GROWTH HORMONE RELEASING HORMONE: SPECIES DIFFERENCES AND POLARITY OF CHLORIDE SECRETORY EFFECTS IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

Florian N. Plesch[1,2], Martha Ratner[4], Nicholas W. Stine[6], Cinda P. Scott[3], Sarah Decker[5], Suzanne Forrest[1], Stephen G. Aller[1], and John N. Forrest, Jr.[1]

[1]Dept. of Medicine, Yale University School of Medicine, New Haven, CT

[2]Medizinische Hochschule Hannover, Hannover, Germany

[3]Columbia University, New York, NY; [4]Wheaton College, Norton, MA

[5]Middlebury College, Middlebury, VT; [6]Dartmouth College, Hanover, NH

Growth hormone releasing hormone (GHRH) is a neuropeptide of the secretin/glucagon/vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase activating polypeptide (PACAP) family. In addition to mammalian VIP and PACAP, rat GHRH activates salt secretion in the shark rectal gland (Epstein et al., Bull. MDIBL, 26:23-24, 1986). Recently, we reported the cloning of the proposed receptor for GHRH from the rectal gland (Plesch et al., Bull. MDIBL, 38:110-12, 1999) as well as the cloning of the native shark GHRH/PACAP peptides (Plesch et al., this issue). The present study further characterizes the physiological effects of mammalian GHRH peptides in the shark rectal gland.

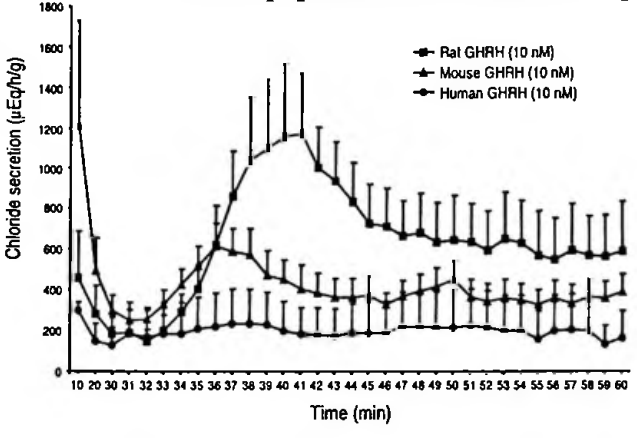


Figure 1. Comparison of the potency of three mammalian forms of GHRH (rat, mouse and human) in the perfused shark rectal gland.

In perfusion experiments, we compared the potency of three mammalian GHRH peptides. Figure 1 illustrates that rat, mouse and human GHRH at equimolar concentrations have different effects on salt secretion in the rectal gland. Whereas 10 nM rat GHRH stimulates chloride secretion to a maximal value of 1395±426 µEq/h/g, the mouse peptide gave a maximum secretion rate of 618±111 µEq/h/g and the human peptide had no effect at 10 nM (figure 1) or 50 nM (data not shown).

A dose response of the most potent secretagogue, rat GHRH, is summarized in Figure 2. The lowest concentration of peptide stimulating salt secretion was 5 nM (630±567 µEq/h/g). The response of the rectal gland to rat GHRH was concentration dependent up to 10 nM. Perfusing with 25 nM (1210 ± 116 µEq/h/g) of rat GHRH resulted in a secretory activity that is comparable to 10 nM (1395 ± 426 µEq/h/g). This data indicate a maximal stimulation response to rat GHRH that is lower than that reported for rat VIP (~2500-3000 µEq/h/g).

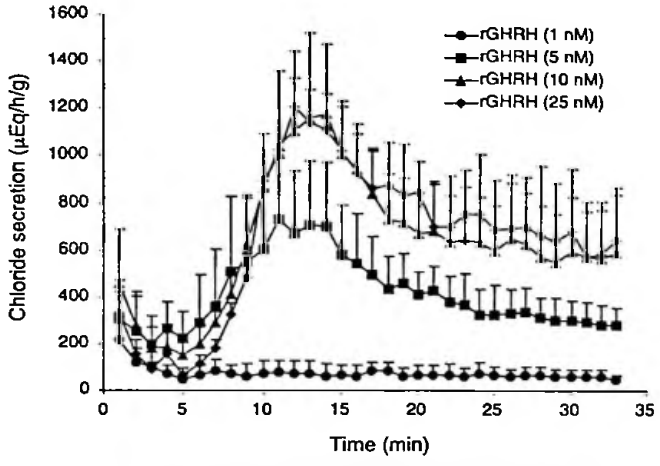
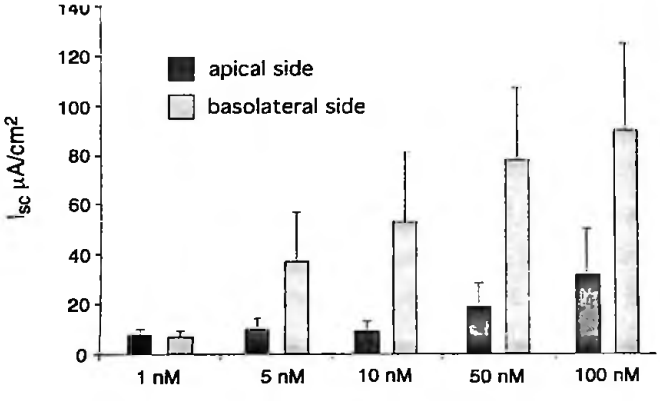


Figure 2. Dose response to rat GHRH (1 to 25nM) in the isolated perfused rectal gland of Squalus acanthias (n=3 to 5 per group).

In short circuit experiments of primary monolayers of cultured shark rectal gland cells, we examined the dose response to rat GHRH and determined the polarity of this effect.

Figure 3 shows the mean I_{sc} for different concentrations of rat GHRH applied to either the apical or basolateral side of the monolayer. Stimulation of GHRH in a dose dependent manner was primarily localized to the basolateral surface. However, in contrast to the perfusion studies, secretion rates continued to rise in the presence of 50 μM and 100 μM GHRH. A low stimulation of I_{sc} was also detected at very high concentrations of rGHRH on the apical side.



3. Polarity of rat GHRH in Isc of monolayers from primary cultures of shark rectal gland cells (n=3 to 4 per group).

In a series of perfusion experiments, we found that the action of GHRH on Cl^- secretion was additive but not synergistic with the related peptide VIP (data not shown).

It is unclear whether stimulation by rat GHRH and VIP is occurring through the same (e.g. a VIP-1 like receptor) or different G-protein coupled receptors (including a GHRH-like receptor). We have cloned a shark GHRH-like hormone from the brain (see abstract in this bulletin) that has 21.4% amino acid sequence homology to human VIP and 20.9% amino acid sequence homology to rat GHRH. To study ligand and receptor specificity, it will be necessary to synthesize the shark specific peptides and determine their affinity for specific shark receptors in expression studies.

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