Differential Expression of CFTR and three G protein coupled receptors in tissues of *Squalus acanthias*

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In the shark rectal gland, chloride secretion through CFTR is regulated by several G protein coupled receptors, including the vasoactive intestinal peptide receptor (VIP-R), the A_0 adenosine receptor, and the growth hormone releasing hormone (GHRH) receptor. The shark CFTR chloride channel was first sequenced by Marshall et al.[4] and has been extensively studied by patch clamp[2] and oocyte expression studies[1,3]. Our laboratory has cloned and sequenced the A_0 adenosine receptor, the VIP-R and GHRH-R in this tissue[6,7]. Little is known about the expression of these proteins in other shark tissues. In the present studies, we used shark specific primers in quantitative PCR to examine the level of mRNA expression of these membrane proteins in 11 shark tissues.

To compare the tissue expression levels of these membrane proteins in tissues of *Squalus acanthias* we applied the *Stratagene Brilliant SYBR Green QPCR System* using the Stratagene MX4000 Real-Time PCR instrument at MDIBL. Total RNA was extracted from tissues using Trizol reagent (Invitrogen). Prior to cDNA synthesis, RNA samples were DNase digested to avoid contamination with genomic DNA (Ambion). RNA quality was analyzed using a Agilent 2100 Bioanalyzer. A total of 3μg RNA was reverse transcribed using SuperScript First-Strand cDNA Synthesis system (Invitrogen). 1μl of cDNA was amplified with primers designed to produce an amplification of approximately 300bp.

CFTR sense: TCTCTGCCTTGGACGAATAATAGC
CFTRantisense: CACTGCCACGCCCTCATCA

VIPR sense primer: GTCCTGAGGGCCATCGCTGTCTT
VIPR antisense primer: GGGCCCGAATGATCCACCAATAC

A_0 adenosine receptor sense: AGCCGAGCGCCACATCAACATCAG
A_0 adenosine receptor antisense: CGGGGTACGGGAGGCAGGAGAC

GHRHR sense primer: ACAGAGCAAGGGTGGAGTGAAT
GHRHR antisense primer: TGGCGCGGAGAATGAAGGAC

beta actin sense primer: CTGGCATTGTGCTAGATTCTGGTG
beta actin antisense primer: AAGAGCTAGCCGTCTGCATCTCAG

Primer specificity was tested by conventional PCR and all primer pairs yielded a single band. Samples were prepared in triplicate and relative expression levels were calculated using the comparative threshold cycle (Ct) method. By subtracting the average beta-actin Ct value from the average target gene Ct-value, the expression levels of the target gene were normalized to the expression level of the reference gene (beta-actin). We picked one tissue from one shark as the standard and set its expression level as one. Expression levels in other tissues and sharks (n=6) were set in relation to this standard. The expression level was calculated using the following equation:

$$2^{-(\text{target gene[sample] Ct} - \text{Actin[sample] Ct}) - (\text{target gene[standard]})}$$

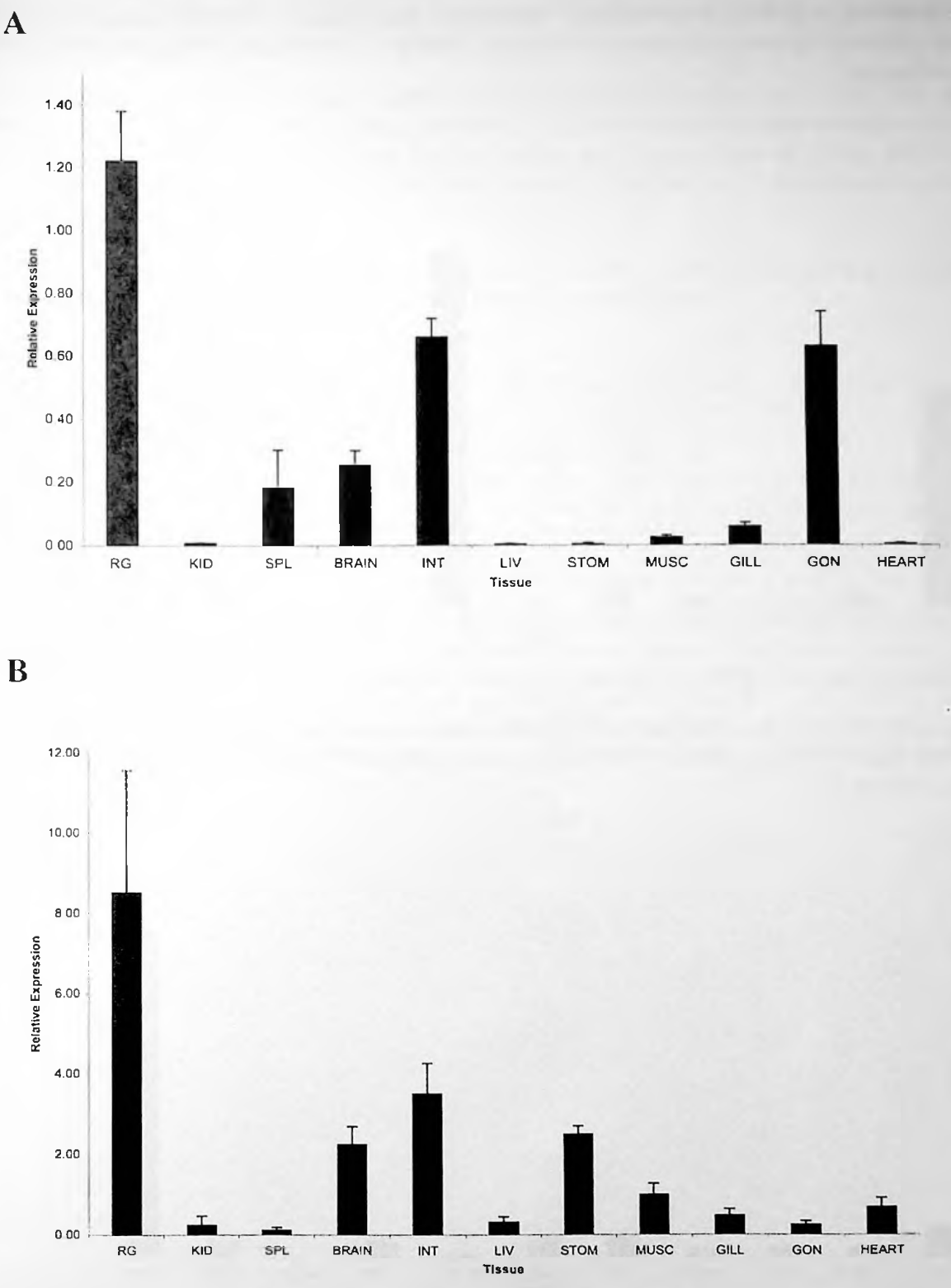

Figure1. Relative expression of CFTR (Panel A) AND VIP-R (Panel B) in shark tissues. Error bars indicate SE. N=6.

Figure 1 (Panel A) shows the relative expression of CFTR in 11 shark tissues. The highest level of expression was in rectal gland, followed by intestine and gonads. CFTR is well established as the pathway for chloride exit in rectal gland cells. Modest levels were seen in brain and spleen. There was

minimal expression in kidney, liver, stomach and heart. Expression of VIP-R was also highest in rectal gland, followed by intestine, stomach and brain. There were low levels of expression in kidney, liver, gill and gonad.

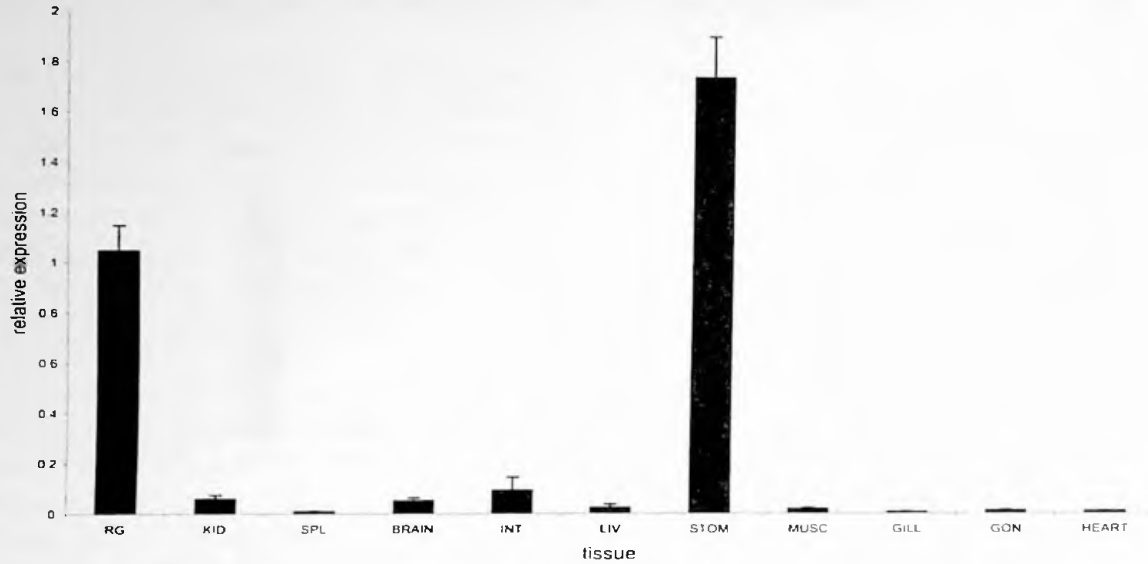

Figure 2. Relative expression of A_0 adenosine receptor in 11 shark tissues. Error bars indicate SE.

The A_0 adenosine receptor is known to be highly expressed in shark rectal gland. Unexpectedly, we also observed high levels of mRNA expression in shark stomach, with low levels of expression in other tissues (Figure 2).

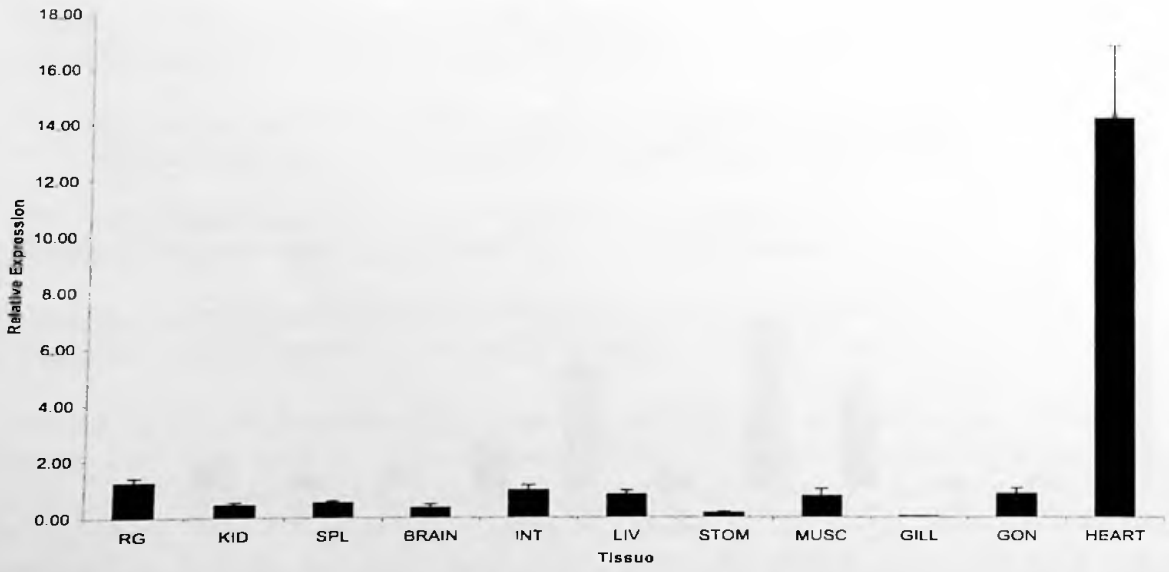

Figure 3. Relative expression of GHRH-R in 11 shark tissues. Error bars indicate SE, N=6.

The results show very high expression of GHRHR in heart, which is 40 times higher than brain and 14 times higher than rectal gland. This result is consistent with human tissue, where myocardium

shows the largest number of ligand binding sites for growth hormone peptides[5]. The next highest level of expression was seen in rectal gland (Figure 3).

These studies provide the first measurements of mRNA expression of CFTR, VIP-R, A_0, and GHRH-R in multiple shark tissues. High expression levels of these membrane proteins are consistent with their known function in mediating NaCl secretion in the rectal gland. Their high expression levels in other tissues, particularly epithelia, brain and heart, suggest likely physiological roles in these tissues.

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