Caffeine and oxidative stress: Optogenetic analysis of hyperexcitable Drosophila mutants

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Background

- One byproduct of the electron transport chain in the mitochondria are Reactive Oxidative Species (ROS), a type of free radical.
- Free radicals are reactive molecules with one or more unpaired electrons and are known to cause damage to proteins, cellular membranes, and DNA.
- Glutathione S-Transferase D (GstD) is a member of a family of multifunctional proteins known for protecting cells from oxidative stress, such as that caused by ROS.
- Antioxidant Response Element is in the promoter region of the GstD gene and up-regulates transcription in response to increased levels of ROS.
- Green Fluorescent Protein (GFP) is a protein extracted from jellyfish and is commonly used as a reporter of expression.

Objectives

- Observe the effects of caffeine and temperature on ROS levels and determine phenotypic differences between different genotypes of flies (ARE/+ vs ARE/Δ) when their metabolism is manipulated, thereby affecting ROS levels and GFP production.

Results

- Figure 1: Model of the ARE promoter region of DNA that is responsible for GstD production. The cloned ARE promoter codes for GFP instead of GstD. Because the body cannot differentiate between the two promoters both are activated when ROS are present which results in GFP transcription. Adapted from http://www.nature.com/scitable/topicpage/gene-expression-1412169

Methods

- Flies were crossed to acquire ARE/+ (wild-type control for the Shaker mutation) and ARE/ShΔ133/+ flies (see Figure 2). ARE/+ flies were also crossed to serve as a genetic control for GstD-ARE-GFP mutants.
- 10 flies of each genotype were put into various conditions including control food, without caffeine, at 23°C, 5 mM caffeine food at 23°C, control food at 29°C, and 5 mM caffeine food at 29°C.
- Flies were anesthetized using ether, then fluorescent "before" photos were taken using a GFP filter cube. The flies were then put in their respective conditions for 3 days, after which the experimental fluorescent photos were taken.

Figure 2. Timeline of the fly imaging process. The flies were kept in their conditions from Day 3 through Day 6.

- For the purpose of acquiring quantitative data, MatLab was used to collect the average intensity of fluorescent light from the flies' abdomens. The intensity was measured on a scale ranging from 0-255, 0 being black and 255 being green.
- A one-way ANOVA test was performed on the above data. Flies ARE/+ and ARE/ShΔ133/+ in Caffeine food at 29°C were found to be significantly brighter than both control and Caffeine flies at 29°C, while flies in control food were found to be significantly different (**).

Conclusions

- A significant difference in brightness was found between ARE and ΔARE, so we now have a noninvasive tool for directly measuring oxidative stress in flies. Caffeine and 29°C are involved in causing increased oxidative stress.

Future Studies

- Continue the experiment with different genetic crosses.
- Utilize this method of measuring ROS levels with Super Oxide Dismutase (Sod) flies. These flies struggle to cope with oxidative stress, but it has been shown that housing them with non-Sod mutants produces a "helper effect" by increasing Sod flies' stress resistance and motor coordination. It is thought that the helper effect also decreases oxidative stress in Sod flies; therefore this tool can be utilized to confirm this.

Sources


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