CLONING OF A GHRH-LIKE RECEPTOR FROM THE SHARK RECTAL GLAND AND FUNCTIONAL EXPRESSION IN *XENOPUS LAEVIS* OOCYTES

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The neuropeptide growth hormone releasing hormone (GHRH) of the secretin/glucagon/ _____#tive intestinal peptide/pituitary adenylate cyclase activating peptide/ family is known to stimulate chloride secretion in the shark rectal gland (Epstein et al., *Bull MDIBL*, 26:23-24, 1986). Short circuit current experiments of primary monolayers localized the site of action of GHRH to the basolateral surface of the rectal gland cell and perfusion experiments in our lab have further demonstrated a difference in the potency of GHRH from three different mammals (Plesch et al., *Bull MDIBL*, 38: 129-130, 1999). GHRH from rat was most potent in stimulating chloride secretion, whereas mouse GHRH was approximately 50%-60% less potent and the human peptide had no effect at equimolar concentrations (10nM) in the perfused gland (Plesch et al., 38: 129-130, 1999). Furthermore, our laboratory reported the partial cloning of a shark GHRH-like receptor (sGHRHR) from shark rectal gland and the identification and cloning of a GHRH-like hormone/PACAAP precursor polypeptide from shark brain. (Plesch et al., *Bull MDIBL*, 38:110-11,1999; Plesch, *Bull MDIBL*, 38:126-127,1999). We now report the complete sequence of the shark rectal gland GHRH-like receptor and its expression and characterization in *Xenopus laevis* oocytes.

The degenerate PCR product previously identified in our laboratory was labeled with [α-^{32}P]dCTP and used to screen a SRG cDNA library. Library screening resulted in identification of a single 2395bp clone with an ORF of 454 amino acids, 485bp of 5' untranslated region and 549 bp of 3' untranslated region containing the poly-A-tail. The deduced amino acid sequence of the shark receptor was 49% identical to the goldfish [*Carassius auratus*] growth-hormone releasing hormone-like peptide receptor and 47% identity to human. Sequence analysis of sGHRHR revealed highest homology to the goldfish receptor within amino acids 134-391, predicted by the domain-prediction programSimple Modular Architecture Research ToolSMART (hultz, J., et al. PNAS, 95, 5857-5864, 1998) to be the seven-transmembrane region (7TM2), with an identity of 66% to goldfish. Greatest diversity (only 25% identical) was seen in the putative ligand binding domain/extracellular domain of shark GHRHR compared to other species, suggesting that the shark receptor may have different agonist binding characteristics.

Start-to-stop PCR was performed, using forward and reverse gene specific primers with restriction enzyme sites for HindIII and XbaI, respectively. This product was cloned into the pCDNA3.1 vector, the vector was linearized with KpnI, and cRNA synthesis was performed using the Ambion®-T7-message machine (Austin, TX). Stage 6 oocytes were harvested from adult *Xenopus laevis* frogs, treated with collagenase prior to manual defolliculation. On day two they were injected with either water, human cystic fibrosis transmembrane conductance regulator CFTR (hCFTR) cRNA only or coinjected with hCFTR and sGHRHR cRNA. Two-electrode voltage clamping (TEVC) was performed under constant perfusion of the oocyte with Frog Ringer's (FR) solution and addition of different GHRH-peptides or 10uM Forskolin + 1mM IBMX.

To determine the different potencies in receptor activation by three different mammalian GHRH-peptides, each peptide was added to the perifusate at a concentration of 5nM. Experiments were performed as follows: oocytes were clamped at -30mV and subsequent stimulation was followed by a short period of perifusion with FR to wash out the agonist, before the next agonist was added. Each experiment was finished by a stimulation with forskolin 10μM+IBMX 1mM to assess the expression level of hCFTR. As a control, hCFTR only injected oocytes were stimulated with 10nM rGHRH, and subsequently with Forskolin+IBMX. Figure 1A shows control oocytes, stimulated with 10nM rGHRH. Baseline conductance in sGHRH+hCFTR coinjected oocytes perifused with Frog Ringers alone was 2.7±1.6μS. On subsequent stimulation with 5nM h-, m-, and ratGHRH conductance increased to 9.8±3.66, 20.1±8 and 87±6.5μS, a 3.4-, 7.5- and 32- fold stimulation in conductance by the three different agonists. (Figure 1B). Stimulation by rGHRH was not different from maximum stimulation following forskolin+IBMX (data not shown). I/V plots from a representative experiment are shown in Figure 2.

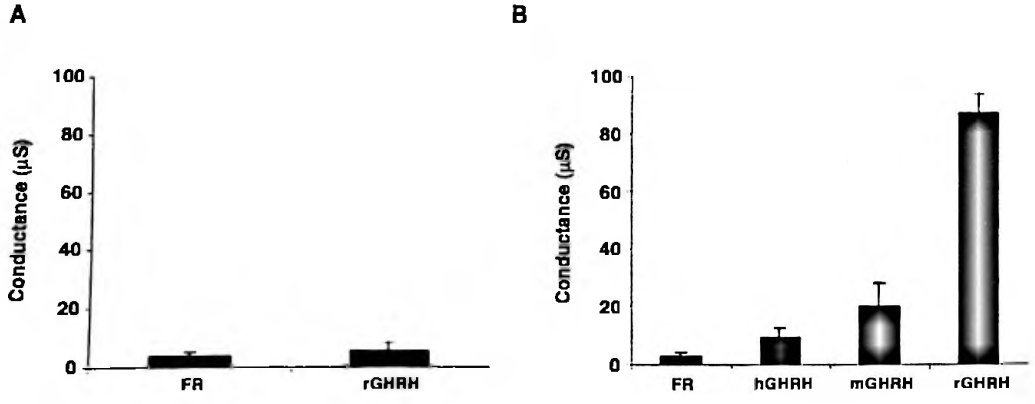

Figure 1: Stimulation of chloride conductance by different GHRH-peptides. Panel A: Control oocytes, injected with hCFTR only and perifused with ratGHRH(10nM) n=6.Panel B: Mean maximum chloride conductance after

stimulation with 5nM human(h)-, mouse(m)-, or rat(r)-GHRH (n=4 each) (FR=Frog Ringers). Chloride conductance was increased 3.4, 7.5, and 32-fold by h-,m-, or r-GHRH.

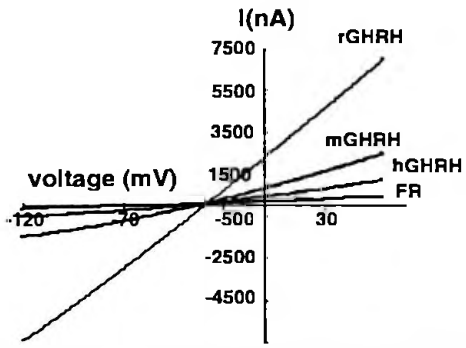

Figure 2: I/V-plot of an oocyte coexpressing sGHRHR and hCFTR stimulated with 5nM human(h)-, mouse(m)-, or rat(r)-GHRH (FR=Frog Ringers)

In conclusion, we report the full length cloning of a shark GHRH receptor which displays the typical structural characteristics of a 7-TM G-protein coupled receptor. When sGHRHR is coexpressed with hCFTR in *Xenopus* oocytes, chloride conductance is increased by GHRH peptides from three different species in a unique order of potency that is identical to that observed in the perfused shark rectal gland. These findings suggest that the cloned receptor is the target for GHRH stimulation of chloride secretion in the intact rectal gland.

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