The Effects of Specific Mitochondrial Inhibitors on Respiration and ROS Production

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Introduction

Mitochondrial Reactive Oxygen Species (mtROS) are chemically reactive species containing oxygen. Examples include peroxides, superoxide, hydroxyl radical, and singlet oxygen.

Although mtROS are thought to be the cause of ageing and age-related diseases, such as Parkinson`s and Alzheimer, in the last twenty years has been shown that they have a key role in **maintaining cellular homeostasis**.

Their dual nature as both signalling and damaging molecules, forced us to rethink the current hypothesis where only the amount of ROS is responsible to define their biological function. In fact, has been reported that increasing ROS level by inducing **Reverse Electron Transport (RET)** at **specific SITE** in the respiratory complex I (CI) can extend lifespan and preserve mitochondrial function (Figure 1).

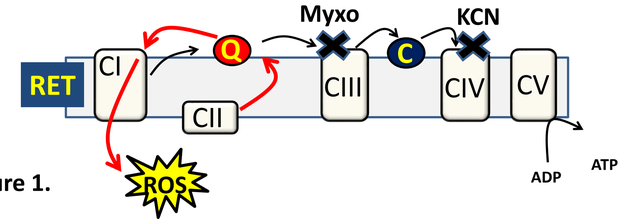


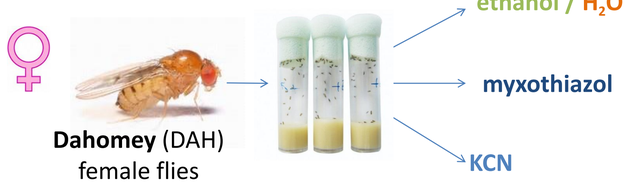
Figure 1.

Aim

The aim of this project was to determine if the inhibition of respiratory Complex III (CIII), by using **Myxothiazol** (Myxo), or Complex IV (IV) with **potassium cyanide** (KCN), was able to induce an over-reduction of the Ubiquinone pool (Q) and increase ROS production induced by RET (Figure 1).

Methods

Fruit flies were feed with different concentrations of **myxothiazol, potasium cyanide** and/or **ethanol/H_2O** (control) added in the food. Mitochondrial respiration and ROS levels where then measured in fly heads by using **high resolution respirometry** and **confocal microscopy** respectively.



Dahomey (DAH) female flies

Results

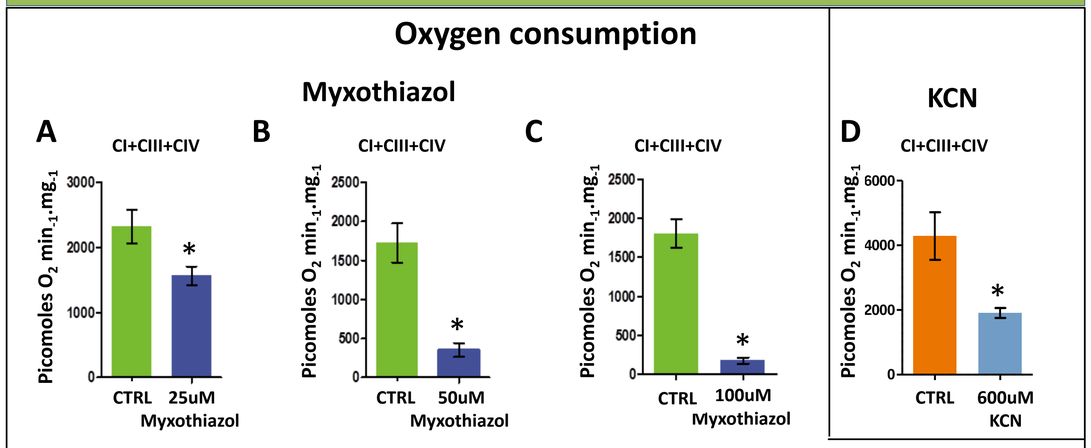


Figure 2. Respiration levels of mitochondria in fly heads following specific myxothiazol and KCN treatment. Flies heads were separated from the bodies and gently homogenised by using a pestle and mortar. The flies heads homogenate was filtered and the respiration was measured by adding 100uL of the homogenate into the chamber of a OROBOROS Oxygraph-2K . The respiration values were than normalized to protein concentration in fly homogenate. (A,B,C) Respiration after Mixothiazol feeding. (D) Respiration after KCN feeding. (*Mean +/- SEM)

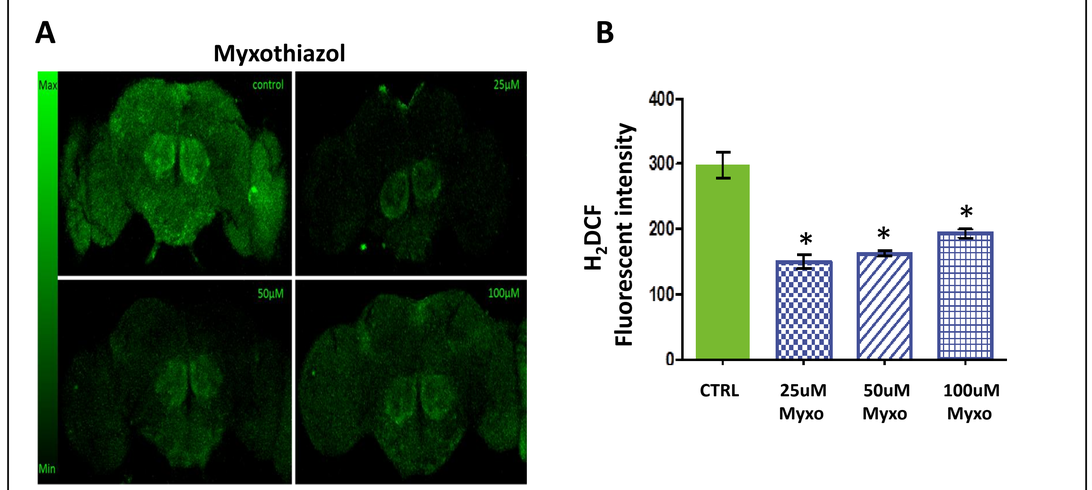


Figure 3. Levels of Reactive Oxygen Species in *D. melanogaster* brains after specific treatment with myxothiazol. Flies brains were dissected and incubated with 30uM H_2DCF in PBS1X for 10 minutes. After the incubation, the brains were washed and imaged by using an inverted confocal microscope. (A) Representative images of fly brains for each Myxothiazol treatment. (B) Quantification of H_2DCF fluorescence (minimum 5 brain per group). (*Mean +/- SEM)

Conclusion

As shown in figure 2A, B, C, mitochondrial respiration **decreased significantly** after myxothiazol feeding. It can be seen that the inhibition of mitochondrial respiration was proportional to the amount of inhibitor added in the food, proven that the feeding experiment was robust and effective. Same result was obtained with the Complex IV inhibitor KCN. Indeed, **KCN** resulted in **56% inhibition** of mitochondrial respiration (Figure 2D).

The H_2DCF staining demonstrated that the inhibition of mitochondrial respiration was followed by a **reduction in ROS production** with the three myxothiazol concentrations tested. This result provide evidence that inhibition of Complex III was not enough to induce both an over-reduction of the ubiquinone pool and a ROS generation via RET (Figure 3).

Discussion

RET has been shown to be an **important signaling mechanism** that can regulate many physiological process and even induce an **increase in longevity**. Understand the mechanism by which this signal is generated is of crucial importance since could be used as therapy for aging and age related diseases, such as Parkinson`s and Alzheimer.

I tested the hypothesis that the inhibition of CIII, by feeding flies with myxothiazol, could have caused an over-reduction of the ubiquinone pool and increasing ROS production via RET.

My experiments show a strong **inhibition in respiration after myxothiazol feeding** (Figure 2) **that was followed by a reduction in ROS production** (Figure 3), ruling out CIII as potential target to induce the RET signal. KCN feeding also resulted in a strong inhibition of mitochondrial respiration and further ROS experiment need to be performed to elucidate its involvement in the RET generation.

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