Gastric inhibitory peptide, glucagon, and serotonin are potent chloride secretagogues in the rectal gland of the skate (*Raja erinacea*) but not the shark (*Squalus acanthias*)

Catherine A. Kelley[3], Sarah E. Decker[1,2], Martha A. Ratner[2], Max Epstein[4], and John N. Forrest Jr.[1,2]
[1] Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510
[2] Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672
[3] Skidmore College, Saratoga Springs, NY 12866 [4] American University, Washington DC, 20016

The Epstein and Silva laboratory previously found that the rectal gland of the little skate, *Raja erinacea*, secretes chloride (Cl^-) in response to forskolin and dibutyryl cyclic AMP/theophylline, all activators of the cAMP messenger pathway, and to CNP, an activator of the cyclic GMP pathway[1,2]. However, the primary hormone(s) in the skate activating secretion through a G protein coupled receptor (GPCR)-cyclic AMP pathway have not been identified. We sought to identify the secretagogues in the skate responsible for Cl^- secretion via a GPCR-cAMP second messenger pathway.

Modifications by our laboratory to the perfusion technique for skate rectal glands included: 1) use of larger PE tubing for cannulation of artery (PE 30) and duct (PE 50); 2) during cannulation, glands were perfused briefly with 10 μM forskolin to increase duct flow rate sufficiently to check for effective perfusion; 3) when this was verified, the perfusate was changed to basal Ringer's solution; 4) glands were then perfused with basal Ringer's for 30 min, and the experimental secretagogue was then perfused for an additional 20 min with one min readings of chloride secretion. When the experimental secretagogue failed to stimulate, forskolin 10 μM and IBMX 100 μM were added at the end of the experiment to ensure that the lack of activation was not due to systemic error.

Thirty skate rectal glands were canulated and perfused with this protocol and effective perfusion was established in nineteen glands. Perfusion of VIP (10 nM), adenosine (100 μM), PHI (50 nM), PACAP (50 nM), and GHRH (50 nM), all potent activators of chloride secretion in the shark (*Squalus acanthias*) rectal gland, failed to stimulate chloride secretion in the skate rectal gland. In agreement with previous studies[2], CNP (10 nM) stimulated chloride secretion from basal levels to 500 ± 132 μEq/h/g.

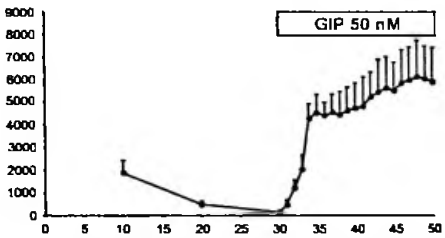

Figure 1. Stimulation of chloride secretion by GIP (50 nM) in the perfused skate rectal gland. N=5, Data are mean ± SE.

Perfusion of Gastric Inhibitory Peptide (GIP) (50 nM) stimulated chloride secretion in the skate dramatically, from 144 ± 56 μEq/h/g to 5893 ± 1510 μEq/h/g (Figure 1). Glucagon (50 μM) and serotonin (50 μM) also stimulated chloride secretion in the skate rectal gland from 176 ± 27 to 1210 ± 420 μEq/h/g and 321 ± 57 to 2622 ± 475 μEq/h/g respectively. Neither GIP, glucagon, or serotonin stimulated chloride secretion when perfused in the shark rectal gland. These studies establish GIP, glucagon, and serotonin as unique specific secretagogues in the skate rectal gland, and suggest that G protein coupled receptors responsive to the agonists are present in the skate, but not the shark gland.

This work was supported by NIH grants DK 34208, NIEHS 5 P30 ES03828 (Center for Membrane Toxicity Studies), and NSF grant DBI-0139190 (REU site at MDIBL). The authors thank Dr. Patricio Silva for technical advice, and the Yale School of Medicine 2005 IPE course for experimental assistance.

1. **Fletcher, L., P. Silva,** and **F.H. Epstein**. Rectal gland function in the little skate *Raja Erinacea*. *Bull. Mt. Desert Isl. Biol. Lab.* 23: 12-13, 1983.
2. **Silva, P., R. Solomon, K. Spokes,** and **F.H. Epstein**. The secretion of chloride by the rectal gland of the little skate, *Raja Erinacea*. *Bull. Mt. Desert Isl. Biol. Lab.* 35: 36, 1996.