

Serotonin Transporter mRNA Expression and Hemolymph Serotonin Concentrations in *Aplysia californica*

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Introduction

- Serotonin (5-hydroxytryptamine; 5-HT) is an important neurochemical that affects both the central nervous system as well as the periphery.¹
- The serotonin transporter (SERT) is responsible for the uptake of extracellular 5-HT and the control of circulating 5-HT concentrations.²
- 5-HT in teleost fish like the Gulf toadfish (*Opsanus beta*) has been observed to be vasoactive and involved in the response to low environmental oxygen levels (hypoxia).²
- Toadfish tissues such as the heart, gill, and brain have higher SERT mRNA expression and higher uptake of 5-HT from the circulation compared to other tissues.²
- Tissue SERT mRNA expression levels have not been quantified in *Aplysia californica*.

Objective

The objective of this study was to quantify hemolymph 5-HT concentrations and the expression of SERT mRNA within different tissue types of *Aplysia californica*. Higher expression of SERT mRNA within the heart compared to other tissues may suggest that SERT plays a similar role in 5-HT regulation as measured in toadfish.

Methods

- Aplysia* (n=8) were obtained from the National Resource for *Aplysia*.
- All specimens were from the same cohort and selected randomly
- Hemolymph samples were drawn and then *Aplysia* were anesthetized with MgCl₂ solution.
- Nervous tissue (N), gill (G), gastrointestinal tract (GI), heart (H) and hepatopancreas (L) were collected.
- Gene-specific primers for qPCR were created using Primer3web and the *Aplysia* SERT sequence (Accession # AF402096.3)
- cDNA was synthesized from the samples using RNA created through total RNA isolation.
- Quantitative PCR was run with the cDNA with the designed primers.
- The 2^{-ΔΔCt} method was used for each gene of interest to determine fold changes from the control of each time point.³
- Mean mRNA expression values were rescaled to nervous tissue.
- An ANOVA test was performed with tissue type as the main factor.

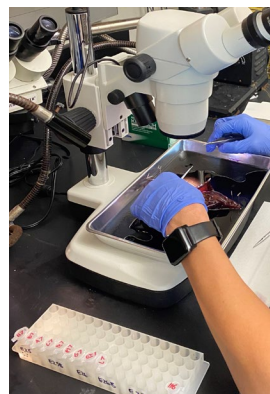


Figure 1. Demonstration of the dissection process to remove tissue samples using a dissection scope and pre-labeled bullet tubes that were then placed into liquid nitrogen

Results

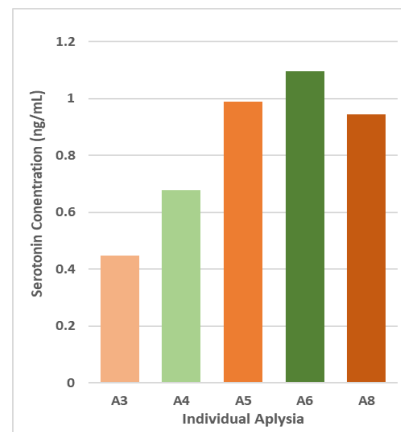


Figure 2. 5-HT concentrations measured in the hemolymph of individual *Aplysia*

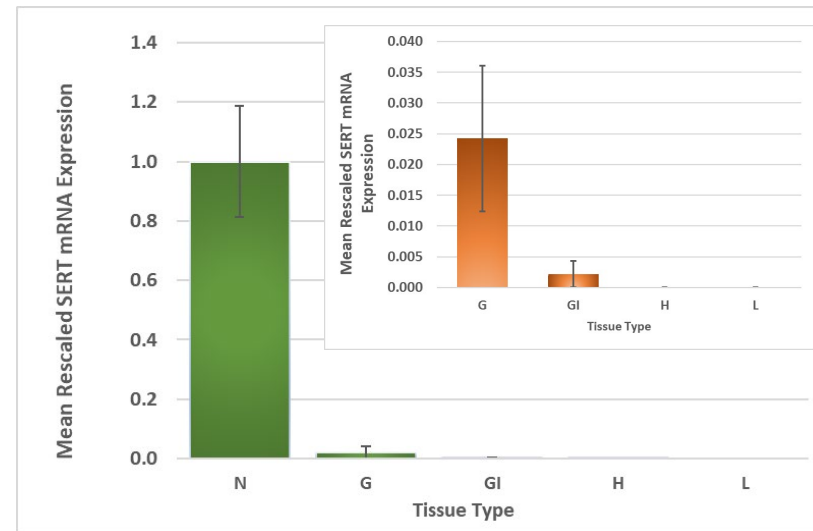


Figure 3. Average SERT mRNA expression in *Aplysia* tissue. N = nervous tissue, (n = 5); G = gill tissue, (n = 6); GI = gastrointestinal tissue, (n = 7); H = heart tissue, (n = 5); L = hepatopancreas tissue, (n = 3). Inset shows tissues with lower SERT mRNA expression. Values are means ± SEM

Discussion

- 5-HT levels within the hemolymph are close to concentrations found in previous *Aplysia* studies, albeit with a slightly higher average concentration (Figure 2).⁴
- Higher values may be attributed to time of sampling and stress induced upon the animals when withdrawing hemolymph.^{4,5}
- Concentrations of 5-HT found were also comparable with plasma 5-HT concentration measured in both fish and mammals.^{6,7,8}
- SERT mRNA expression is higher in nervous tissue than any other tissue type (Figure 3).
- This suggests that *Aplysia* utilizes 5-HT primarily within sensory neurons motor neurons consistent with sensitization studies.⁴
- It is surprising to see no measurable mRNA expression of SERT within the cardiac tissue since it is prominent within teleosts species like *Opsanus beta*.²

Future Work

- Establish physiological role of hemolymph 5-HT and gill SERT in *Aplysia*
- Investigate the relationship between hemolymph 5-HT, SERT mRNA expression, and environmental change such as hypoxia

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