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Effects of the Msr system in Drosophila Melanogaster cell cultures; on dopamine levels in the organism as a whole, and the localization of MsrA and MsrB isofomrs

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All organisms rely on protective mechanisms in response to cellular oxidation due to free radical reactions. Methionine sulfoxide reductase and superoxide dismutase (SOD) are two enzymes that protect the cell from oxidative damage by a reversal of the damaging reaction via a redox reaction. Oxidative damage ages the organism due to the reaction of oxygen with key cellular components such as proteins.
The overall goal is to investigate the role of the Msr proteins in cells damaged due to oxidative stress. There is, therefore, a pressing need for conducting a study on an embryonic mutant cell culture for in vitro investigation of how the Msr system works in Msr null mutants. In addition to biochemical cell studies, a preliminary analysis of dopamine levels has shown that it is possible that the Msr system could play an important role in maintaining dopamine levels at a necessary physiological range. Finally, the cellular locations of the various isoforms are currently unconfirmed. Four isoforms have been subcloned into an expression vector and will be used to visualize the protein's respective location.

Does Methionine Sulfoxide Reductase Have A Role In Maintaining Adequate Dopamine Levels in *Drosophila Melanogaster*?

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Abstract

•The overall goal is to investigate the role of the Msr proteins in dopamine production. A preliminary analysis of dopamine levels has shown that it is possible that the Msr system could play an important role in maintaining dopamine levels at a necessary physiological range.

Introduction

•All organisms rely on protective mechanisms in response to cellular oxidation due to free radical reactions. Methionine sulfoxide reductase is an enzyme that protects the cell from oxidative damage by reversing oxidative damage via redox reaction. Oxidative damage ages organisms due oxygen reacting with key cellular components such as proteins.

•The Msr proteins have specificity for oxidized methionines. In mammals there is one MsrA gene and three MsrB genes. However, in drosophila melanogaster, there is one gene for MsrA and one gene for MsrB. Genetic crosses were done to obtain flies with both MsrA and MsrB knocked out; another with only MsrA knocked out; and yet another with only MsrB knocked out.

•The importance of studying Msr mutants is that it may elucidate the contributions of the Msr system on the production and/or maintenance on dopamine levels. It can be reasoned that increases in oxidative damage to proteins may have an effect on the levels of dopamine production. The rate-limiting step in the dopamine synthesis pathway is tyrosine hydroxylase (TH), which is also the first enzyme in the pathway. If TH damage due to protein oxidation occurs, a drop in dopamine synthesis could be inferred.

•If it can be shown that there is a loss of dopamine levels in the MsrA^{-}B^{-} mutants at 35-40 days of age, then it would be reasonable to assume that the Msr genes play a protective role in degeneration of dopaminergic neurons.

Materials and Methods

• Total fly extracts have been taken from the wild-type flies and MsrA^{-}B^{-} null mutant flies. The data suggests that at 35-40 days of age, the mutants have less dopamine than the wild-type strain. To confirm that dopamine is the peak that is being analyzed in the fly extract, a standard solution containing three concentrations of dopamine was made. The standards and the extracts are injected into an HPLC column.

Results

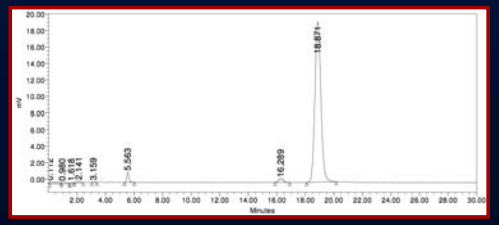

Figure 1. A 50 pmole dopamine standard is injected into an HPLC column and has a retention time of 18.87 minutes.

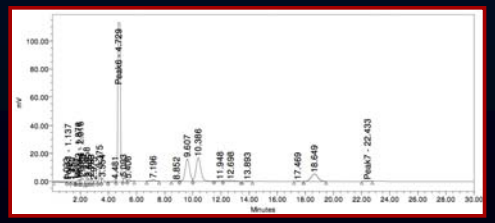

Figure 2. An Extract from the wild-type fly line was injected into an HPLC column. The peak at 18.649 minutes corresponds to the peak of the dopamine standard shown above.

Results

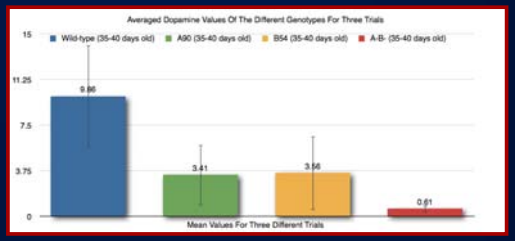

Figure 3. The chart shows the difference in the levels of dopamine between each of the genotypes used. The two middle bars show the dopamine levels for the single mutants, A^{-} and B^{-} from left to right. The data is based on the average dopamine levels from three trials. Although there does not seem to be a significant difference between the Wild-type and the single mutants, there does appear to be a difference between the wild-type and the double mutants.

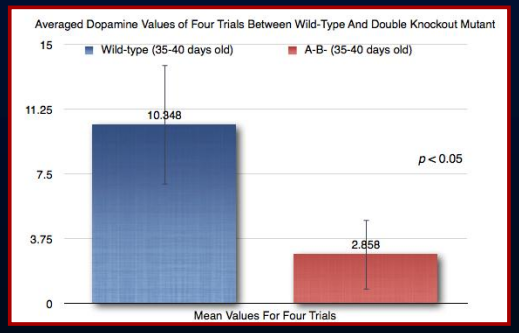

Figure 4. The chart shows the difference in the levels of dopamine between the wild-type flies and the MsrA^{-}B^{-} flies. The differences between these two genotypes will be focused on more so than the difference between all four genotypes used. The chart represents data on the dopamine levels averaged from four trials. It is shown that there appears to be a significant difference in the levels between these two genotypes.

Future Studies

•Role of the Msr system in Dopamine production:
- Could a lack of Msr hinder dopamine production, in turn, causing neurons dependent on dopaminergic output to cease functioning?
- A possible path of inquiry could be to study the Tyrosine Hydroxylase pathway with respect to the Msr system.
- Transgenic fly lines that would rescue the Msr mutant phenotypes are currently being made. Would dopamine levels be rescued?
- Are the mRNA levels of TH decreased in an Msr mutant? PCR may answer…
- Is the enzymatic activity of TH being compromised in the Msr mutants?
- Are the TH protein levels lowered in the Msr mutants? Western Blotting may answer…

References

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