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 2ΔΔCT method was used for each gene of interest to determine fold changes from the control of each time point.2
• Mean mRNA expression values were rescaled to nervous tissue.
• ANOVA test was performed with tissue type as the main factor.

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).

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.4,7
• 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.6
• 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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References

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