Partial sequence of type 3 phosphodiesterase in the rectal gland of the dogfish shark (*Squalus acanthias)*

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The shark hormone C-type natriuretic peptide stimulates chloride secretion in the rectal gland through increased production of the second messenger cGMP. As part of this signaling pathway, intracellular cGMP levels are regulated by the activity of type 3 phosphodiesterase. This abstract describes the partial sequence of a 290 base pair region of type 3 phosphodiesterase and the likely identification of this protein by Western blotting in dogfish shark rectal gland tissue.

C-type natriuretic peptide (CNP), the dominant peptide in the shark heart, strongly activates Cl^- secretion through CFTR channels in shark rectal gland (SRG) tissue[4]. This signaling pathway is initiated by CNP binding to its receptor, NPR-B, to activate guanyl cyclase[1] and thus raise intracellular cGMP levels. Previous experiments in our lab in the perfused SRG[3] and voltage clamped SRG epithelial cells[2] indicate that type 3 phosphodiesterase (PDE-3) is involved. High levels of cGMP inhibit PDE-3 hydrolysis of the second messenger cAMP. Thus, high cGMP levels maintain high cAMP levels, which allows cAMP to activate PKA phosphorylation of the CFTR channel.

To advance our understanding of PDE-3 in this signaling pathway, we sought to clone SRG PDE-3, expected to be about 1100 amino acids in size. A shark rectal gland was homogenized in TRIzol lysis buffer with a mortar and pestle to extract RNA. Single strand cDNA was reverse transcribed from the SRG RNA with the Invitrogen SuperScript III cDNA synthesis kit.

The NCBI protein EST database was searched for ESTs of SRG PDE3. One 583 bp EST with accession number EE886450.1 appeared to be the most promising and coded for ~190 amino acids at the 5' end of the gene. The EST was scored as most homologous to PDE3B. Degenerate primers were designed by locating conserved regions in protein sequence alignments of PDE3B in human, cow, mouse, rat, chicken, and zebrafish species. The forward primer MJB17f: 5'GAYGTIYTICAYGCIGTITGG-3' and reverse primer MJB13r: 5'-GCNGCRTGRTGRTTYTCIAR-3' produced a band of expected size of 290 bp (Fig 1, right).

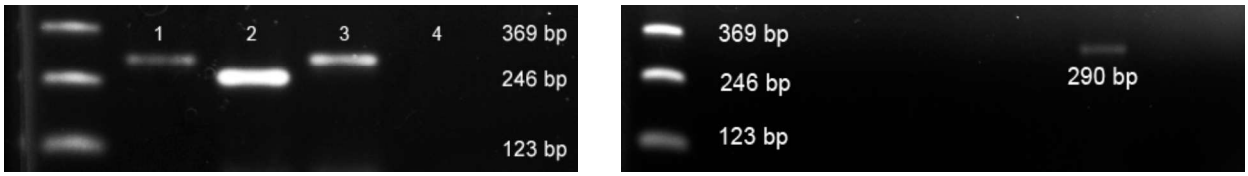

Figure 1. Positive and negative controls (left) and product (right) from the same round of PCR were run on different gels. (Left): positive controls in lane 1: SRG CFTR, lane 2: SRG β-Actin, lane 3: a fragment of the SRG EST, lane 4: negative control (without cDNA). (Right): the 290 bp PCR product.

The product was sequenced as:
5'CTGCAGACTACAATGAGCGATCACGGCTCAATGAGCGATTCAGATTCNNACAGTGGAATCACTC
ATGGACACATGGGTTATGCCATATCGAAAACCTACAGTGTGACCGAGGACAGATATGGCTGTGTT
ATTGCCAATGTTCCTGCCTTGGAGCTGATGGCCCTCTATGTCGCTGCGGCCATGCATGATTATGATC
ACCCAGGAAGGACCAATGCTTTTCTGGTGGCAACCAGTGCACCACAGGCTGTTCTGTACAATGACC
GTTCAGTGCTCGAAAACCACCACGCCGC-3'

The 290 bp PCR product was translated 5'3' frame 1 and aligned with the two isoforms of PDE3. The product appears to be from a conserved region shared by both the A (Fig 2) and B (Fig 3) PDE3 isoforms.

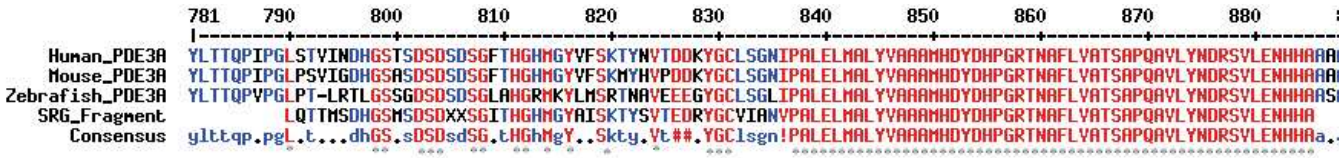

Figure 2. Amino acid alignment of the SRG PCR product compared to human, mouse, and zebrafish PDE3A

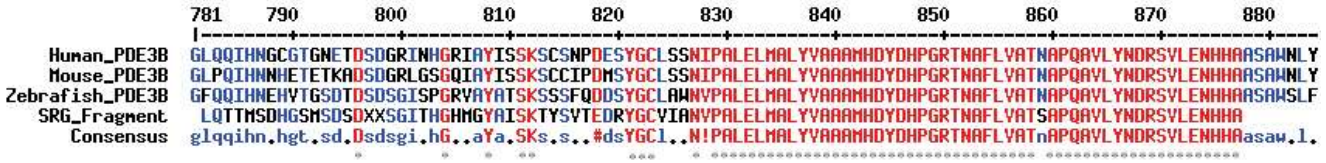

Figure 3. Amino acid alignment of the SRG PCR product with human, mouse, and zebrafish PDE3B

Thus, PDE3B degenerate primers gave limited success, only amplifying a region conserved between the A and B isoforms. Furthermore, a Western blot was run with polyclonal PDE3B antibodies and did not produce any bands, while a similar Western blot run with PDE3A polyclonal antibodies produced a band of 120 kDa (Fig 4), the expected size of full length SRG PDE3A.

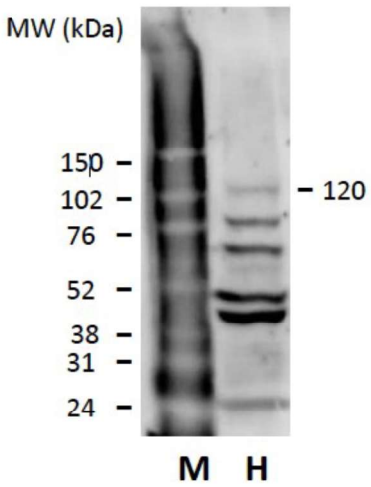

Figure 4. Western Blot of SRG homogenate (M= molecular markers, H=homogenate) using rabbit polyclonal Ab PDE3A (Santa Cruz): 1:200.

It is not certain whether the presence of smaller bands in Figure 4 is due to proteolysis of PDE3A or to non-specific binding of the antibody. Thus, these sequencing and Western blot results do not provide conclusive evidence as to which isoform is present in the SRG. PDE3A degenerate primers and RACE PCR may yield more results in future experiments.

This work was supported by NIH grants DK 34208, NIEHS 5 P30 ES03828 (Dr. Forrest) and an NSF grant DBI-0453391. Thanks to Chris Smith at the MDIBL DNA sequencing center.

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