CLONING AND PARTIAL SEQUENCE OF A GROWTH HORMONE RELEASING HORMONE-LIKE RECEPTOR FROM THE RECTAL GLAND OF THE SPINY DOGFISH, *SQUALUS ACANTHIAS*

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The rectal gland of the dogfish shark is an organ specialized for salt secretion and highly regulated by hormones. Vasoactive intestinal peptide (VIP) and somatostatin (SRIF) represent classic activators and inhibitors of salt secretion in the rectal gland, respectively. These hormones act through G-protein coupled receptors (GPCR), a membrane protein family with seven transmembrane regions. VIP is grouped with a number of structurally related peptides including PACAP, PHM, secretin, and growth hormone releasing hormone (GHRH). Of these, only VIP, PACAP and GHRH stimulate secretion in the shark rectal gland. GHRH stimulates chloride secretion at concentrations about 10-fold higher than the concentrations of VIP needed to produce the same effect (Epstein et al., *Bull. MDIBL*, 26:23-24, 1986).

In 1992, the first teleost GHRH was purified from carp and sequenced. In both mammals and goldfish, GHRH stimulates the release of growth hormone from the pituitary. As in mammals, it stimulates the release of growth hormone in the pituitary of goldfish. Teleost GHRH has a remarkably low homology (57% amino acid identity) to its mammalian counterpart (Vaughan et al., *Neuroendocrinology*, 56:539-549, 1992). In 1998, the first marine GHRH-like receptor was cloned from the pituitary tissue of the goldfish. Like the carp hormone, its sequence displays a rather distant relation to the mammalian receptor.

In the present work, we have partially cloned a dogfish shark GHRH-like receptor from rectal gland cDNA using partially nested PCR. The cloning of this receptor from the rectal gland coupled with the cloning of a specific rectal gland VIP-1 receptor (reported elsewhere in this Bulletin) suggests that endogenous GHRH and VIP-like substances may stimulate chloride secretion by activating separate receptor proteins in the gland. This is the first suggestion that GHRH receptors may be regulatory proteins in chloride secreting epithelia.

cDNA was prepared from fresh shark rectal gland tissue as described (Plesch et al, this issue). Semi-nested PCR was performed using degenerate primers to the transmembrane regions 2 (TGCAYTGYACNMGNAAYTAYATYCA), 6 (AGSGGGATSAGSRKNAGNGTGGAYTT), and 7 (TGSACCTCNCCRTTNASRAARCARTA) of the secretin receptor family. After the first set of PCR cycling using 2 and 7 as primers, 1µl of the products was amplified in the second PCR using nested primers 2 and 6. Both sets of PCR were performed for 30 cycles of 1 min at 94 °C, 58 °C, and 68 °C. The reaction products (ranging in size from 450 bp to 800 bp) were cloned (TA-cloning kit, Invitrogen) and sequenced (DNA Sequencing Facility, University of Maine, Orono).

The sequencing of four identical clones yielded 542 bp of nucleotide sequence with an open reading frame of 180 amino acids. Figure 2 displays an amino acid alignment of shark GHRH-like receptor to human and goldfish counterparts. The identity between shark and goldfish GHRH-like receptors is 65.9%, and between shark VIP-1 receptor and shark GHRH-like receptor is 60.6%. Among mammalian GPCRs, shark GHRH-like receptor had highest homology with rat VIP-1 receptor (60.3%), and was only 46.9% identical to human GHRH receptor and even lower for the rat GHRH receptor (44.9%). Nevertheless, a phylogenetic analysis (Figure 3) shows that fish GHRH-like receptors are more closely related to the mammalian GHRH receptors when compared with other members of the gene family. Four cysteine residues that are conserved in all GHRH, PACAP, and VIP-1 and VIP-2 receptors can also be found in the shark fragment (C^{2}, C^{38}, C^{45},

C^{115}). In addition, there is one cysteine in TM3 (C^{55}) that only exists in fish GHRH-like receptors and one in TM4 that is unique in shark (C^{89}). These cysteines are possible candidates for disulfide bonding which may direct the three dimensional structure of the protein.

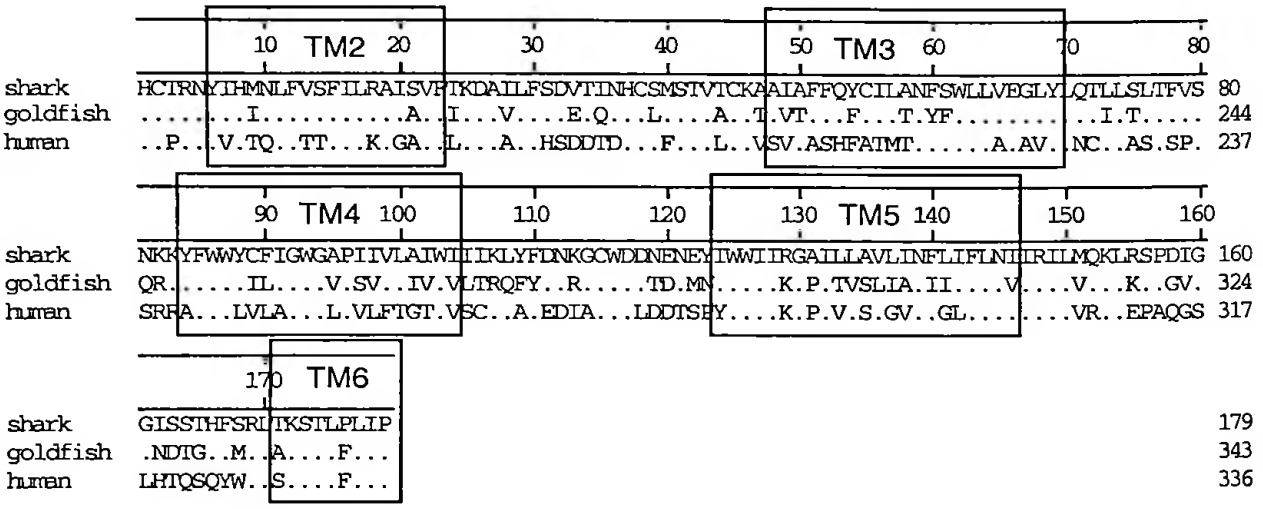

Figure 2. Alignment of the deduced amino acid sequence of dogfish shark, goldfish and human GHRH receptors. Identical residues are represented by dots. The proposed transmembrane regions are indicated by boxes.

In contrast to mammals where GHRH and PACAP are encoded by different gene loci, the two hormones are synthesized from a single precursor polypeptide in fish (McRory et al., *Mol. Cell. Endocrinol.*, 108:169-177, 1995). Sequence identities between marine and mammalian GHRH are very low (31-40%). Carp GHRH is highly related to mammalian VIP (Vaughan et al., *Neuroendocrinology*, 56:539-549, 1992). Our findings suggest that there are different endogenous physiological ligands in the shark for the new and separate receptors (VIP-1 and GHRH-like) we have identified in the gland.

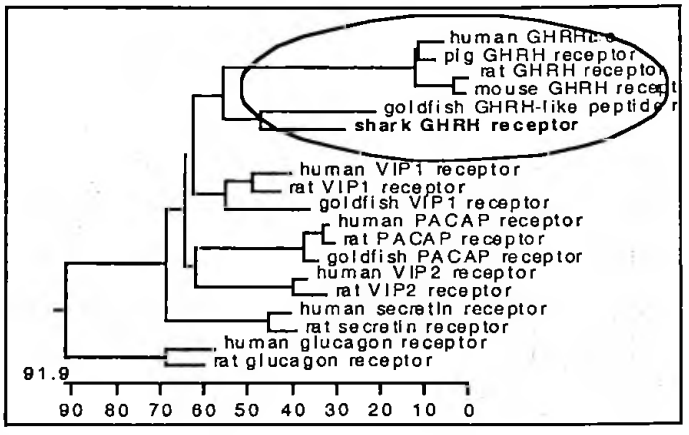

Figure 3. Phylogeny of receptor fragments of the secretin GPCR family from human, rat and goldfish aligned with the shark GHRH-like receptor. The alignment was done using partial protein sequence fitted to the sequence obtained from the dogfish shark (from LHCTRN motif in TM2 to LLLIP motif in TM6). The tree was generated by the MegAlign module of Lasergene biosoftware.

Although GHRH receptors are known to be expressed in several organs apart from the brain (e.g. human and rat kidney (Fujinaka et al., *FEBS Lett.*, 394:1-4, 1996), the physiological role(s) of such receptors in epithelial tissues have not been studied. The present work suggests that GHRH receptors may regulate ion transport in certain epithelial cells.

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