Effects of Sulfur Dioxide on abdA Gene Expression and Larval Development in Drosophila melanogaster

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: Sulfur Dioxide toxicity
Gene: abdA: Abdominal A (abdA) is a homeobox-containing transcription factor that contributes to the development of embryonic segments.

1 Introduction

In this experiment, we tested the effects sulfur dioxide exposure had on abd-A gene expression and larval development in Drosophila melanogaster. Previous studies showed that sulfur dioxide exposure inhibits development of gonads in adult flies and increases development time. We hypothesized that expression of abd-A would decrease in developing Drosophila exposed to sulfur dioxide. The abd-A gene is in the Hox gene family, which functions in the development of body segmentation. The abd-A gene is specific to the abdominal region of the fly, and is also important to the development of gonads and fat bodies.

To test our hypothesis, we randomly selected three sets of mating pairs and allowed them to lay eggs in larval culture dishes. The experimental group of larvae was subjected to sulfur dioxide continuously for seven days, while the control group was not exposed to sulfur dioxide, but kept under the exact same conditions. After the exposure, fly larvae were extracted from their culture dishes, sorted by developmental stage, and counted. RNA was then extracted from the larvae after counting. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) was performed to determine abd-A expression in experimental and control groups.

Due to sulfur dioxide having detrimental effects on gonad and larval development, it was predicted that, in the presence of sulfur dioxide, abd-A gene expression would decrease, and developmental time course of larval development would be delayed.

2 Methods

Primer synthesis: Primers for abd-A were designed using Flybase and Integrated DNA Technologies PrimerQuest. The sequences of the forward and reverse primers targeting abd-A are as follows: GCACAGTTCGCTCAGTTCTATC (Forward) and GTCGCGCCTGGTCATTTATTTC (Reverse). The sequences of the control Gapdh primers are as follows: CGCCAAGAAGGTCATCATCTC (Forward) and CCTCGACCTTAGCCTTGATTTC (Reverse).

Culturing Drosophila: Wild type Drosophila cultures were purchased from Carolina Biological and maintained in Formula 4-24 Instant Drosophila Medium in culture vials as per the manufacturers protocol (Carolina Biological). Briefly, equal volumes of water and dry media were added to the culture tubes along with several grains of bakers yeast (Red Star Yeast). The cultures were incubated at room temperature and were sub-cultured every two weeks. The anesthetizing of the flies was accomplished using CO2 FlyBeds (Azer Scientific) and The Flowbuddy CO2 regulator (Flystuff.com). For the experiment, wild type flies were given 24hrs to mate in their respective experimental chambers. The adults were then removed and the experimental larval cultures were exposed to SO2 by placing them in a sealed beaker with solid Sulfur for 4 days while control larval cultures were placed in the same conditions, excluding the sulfur.

RNA extraction: RNA was extracted from control and experimental Drosophila larvae after 72hrs using Qiagens RNeasy Mini Kit as per the manufacturer's instructions. To prepare for RNA extraction, the larvae were transferred, using forceps, into 35mm petri dishes filled with 1x PBS (ThermoFisherScientific). The larvae were then placed in pre-chilled Eppendorf tubes and incubated in the freezer for 10 minutes. After freezing, a 1000uL micropipette tip was used to grind the larvae for 1 minute. Following the addition of Buffer RLT, the larval tissue was gently passed through an 18 gauge-needled syringe 10 times followed by a 25 gauge-needled syringe 10 times. During RNA extraction, the samples were subjected to on-column DNase treatment using an RNase-free DNase kit (Qiagen).

RT-PCR: cDNA was synthesized using the RevertAid RT kit (ThermoScientific) and following the manufacturer's protocol. PCR was performed using GoTaq Green PCR Master Mix (Promega) using the manufacturer's protocol. PCR amplfication of Gapdh cDNA was used as a positive control for the Drosophila RT-PCRs while the RevertAid Gapdh was used as a positive control for the entire RT-PCR experiment as the reagents for this sample were provided in the RevertAid kit. The No Template Control (NTC) reactions contained all reagents for the RT-PCR reaction, except nuclease-free water was added in place of RNA template.

Gel electrophoresis: The RT-PCR reactions were electrophoresed on a 1% agaorse gel (BioRad) in 1xTBE (VWR). The ImageJ gel analysis program was used to determine the relative intensities of the PCR products for semi-quantitative analysis.

Larval instar counts: Following 96hrs of exposure to SO2, the numbers of $1^{st}/2^{nd}$ and 3^{rd} instar larvae were determined by manual counting under a dissecting microscope. Briefly,

control and experimental flies were transferred, using forceps, to their respective 35mm petri dish w/1x PBS prior to counting. These same larvae were then used for the RNA extraction procedure described above.

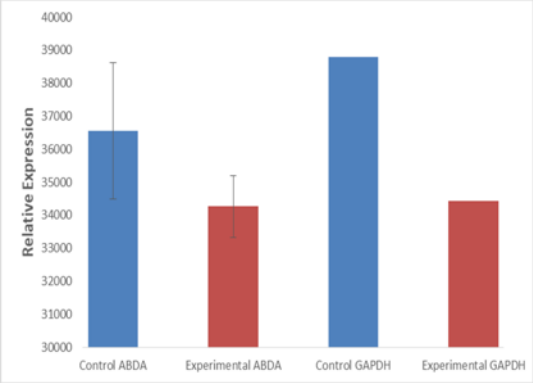
3 Results

Our results indicate that exposure of larvae to environmental SO2 resulted in no major change in expression of abd A, as seen in Figures 1 ($p = 0.53$). Furthermore, SO2 exposure did not affect the number of 1st/2nd or 3rd instar larva when comparing the experimental and control groups ($p=0.28$ for 1st/2nd instar and $p=0.77$ for 3^{rd} instar) . Based on our results, exposure of Drosophila larvae to SO2 affects neither expression of the abdA gene nor the viability of the larvae.

Future experiments could be improved upon by increasing sample size and by developing a more accurate system for exposing the Drosophila larvae to SO2 during development. Furthermore, a more quantitative measure should be performed to better determine whether Sulfur Dioxide affects expression of the abdA gene.

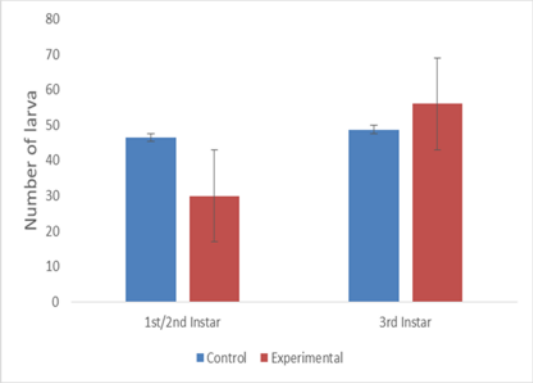
4 Figures

4.1 Relative Expression of abdA in Control and Sulfur Dioxide-treated Drosophila Cultures



The relative expression of abdA compared to the control gene Gapdh was measured using semi-quantitative RT-PCR across four control and four SiO2-treated Drosophila cultures. The error bars represent the standard error of the means for each condition. A student's t-test was performed to determine the significance of changes in gene expression between the control and experimental cultures, with $p=0.53$ for abdA and $p=0.10$ for Gapdh.

4.2 Larval Counts of Control and Sulfur Dioxide-treated Drosophila cultures



The average number of larvae in the 1st/2nd and 3rd instar stages across four control and four Sulfur Dioxide-treated cultures was found not to be significantly different (p=0.28 for 1st/2nd and p=0.77 for 3rd). The measure of statistical significance was determine using a two-tailed t-test assuming unequal variance and the error bars represent the standard error of the means.

5 Acknowledgements

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