CLONING AND IDENTIFICATION OF A VIP-1 RECEPTOR EXPRESSED IN THE RECTAL GLAND OF THE SPINY DOGFISH, *SQUALUS ACANTHIAS*

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Stimulation of salt secretion in the rectal gland of the spiny dogfish by vasoactive intestinal peptide was described more than twenty years ago (Stoff et al., *Bull. MDIBL* 16:95-98, 1976; Stoff et al., *Am. J. Physiol.*, 237:F138-44, 1979). Since then, VIP has been the most thoroughly examined native activator of Cl^- secretion in this tissue. VIP has been shown to act on three messenger pathways: (1) activation of adenylyl cyclase, increasing cellular cAMP levels (Stoff et al., *Bull. MDIBL*, 16:95-98, 1976), (2) increases in intracellular Ca^{2+} (Kelley et al., *Bull. MDIBL*, 27:129-137, 1988), and (3) release of inositol phosphates (Ecay et al., *J. Cell. Physiol.*, 146:407-16, 1991). Binding studies in rectal gland membranes revealed a single high affinity binding site for VIP (Silva et al., *Am. J. Physiol.* 249:R329-R334, 1985). Despite this detailed study, the molecular characterization of the rectal gland VIP receptor has not been determined. In this study we report the partial cloning of a rectal gland VIP receptor that has homology to previously cloned VIP-1 receptors of other species.

Total RNA was prepared from fresh shark rectal gland tissue using TRIZOL® Reagent from Gibco. Reverse transcription was carried out with oligo dT primers (Clontech Advantage™). For a semi-nested PCR approach (Chow et al., *Gen. and Comp. Endocrin.*, 105: 176-85, 1997), degenerate primers to the transmembrane (TM) regions 2 (TGCAYTGYACNMGNAAYTAYATYCA), 6 (AGSGGGATSAGSRKNAGNGTGGAYTT) and 7 (TGSACCTCNCCRTTNASRAARCARTA) of the secretin receptor family were synthesized. After the first PCR using primers from TM2 and TM7, 1 µl of the products was amplified in a second PCR using nested primers from TM2 and TM6. Reaction products ranging in size from 450 bp to 800 bp were cloned (TA-cloning, Invitrogen) and recombinants were screened utilizing blue-white selection. Clones were then sequenced by automated techniques (DNA Sequencing Facility, University of Maine, Orono).

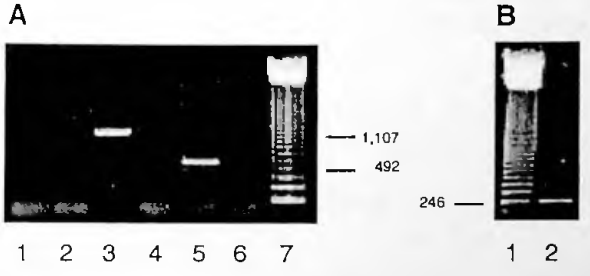

Figure 1. Gel electrophoresis of PCR products. Panel A displays amplification products of the semi-nested degenerate PCR on shark rectal gland cDNA: PCR with primers TM2 and TM7 (lane 1), PCR with primers TM2 and TM6 (lane 2), positive control using A_0 adenosine receptor primers (lane 3), negative control for first PCR (lane 4), PCR using primers 2 and 6 and 1 µl of the PCR reaction of lane 1 as template (lane 5), negative control for second PCR (lane 6), 123 bp ladder (lane 7). A distinct band is obtained only with semi-nested PCR. Panel B shows a 123 bp ladder (lane 1) and a 234 bp fragment of shark VIP-1 receptor transcript amplified with specific primers (lane 2).

Sequencing of two identical clones yielded 537 bp of nucleotide sequence coding for 179 amino acids. Both sequences had highest homology to VIP-1 receptors of higher species and lower scores for other members of the secretin receptor family. The identity of the shark VIP receptor to its counterparts in human, rat and goldfish was 75.4%, 74.9% and 69.3%, respectively. Phylogenetic analysis (Figure 2) shows the shark receptor as a progenitor protein forming the first branch of the VIP-1 receptor family.

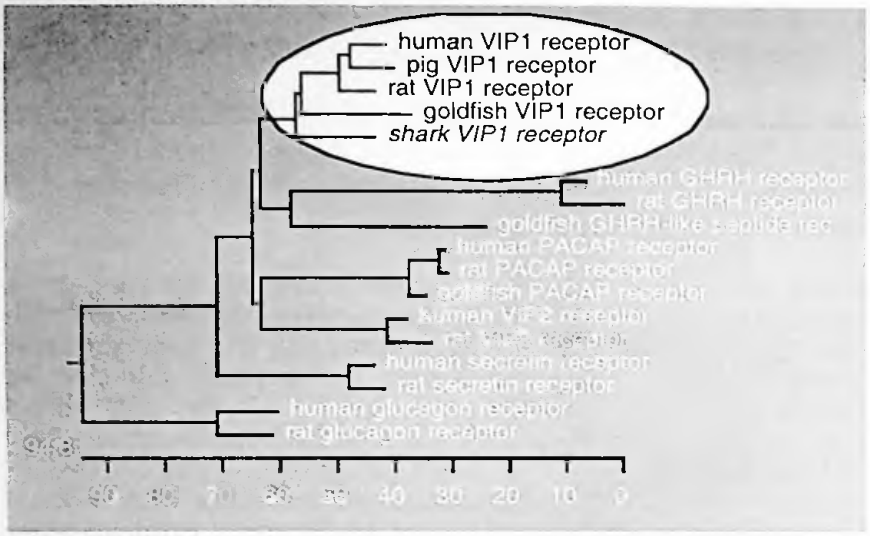

Figure 2. Phylogeny of receptor fragments of the secretin GPCR family from human, rat and goldfish aligned with shark VIP-1 receptor. The alignment was done using partial protein sequence fitted to the sequence obtained from the dogfish shark (from LHCTRN motif in TM2 to LLLIP motif in TM6). The tree was generated by the MegAlign module of Lasergene software.

The agonist potency order for human VIP-1 receptors has recently been determined to be VIP = PACAP-38 = PACAP-27 > helodermin > secretin (Ulrich et al., *Gastroenterology*, 114:382-397, 1998). Based on chloride secretion and adenylyl cyclase activity, Silva et al. (*Bull. MDIBL*, 34:39-41, 1995) proposed a different potency order for the shark receptor (helodermin > VIP >> PACAP). Structural analysis of the shark VIP receptor suggests residues that could account for such species differences. Two residues in human VIPR-1 (Q-207, and G-211) have been proposed to be involved in ligand selectivity (Laburthe et al., *Ann. N.Y. Acad. of Sci.* 805:94-109, 1996). The corresponding residues in shark VIP-1 receptor are different from the human isoform (H and V, respectively). These residues are targets for future mutagenesis studies to determine their role in species-dependent agonist binding.

In summary, we report the partial cloning of a shark rectal gland VIP-1 receptor that is a candidate protein for mediating the chloride secretory effect of endogenous VIP-like substances in the shark.

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