Assessing the Impacts of Various Substrates on Mitochondrial Superoxide Flashes

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BACKGROUND

Reactive oxygen species (ROS)
- class of radical or non-radical oxygen containing molecules
- display high reactivity with organic molecules
- generated as by-products of mitochondrial respiration
- disproportionation reaction to convert to hydrogen peroxide.

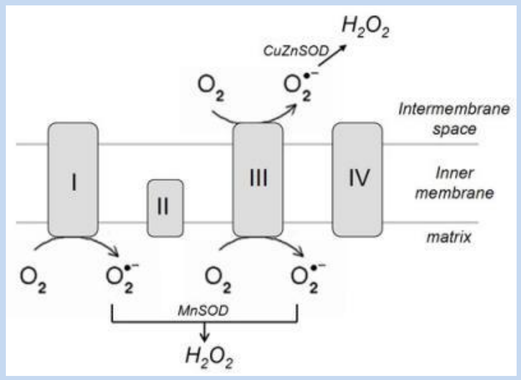


Figure 1: Reactive Oxygen Species produced as by-products of electron transport chain.

Significance: Homeostatic levels of ROS are important for:
- Cell metabolism
- Ion channels /transporter,
- Protein kinase/phophatase activation
- Gene expression

When ROS production exceeds anti-oxidative defense mechanisms, oxidative stress and damage ensue. High levels of ROS can lead to cell death and are linked to diseases including Alzheimer's, diabetes and cancer. Further studies on ROS will assist in understanding their roles in reactive oxygen signalling and whether they are linked to metabolic diseases.

INTRODUCTION

Rigorous well controlled studies of the role of ROS in mitochondrial dysfunction and insulin resistance had not been possible due to the lack of highly specific and sensitive ROS biosensors. Recent studies at University of Rochester identified a sensor mt-cpYFP which can measure superoxide. They noticed that superoxide is produced in 'flashes' and named this phenomenon mitochondrial superoxide flashes (mSOF). They are stochastic, quantal bursts of superoxide in the mitochondria with a range of periodicities (11-20s) occurring in a wide variety of cell types.

This study investigates:
- the effect of different metabolic substrates and inhibitors on the frequency and duration of the mSOF
- identify if they are metabolically linked

This study will be carried out in muscle fibers isolated from mice that express the mt-cpYFP protein in muscle mitochondria. After the muscle fibers are isolated and treated with specific substrates, they are viewed under the microscope and images are taken for specific time intervals. The flash events of the control fibers will be compared to the treated fibers.

In this particular study, the control samples will be compared to fibers that are treated:
1. Oligomycin (5 uM)
2. Antimycin A (2.5 uM)
3. Exposure to high glucose (25 mM) media.

MATERIALS AND METHODS

Flexor Digitorum Brevis (FDB) Dissection
1. Cull mouse, cut off feet at the ankle and place into ringer solution
2. Cut away skin from the bottom of the foot pad to reveal tendons and muscle
4. Cut large tendon at the ankle joint and pull FDB upwards and off, cutting tendons at toes
5. Put both FDBs into a 15 ml falcon tube in 7 ml Ringer

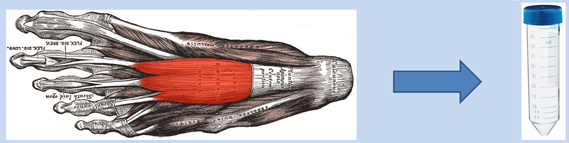
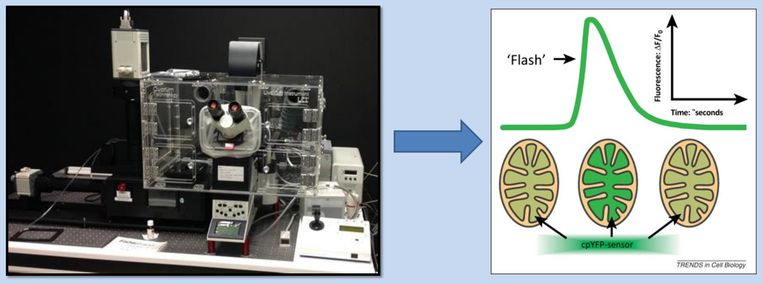


Figure 2: Location of the Flexor Digitorum Brevis.

Seperating Individual Muscle Fibers
1. Collagenase A to digest connective tissue (1mg/ml)
2. Shake at 37°C for 45 min
3. Wash with 3 x Ringer or F12 media to remove collagenase A
4. Add 3ml ringer (1.5ml/foot)
5. Gently triturate muscle to break apart and release muscle fibers into solution using a glass blown pipet.

Imaging
1. Transfer solution of muscle (without the tendon) with a pipette to the imaging dishes
2. Allow fibers to adhere to the dish for 1 hour at room temperature
3. Image using the Quorum Spinning-disk Confocal Microscope



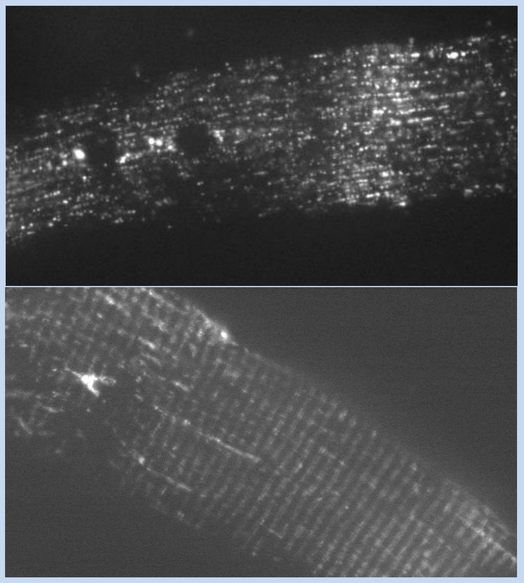


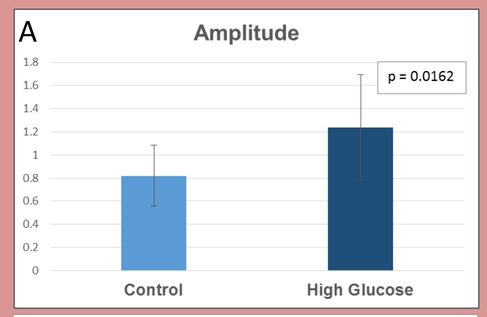
Figure 4: Muscle fibers viewed under the microscope (x63). Comparison of a fiber undergoing apoptosis which we would not image (top) and a suitable fiber (bottom)

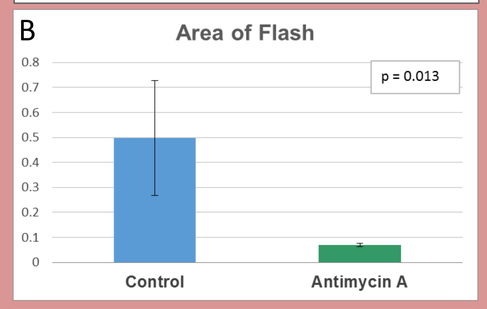
ANALYSIS

After the images were captured using the Quorum Spinning-disk Confocal Microscope, they were analysed using MATLAB to detect the flash events. MATLAB detects the number of flashes, the duration, the time to peak, the area and the amplitude. Data were analysed using a Student's T-test.

RESULTS

Significant results were observed for high glucose (25 mM) treated fibers compared to control (5 mM glucose) fibers. The amplitude of flashes for high glucose is 1.239±0.46 um compared to 0.820±0.26 um for control fibers (P=0.0162).





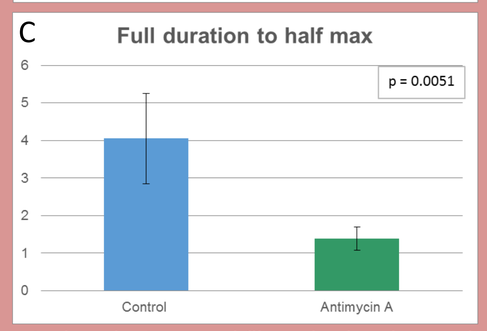


Figure 5: (A) Comparing the flash amplitude of the control fibers and the high glucose treated fibers. (B) Comparing the full duration to half max and (C) area of flash of control and antimycin treated fibers. Data expressed as Mean ± standard deviation (SD).

Antimycin-A treated fibers had a shorter time to half max, 1.383±0.31 s, compared to 4.049±1.20 s for control fibers (P=0.0051). The area of the flash differed significantly for treated fibers, 0.0684±0.007 um^2, and controlled fibers, 0.4984±0.23 um^2 (P=0.013).

CONCLUSIONS

Prior to the experiment, it was predicted that the mitochondrial flashes are linked to cellular metabolism.

For high glucose treated cells:
- more cellular metabolism and more ROS to be produced
- more flashes or higher amplitude of flashes are expected
- prediction is supported based on amplitude comparisons

Cells treated with antimycin-A and oligomycin:
- both inhibitors of the electron transport chain (ubiquinol and ATP sythase) and disrupt production of ATP
- Fewer flashes are expected compared to the controlled cells.
- Antimycin-A resulted in a shorter time to half max and the flash area was smaller

These results suggests that mSOF are linked to cellular metabolism. Further research will lead to a greater understanding of the mechanisms involved in producing these differences.

REFERENCES AND ACKNOWLEDGMENTS

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