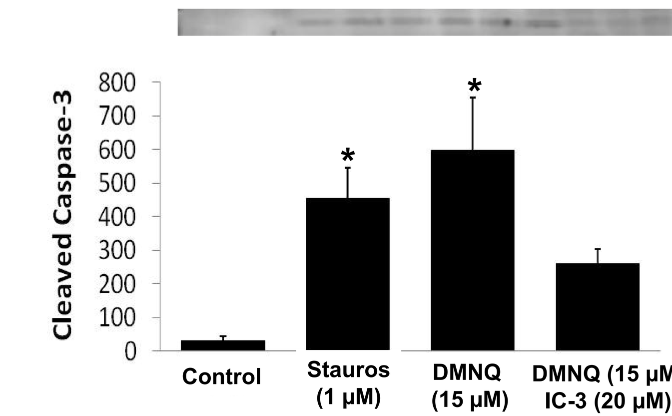


Fig. 3. DMNQ induces caspase-3 activation. Inhibitor of caspase-3 (20µM) was added to cells 30 min prior to 4hr treatment of DMNQ. Staurosporine positive control was used for Western Blotting analyses. All data shown are means SEM, n≥3; *p<0.05 vs. control.

SHSY5Y cells

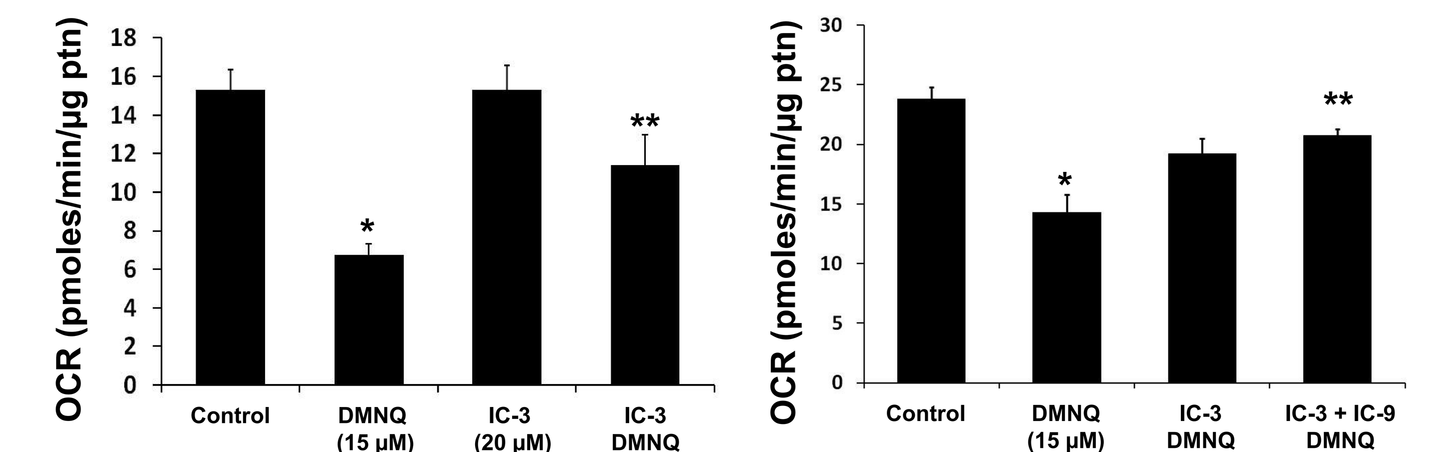


Fig. 4. Basal OCR was partially restored by caspase-3 and caspase-9 inhibition. SHSY5Y cells were treated with caspase-3 inhibitor (IC-3) and caspase-9 inhibitor (IC-9) for an hour and 15 µM DMNQ for an additional of 3 hours prior to bioenergetics measurement. All data shown are means SEM, n≥3; *p<0.01 vs. control and **p<0.05 vs. DMNQ treated cells.

Summary

- In both endothelial and neuroblastoma cells, we found that ROS were cytotoxic and associated with the activation of caspase-9 and caspase-3. The maximal and reserve capacities of the cells were decreased with the addition of ROS in the same concentration-dependent manner.
- Treatment with the caspase-3 inhibitor prior to DMNQ exposure resulted in the partial restoration of the reserve capacity, as well as a decrease on the extent of proton leak compared to cells treated with DMNQ alone. A commensurate decrease in cleaved caspase-3 was confirmed via Western blot analysis.
- Taken together, our results suggest that H_2O_2 causes mitochondrial dysfunction, triggering caspase-3 activation, which then induces apoptosis. This probably occurs through interconnected pathways, because inhibition of caspase-3 partially rescues mitochondrial function.

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