Effect of Metabolic Inhibitors and Hypoxia on Cellular Metabolism of Single Human Coronary Artery Smooth Muscle Cells

Background: ATP, the main energy currency of cells, also acts as an important signalling molecule in coronary arteries. It is generally understood that ATP production relies on the delivery of oxygen and is therefore reduced during hypoxia. The change in nucleotide levels has been proposed to trigger dilation of coronary arteries and increase blood flow, but the exact mechanism is still controversial. Here, we investigated whether metabolic inhibition and lower oxygen tension cause changes in cellular metabolism in single human coronary artery smooth muscle cells (HCASMCs).

Methods: Seahorse technique was used to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of HCASMCs. Cellular ATP was measured using CellTiter-Glo Luminescent Cell Viability Assay. Perceval/PercevalHR, fluorescent reporters of ATP/ADP ratio (1, 2), were transfected into HCASMCs. Intracellular pH was monitored using a genetically encoded biosensor, pHRed (3). Changes in mitochondrial membrane were characterized by a mitochondrial membrane potential sensitive dye, Rhodamine123. The fluorescent signal was collected in a confocal microscope. ATP/ADP ratio in the cell membrane micro-domain was examined by TIRF imaging or using Lyn-FUGW-PercevalHR.

Results: Application of 1 µM oligomycin reduced OCR from 5.93±0.43 pmol/min/µg protein to 2.66±0.36 pmol/min/µg protein, which indicated ATP synthase-linked OCR accounts for 55.24% of total OCR at basal level. Basal ECAR in glucose free Seahorse medium was 3.24±0.18 mpH/min/µg protein, application of 10 mM glucose increased ECAR to 85.27±3.22 mpH/min/µg protein, and 1 µM oligomycin application lead to a maximum of 12.18±0.50 mpH/min/µg protein. Application of metabolic inhibitors for 10 minutes decreased ATP by 22.00±1.86% (5 mM 2-deoxy-D-glucose/2-DG, n=4), 5.65±1.65% (6 µM oligomycin, n=4), 36.37±0.56% (2-DG plus oligomycin, n=4). Application of 2-DG decreased Perceval fluorescence by 54.02±15.29% (n=3). Inhibiting oxidative phosphorylation caused a reduction in PercevalHR fluorescence by 16.36±2.32% (1 µM Rotenone, n=58), 53.92±2.38% (1 µM antimycin, n=41), 37.97±2.17% (6 µM oligomycin, n=71), and 33.14±2.76% (1 µM CCCP, n=54). Transient hypoxia (1% O_2) had no effect on ATP/ADP ratio signal. The majority of the cells showed no change in intracellular pH under experimental conditions, indicating the changes in Perceval/PercevalHR fluorescence is not due to the changes of pH. Recording mitochondrial membrane potential showed no statistically significant change under hypoxia when compared to normoxia. Using TIRF imaging or by adding a membrane targeting sequence to PercevalHR allowed to examine energy changes in cell membrane micro-domains.

Conclusion: HCASMCs manifest a relatively oxidative bioenergetic phenotype. Inhibiting glycolysis and oxidative phosphorylation by metabolic inhibitors caused a reduction in cellular ATP and ATP/ADP ratio. 1% O_2 had no effect on ATP/ADP ratio under our experimental conditions.

***References*:**

(1) Berg J et al. (2009). *Nat Methods* **6**: 161-6.
(2) Tantama et al. (2013).*Nat Commun* **4**:2550.
(3) Tantama M et al. (2011). *J AM Chem Soc* **133**:10034-7.