

Serotonin transporter distribution across tissues of lab-reared sheephead minnow, *Cyprinodon variegatus*

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Introduction

Sheepshead minnow in the wild are a historically hypoxia tolerant species that live in euryhaline environments (1, 2).

The neurochemical, serotonin (5-HT), is involved in the hypoxia response of many teleost fish in several ways including the vasoactivity of blood vessels which controls blood flow to tissues (3).

In hypoxia tolerant Gulf toadfish, *Opsanus beta*, and goldfish, *Carassius auratus*, there is high expression of the serotonin transporter (SERT) in the heart (4).

Unlike wild sheephead minnow, lab-reared sheephead minnow were found to have a short time to loss of equilibrium (tLOE), indicating hypoxia intolerance (5).

Objective and Hypothesis

Our objective was to determine relative SERT distribution across the different tissues of lab-reared sheephead minnow.

We hypothesized that there would not be a high presence of SERT in the heart of hypoxia intolerant lab-reared sheephead minnow.

Methods

Sheepshead minnow (n = 5) were over-anesthetized and heart, brain, gill, kidney, liver, and intestine were dissected and preserved in liquid nitrogen.

Preserved tissues underwent total RNA isolation, DNase treatment, and cDNA synthesis.

Hypoxanthine guanine phosphoribosyl-transferase (HPRT; housekeeping gene) and SERT (gene of interest) mRNA expression was analyzed using qPCR.

The values obtained by qPCR were tested for normality. A one-way ANOVA was performed on log-transformed data.

Results

Table 1: Total RNA concentrations across tissues found that not enough RNA was isolated from the heart to proceed with DNase treatment. 260:280 ratios were above the targeted value of ~2.0 indicating good quality RNA in all tissues but the kidney. Values are means \pm standard deviation.

	Heart	Brain	Gill	Kidney	Liver	Intestine
260:280	2.081 \pm 0.198	2.177 \pm 0.195	2.506 \pm 0.307	1.973 \pm 0.377	2.323 \pm 0.334	2.339 \pm 0.307
Total RNA Concentration (ng/μL)	94.5 \pm 49.1	598.0 \pm 325.0	1655.5 \pm 498.5	332.5 \pm 255.8	2399.2 \pm 541.6	2458.6 \pm 334.0

Table 2: Forward and reverse primers for HPRT and SERT used for qPCR.

Primer	Sequence (5' \rightarrow 3')
HPRT forward	CCTTTTGCATCGTGCTCACTTCA
HPRT reverse	TGCAGAACAGCTCAACTCAAAG
SERT forward	GCACTACGGTTTGGATCGAC
SERT reverse	CCAGCACGGTAAAGATGACG

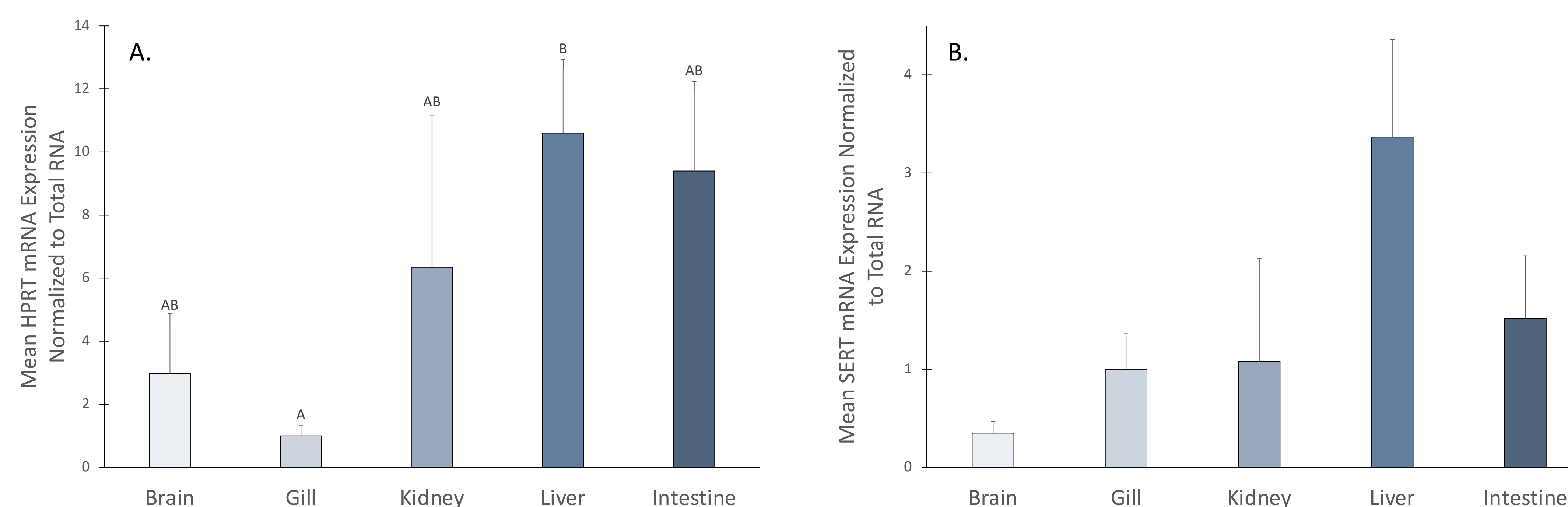


Figure 1: (A) HPRT and (B) SERT mRNA expression in different tissues. There was a statistically significant difference in the HPRT mRNA expression of the gill and liver tissues ($p = 0.044$) and no significant difference in the mRNA expression of SERT across any of the tissues ($p = 0.075$). Values are means \pm standard deviation, $n = 2 - 5$. Different letters denote significant difference.

References

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Discussion

The significant difference between the HPRT mRNA expression in the gill and liver indicates that HPRT is not the housekeeping gene most suited to use in sheephead minnow.

A different housekeeping gene or determining copy number may yield a better baseline for SERT comparisons across tissues.

Our hypothesis that there would not be a higher presence of SERT in the heart could not be tested because the low concentration of RNA in the heart yielded too little product for DNase treatment and following steps.

The lack of significant difference in SERT mRNA expression across tissues could be due to the low n for each tissue type or the small total n of animals sampled.

The tendency for the liver to have higher SERT mRNA expression than the brain has been shown in previous studies of Gulf toadfish (6, 4).

A previous study has suggested that this may imply that the liver is essential for 5-HT synthesis or sequestration in Gulf toadfish which could also be true for sheephead minnow (6).

Future Directions

Explore the mRNA expression of different housekeeping genes in these same tissues to find one that will stay constant.

Analyze SERT mRNA expression in heart by pooling individuals to get a higher quantity of total RNA.

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