The Effect of Serotonin in Hypoxia Induced Hemoglobin Producing Pathway in Water Fleas *(Daphnia magna)*

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Introduction

Environmental stress such as increasing population (Hobæk, Larsson, 1990), decreasing of illumination period (Rider *et al*.,2005) and hypoxia (Zeis *et al*., 2009) affect the phenotype of water fleas (*Daphnia magna*). Increasing hemoglobin amounts in water fleas' that are subjected to hypoxia result in visible red pigmentation (Gorr *et al*., 2004). Furthermore, serotonin (5-HT) shows a reducing effect to methyl farnesoate (juvenile hormone) synthesis which provides hemoglobin production by activating the globin 2 (hb2) gene (Gorr, 2006) by the help of decreasing of Pigment Concentrating Hormone (PCH) and rising of Pigment Dispersing Hormone (PDH) (Landau *et al*., 1989).

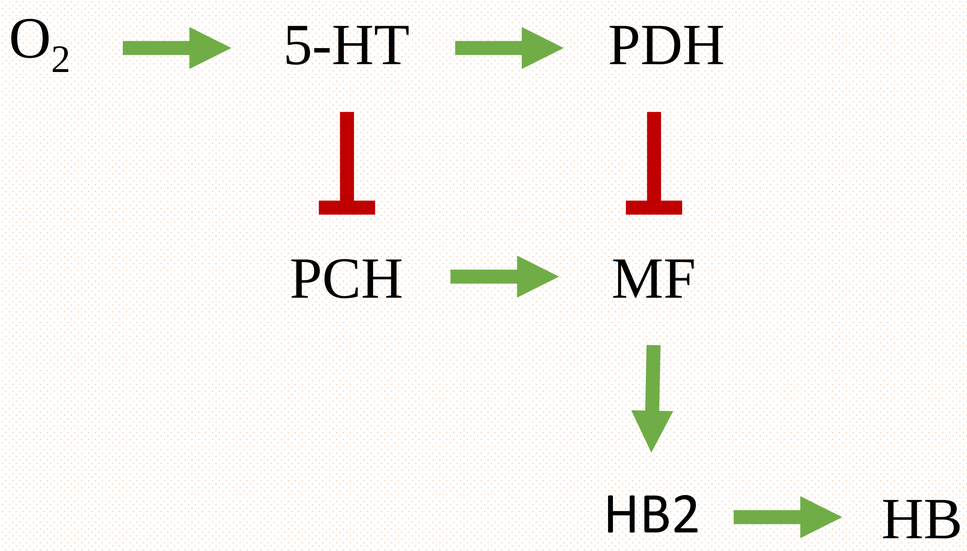

Figure 1. The Pathway of Oxygen, Serotonin and Hemoglobin Level.

(O_2, Oxygen 5-HT, Serotonin; PCH, Pigment Concentrating Hormone; PDH, Pigment Dispersing Hormone; MF, Metyl Farnesoate; HB2, gene of *Daphnia* Hemoglobin 2; HB, Hemoglobin)

Our hypothesis is that serotonin has suppressive effect on hypoxia induced hemoglobin pathway in *Daphnia magna*. Therefore, we carry out an experiment to test our hypothesis with using both agonist (Fluoxetine) and antagonist (Cyproheptadine) to affect serotonin receptor (5HT-2) which is probably related with hemoglobin production pathway on *Daphnia magna*.

Methods

Illumination and other conditions were made in accordance with the OECD (2012) protocol in the *Daphnia* growing section .Ten to fifteen water fleas are kept on 0.8 liter of artificial *Daphnia* medium within 1 liter jars during the experiment. Cyproheptadine (100 µg/L) were used for antogonist of 5-HT_2 (K_i = 1.67 nM). Besides, Fluoxetine (80 µg/L) were used for agonist of 5-$HT_{2.}$ (K_i = 1.00 nM). (Campos, Pina& Barata, 2012)

The experiment lasted for 11 days (Zeis *et al*., 2003). At the end of 11 days, level of hemoglobin was measured by application of the protocol of Yampolsky (n.d.) via spectrophotometer.

Absorbance of hemoglobin quantity peaked at many nanometer ranges. However, we elected the absorbance at 458 nm and 496 nm, which were the most repeated values in all samples. Their relative absorbances were normalized by the absorbance values in the Bradford method which used for detection of total protein content of water fleas.

Results

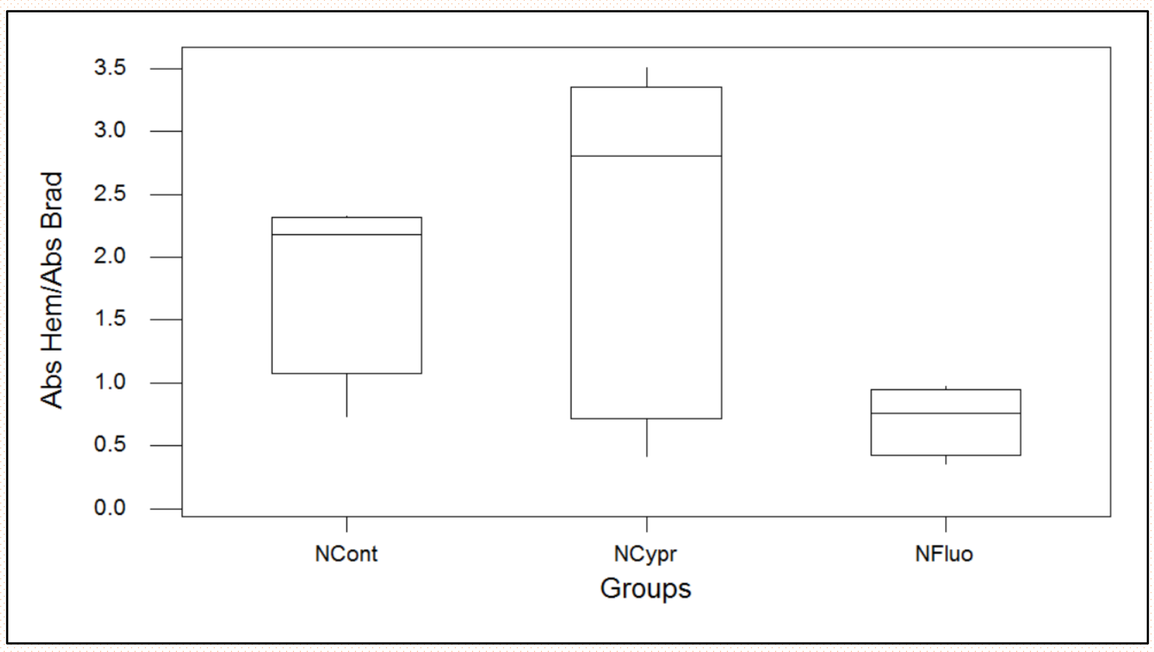

Figure 2. Relative Absorbance of Control, Cyproheptadine and Fluoxetine

(Total sample size of Control, Cyproheptadine and Fluoxetine Groups; Ncont, NCypr, and Nfluo, respectively. Abs Hem/ Abs Brad, ratio of absorbance value of hemoglobin and total protein)

The mean and standard deviation of relative absorbance of control, cyproheptadine and fluoxetine were 1.857 (st.dev= 0.757), 2.278 (st.dev= 1.325) and 0.708 (st.dev= 0.276), respectively. Increase in hemoglobin was observed as a result of suppression of serotonin by Cyproheptadine. Similarly, when fluoxetine triggers serotonin receptors, a significant decrease in relative absorbance of hemoglobin has been noticed. Therefore, these results confirm our hypothesis.

Future Directions

Figure 3. Artificial Hypoxic Environment for the Experiment

Studies have shown that the 5-HT2 receptor works efficiently with fluoxetine and cyproheptadine in water fleas. Our next step is to determine the difference between hypoxic and normal environment in serotonin pathway. With this system, the gas in the bottles will be absorbed and the oxygen content will be reduced to 15.2 pKa. (Zeis *et al*., 2003) (Other variables will be kept constant.)

Conclusion

Experiments have shown that Flouxetine acts as an agonist at the 5-HT2 receptor and Cyroheptadine acts as an antagonist. For this reason, the amount of hemoglobin was reduced by the treatment of Flouxetine, and was increased by the treatment of Cyproheptadine. Future work is expected to show in detail the relationship of oxygen deficiency with serotonin pathway.

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