DIFFERENTIAL EXPRESSION OF SHARK A_0 ADENOSINE RECEPTOR AND CFTR IN TISSUES OF *SQUALUS ACANTHIAS*

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The highly specialized tubular epithelium of the dogfish shark rectal gland (SRG) is a useful model for hormone-regulated chloride secretion through CFTR-like chloride channels. Our laboratory has recently cloned and sequenced a unique A_0 adenosine receptor from the shark rectal gland. This receptor is the oldest known adenosine receptor subtype and regulates chloride secretion through the CFTR-like chloride channel in the shark rectal gland. Shark CFTR is 72% identical to human CFTR (J. Marshall et al., J. Biol. Chem. 266:22749-22754, 1991). To determine the relative tissue expression of both the shark A_0 receptor and shark CFTR, we performed quantitative reverse-transcriptase PCR (RT-PCR) on a series of shark epithelial and non-epithelial tissues.

Total RNA extraction (United States Biochemical REX total RNA extraction kit), reverse-transcription (Invitrogen cDNA cycle kit) and PCR (Schofield et al., Am. J. Physiol. 261(4 Pt 2):F734-9, 1991) were performed according to the protocols. RNA samples were pre-treated with RNAse free DNAse. Control reactions contained either no template RNA (reverse-transcriptase controls) or no cDNA (PCR controls). Duplicate PCR reactions were performed on cDNA from each tissue. One reaction used shark specific histone H3.3 primers and the other used shark specific primers for either A_0 or CFTR. Precautions against contamination included the use of aerosol free pipette tips and performance of each step in separate hoods. Results were verified by repetition of PCR and enzyme cuts in known locations of the sequenced A_0 receptor from the rectal gland. The internal control standard was a shark specific histone H3.3 subtype, cloned and sequenced as reported in this bulletin (G. Hemminger et al., this Bulletin).

We first determined the effect of the number of PCR cycles on the intensity of the resulting product for the shark histone H3.3 subtype (PCR product - 255 bp) and the shark A_0 adenosine receptor (PCR product - 362 bp) on shark rectal gland cDNA. Aliquots were removed at 15, 20, 25, 30, 35 and 40 cycles, run on an agarose gel and scanned by densitometry (Figure 1). When intensity of product is plotted vs. PCR cycle number, an exponential curve is seen. All subsequent RT-PCR experiments were performed at 35 cycles in order to avoid the plateau of amplification.

RT-PCR was then performed with primers for the shark A_0 adenosine receptor (362 bp product), shark CFTR (532 bp product) and our specific shark histone H3.3 primers (255 bp product) on shark tissue cDNA at 35 cycles (Figure 2).

As shown in Figure 2, the highest relative amount of message for the shark A_0 adenosine receptor was detected in three epithelial tissues: rectal gland, stomach and kidney. Rectal gland and stomach had 3 to 4 fold more message than shark kidney. Small amounts of A_0 message were detected in brain, spleen, testis, heart, liver and intestine and none was detected in the gill.

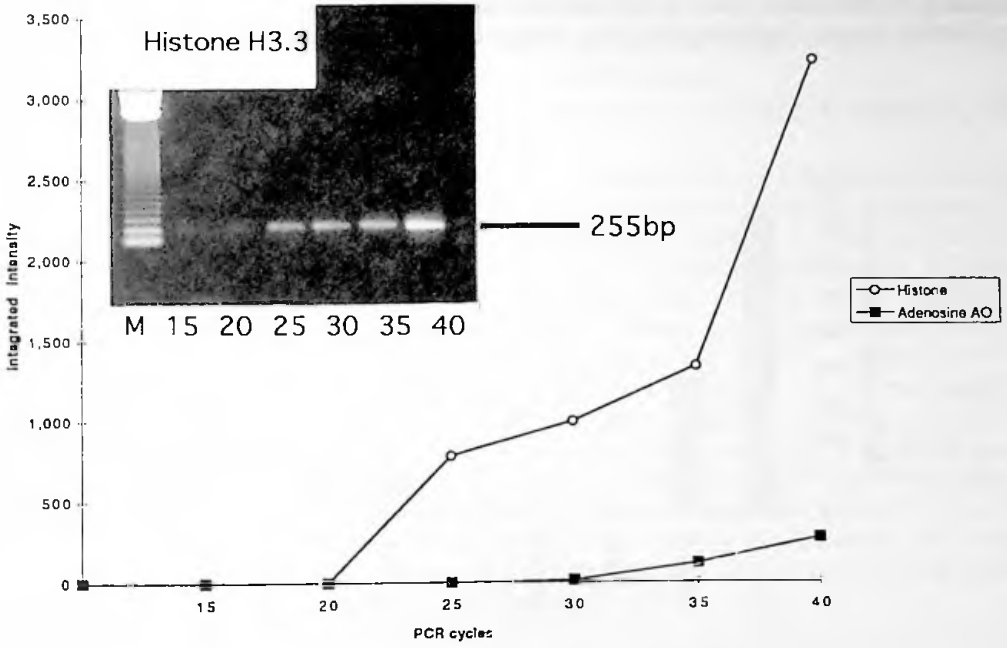

Figure 1. Densitometric scanning of the histone H3.3 and adenosine A_0 receptor RT-PCR products.

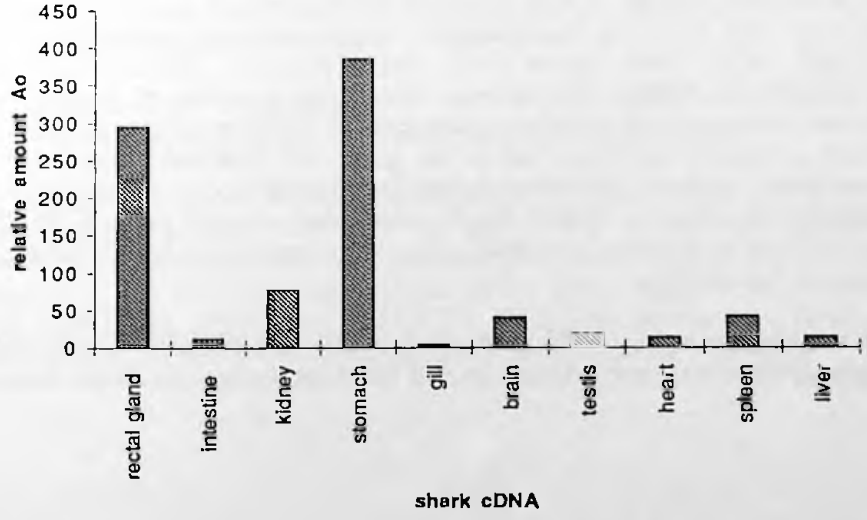

Figure 2. Relative expression of message for the A_0 adenosine receptor in selected shark tissues by quantitative RT-PCR using histone H3.3 as the internal control.

The relative expression of shark CFTR in the same tissues is shown in Figure 3. The highest amount of CFTR message was detected in the rectal gland and the expression in this tissue was two fold higher than intestine and brain, the next highest tissues. Significant amounts was also detected in the testis and a small amount was found in the spleen. CFTR was not detected in three epithelial tissues including kidney, stomach and gill, or in liver.

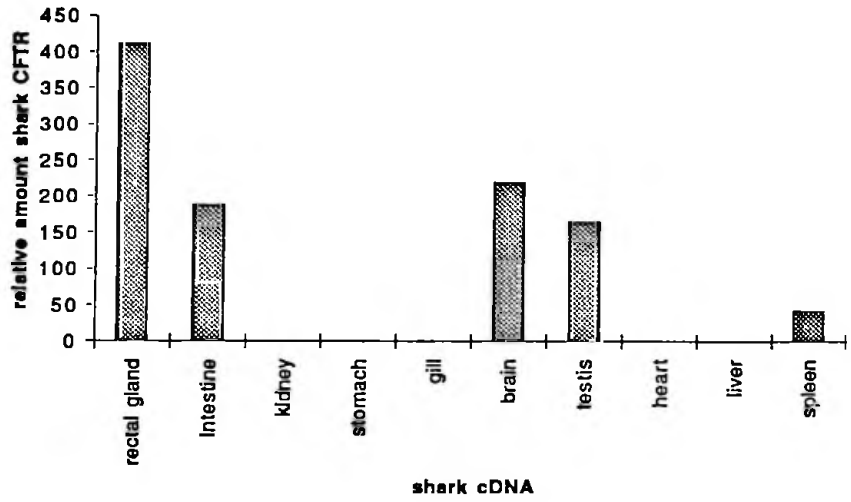

Figure 3. Relative expression of message for shark CFTR in selected tissues by quantitative RT-PCR using histone H3.3 as the internal control.

This study demonstrates tissue specific expression of both the shark A_0 adenosine receptor and shark CFTR in selected tissues. Only the rectal gland contains large amounts of message for both genes, consistent with previous functional and immunohistochemical data (Kelley et al., J. Clin. Invest., 85:1629-1636, 1990; Devor et al., Am. J. Physiol., 268:C70-79, 1995; Lehrich et al., Bull. MDIBL 34:28-31, 1995). There were significant amounts of A_0 receptor message in both shark kidney and stomach and significant amounts of CFTR message in shark intestine, brain and testis. Neither message was detected in the gill. We conclude that there is differential expression of these genes in shark epithelial tissues and that the rectal gland is the single tissue in the shark with abundant expression of both the A_0 adenosine receptor and CFTR. RT-PCR, using the shark histone H3.3 as an internal standard, is a useful technique for detecting tissue specific expression of genes in this species.

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