CLONING AND SEQUENCE ANALYSIS OF A VIP RECEPTOR EXPRESSED IN THE RECTAL GLAND OF THE SPINY DOGFISH, *SQUALUS ACANTHIAS*

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The shark rectal gland (SRG) of *Squalus acanthias* is stimulated to secrete chloride when perfused with the neurotransmitter vasoactive intestinal peptide (VIP), a 28-amino acid polypeptide. Based on studies in the perfused rectal gland, Stoff et al (*Am. J. Physiology* 237: F138-F144, 1979) first proposed that a VIP receptor was present in the rectal gland epithelium. Subsequent studies demonstrated that VIP stimulates chloride secretion in isolated perfused tubules and cultured cells of the SRG (Valentich and Forrest, *Am. J. Physiology* 260: C813-23, 1991). Silva et al (*Am. J. Physiology* 249: R329-34, 1985) demonstrated saturable specific binding of ^{125}I-VIP to rectal gland cells. Effects of VIP in the SRG include: (1) activation of adenylyl cyclase, increasing the cellular cAMP levels (Stoff et al., *Bull. MDIBL*, 16:95-98, 1976), (2) increase in the intracellular Ca^{2+} concentration (Kelley et al., *Bull. MDIBL*, 27:129-137, 1988; Gregor et al., *Pflugers Arch-Euro. J. Physiology* 436:133-40, 1998), and (3) release of inositol phosphates (Ecay et al., *J. Cell. Physiol.,* 146:407-16, 1991).

Although the signal transduction pathways for VIP in the SRG have been thoroughly examined, the specific VIP receptor in this tissue has not been identified. Twenty-five years after Stoff's observation, we report the molecular characterization of a VIP-like receptor cloned from the SRG.

Total RNA was isolated from fresh dogfish rectal gland tissue using a cesium chloride method (Molecular Cloning). RNA was subjected to a DNAse 1 digest and reverse transcription was carried out with oligo(dT) primers (Clontech Advantage™). Shark specific primers were designed from a partial sequence of the SRG VIP-1 receptor previously reported by our laboratory (Plesch et al., Bull. MDIBL 38:116-17). To obtain the full length sequence, SMART RACE-PCR was performed as described (Clonetech SMART RACE cDNA Amplification Kit) and the PCR products were subcloned by TOPO-TA cloning (Invitrogen). The sense primer 5'-CTTCGTCCTGAGGGCCATCGCTGTCTT-3' and antisense primer: 5'- GGGGCCCGAATGA TCCACCAATACG-3' were used to amplify 3' RACE and 5' RACE fragments of shark VIP receptor (Figure 1). PCR was performed for 35 cycles (95 °C for 30 sec; 65 °C for 3 min 30 sec) using Expand™ Long DNA polymerase (Boehringer Mannheim). Figure 2A illustrates bands from 5' RACE-PCR (~250 bp) and 3' RACE-PCR (~1.5kb) which were cloned (TOPO-TA cloning, Invitrogen), and sequenced at the MDIBL Marine DNA Sequencing Facility.

Figure 1: Schematic representation of cloning strategy for SRG VIP receptor using shark rectal gland adaptor-ligated cDNA and SMART RACE Amplification (Clonetech). Adapters are represented as black blocks located at the cDNA ends. Gray arrows represent 5' RACE-PCR primers and black arrows 3' RACE-PCR primers

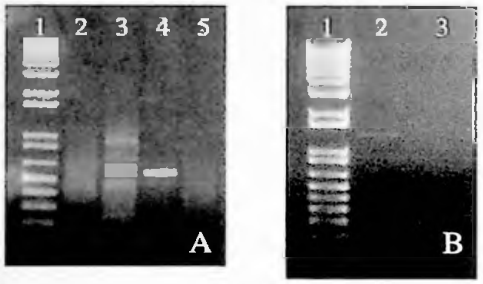

Figure 2: Gel electrophoresis of PCR products. Figure 2A displays produ from 5' RACE-PCR. 1 kb-plus DNA ladder (lane 1); shark specific prim (lanes 2-3); Universal Primer Mix (lane 4); no cDNA as a negative cont (lane 5). The faint 750 bp product in lane 3 that was not amplified w Universal Primer Mix was sequenced and identified as 5' VIP-R. Figure displays the 1.3 kb full length VIP receptor construct prepared for expres studies: 1kb-plus DNA ladder (lane 1), a 1.3kb PCR product from sha rectal gland (lane 2), and negative control with no cDNA (lane 3).

The full-length construct was obtained using a specific sense primer beginning at the start codon and antisense primer ending at the stop codon (Figure 2B). The full-length protein sequence of the shark VIP receptor had only 57% identity to mouse VIP-1 receptor, 56% to human and rat VIP-1 receptor and 48% to goldfish VIP-1 receptor (Lasergene DNASTAR). Phylogenetic analysis of this clone shows the relationship between the shark VIP receptor and other G-protein coupled receptors in the secretin family including growth hormone releasing hormone (GHRH) receptor and PACAP receptor (Figure 3). Predictions of the seven transmembrane spanning regions based on a hydropathy plot are shown in figure 4. The SRG VIP receptor retains the conserved cysteines (6 residues), tryptophan (2 residues), proline (1 residue) and glycine (1 residue) in the N terminal region that are considered essential for ligand binding. However, a glutamic acid residue in the human VIP-1 receptor considered important for ligand binding (Laburthe et al., *Ann N Y Acad Sci.* 805:94-109, 1996) is replaced by an alanine residue in the SRG VIP receptor.

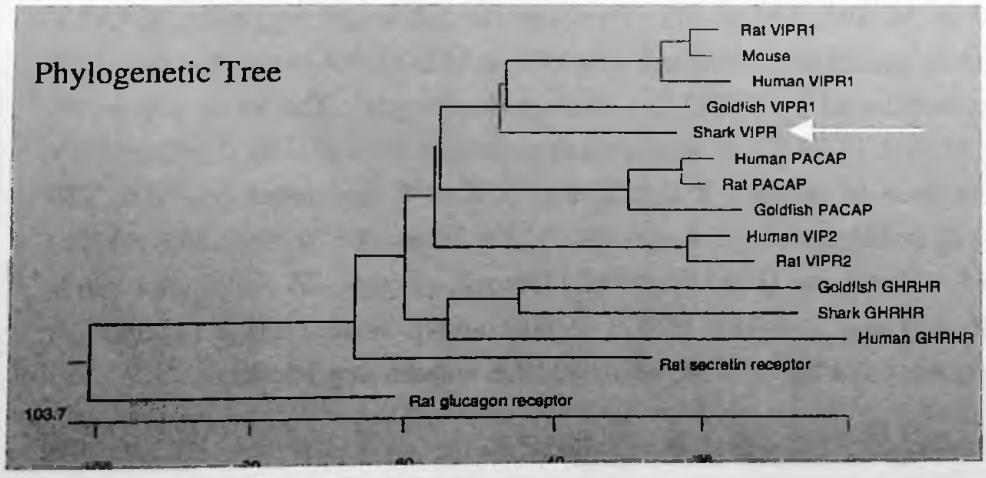

FIGURE 3: Phylogeny of receptors the secretin G-Protein Coupled Receptor (GPCR) family including GHRH receptor and PACAP recepto from human, rat, and goldfish aligne with full length Shark VIP receptor (Lasergene).

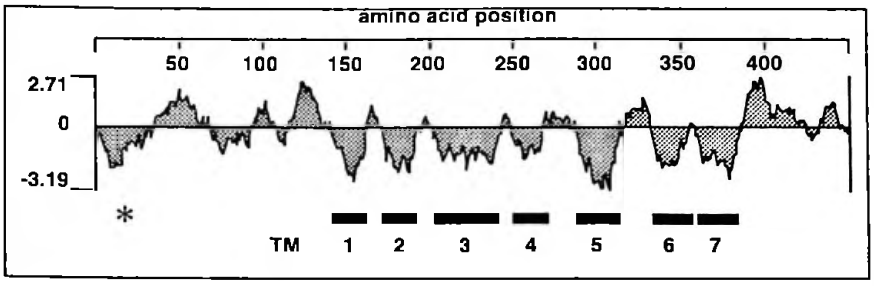

FIGURE 4: Hydropathy plot of the shark rectal gland VIP-1 receptor. The strongly hydrophobic region at the N-Terminus corresponds to the predicted signal sequence (marked with a '*'). The seven predicted hydrophobic regions are marked with numbers and black bars. Horizontal scale represents the amino acid position.

We are now examining the functional properties of the SRG VIP receptor in expression studies in *Xenopus* oocytes and COS-7 cells. In summary, we report the full length cloning from SRG of the oldest VIP-1 receptor identified to date. This receptor is the likely target for VIP and related hormones that stimulate salt secretion in the rectal gland.

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