PITUITARY ADENLYATE CYCLASE ACTIVATING POLYPEPTIDE IS A POTENT CHLORIDE SECRETAGOGUE IN THE SHARK (SQUALUS ACANTHIAS) RECTAL GLAND.

J. D. Stidham[1], D. Opdyke[2], C. Aller[2], M. Ratner[2], G. M. Forrest[2], K. Marks[1], S. Aller[2], and J. N. Forrest, Jr.[2].

[1]Department of Biology, Presbyterian College, Clinton, SC 99999

[2]Department of Medicine, Yale University School of Medicine, New Haven, CT 06510

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a neuropeptide of the secretin/glucagon/VIP/growth hormone releasing hormone family. PACAP and VIP are hypothalamic peptides sharing considerable sequence homology but with differing central nervous system effects in higher species, including humans (Spengler et al., Nature 365:170-175, 1993). Because PACAP has been identified in the human gut, we determined the effects of two molecular forms of PACAP: PACAP-38 and PACAP-27, on chloride secretion in the isolated perfused rectal gland and in cultured monolayers of rectal gland cells.

In membrane binding studies, two distinct classes of PACAP receptors have been defined. The PACAP type 1 receptor is found in the hypothalamus, brain stem, pituitary, adrenal gland and testes and appears specific for PACAP. A PACAP type II receptor has recently been cloned; this receptor does not discriminate between PACAP and VIP (Schmidt et al., Pancreas 8: 476-487, 1993). The following experiments were carried to determine if PACAP is an activator of chloride secretion in the perfused gland, to dertermine if the receptor for PACAP is present on cultured rectal gland cells free of neural elements, and if present, to identify the type of PACAP receptor present in the gland. Chloride secretion was measured as previously described (Kelley at al., J. Clin. Invest. 88: 1933-1939, 1991). All data are mean ± SEM.

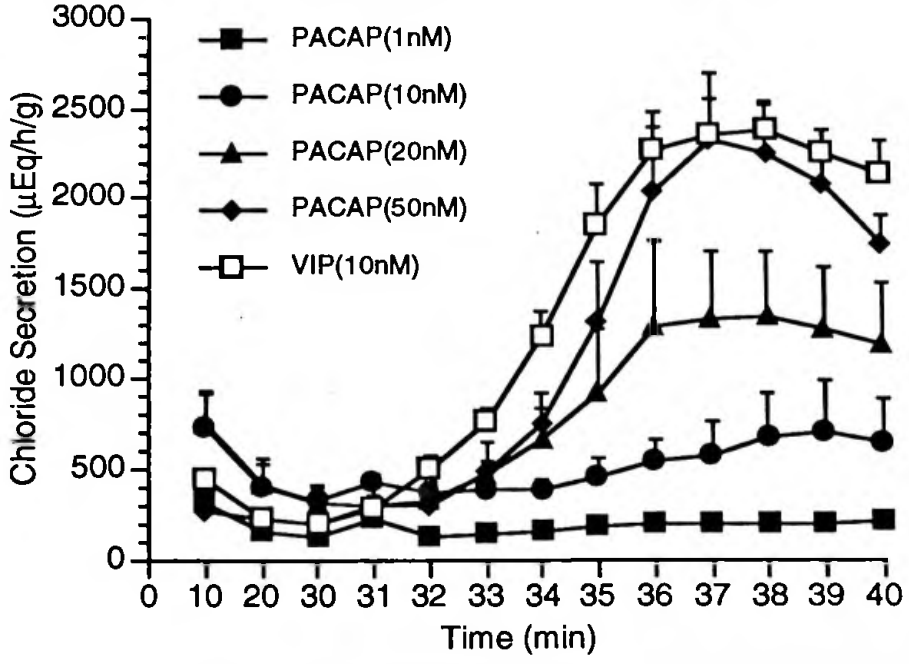

Figure 1. Dose response to PACAP-38 (1 to 50nM) compared to VIP(10nM) on chloride secretion in the isolated perfused rectal gland of Squalus acanthias (n=4-6 per group).

Figure 1 depicts the dose response to PACAP-38 on chloride secretion in the isolated perfused rectal gland. At a concentration of 1 nM, PACAP did not elevate chloride secretion above basal values. At 10, 20 and 50 nM, PACAP elicited a dose dependent increase in chloride secretion.

At a concentration of 50 nM, chloride secretion with PACAP reached 2328±366 μeq/h/g, a near maximal response to secretagogues in the isolated perfused gland. When compared to PACAP, VIP appears to be a more potent secretagogue since the response to 10 nM VIP was substantially greater than 10 or 20 nM PACAP and was equivalent to the response of 50 nM PACAP (Figure 1). The response to PACAP-27 was similar to PACAP-38 (data not shown).

To determine if PACAP can directly activate chloride transport in rectal gland cells free of neural elements, measurements of short circuit current (I_{sc}) were carried out in primary cultures of rectal gland cells prepared as a monolayer on permeable collagen coated nylon mesh supports. When applied to the apical solution, PACAP at concentrations of 5 to 100nM, showed little response. When applied to the basolateral media, both PACAP-38 and PACAP-27 markedly stimulated short circuit current, from 2μA/cm^2 to 85μA/cm^2 and 55μA/cm^2 respectively (Figure 2). Prior exposure to PACAP-38 and -27 did not desensitize the tissue to the subsequent addition of VIP (100nM) to the basolateral solution (Figure 2).

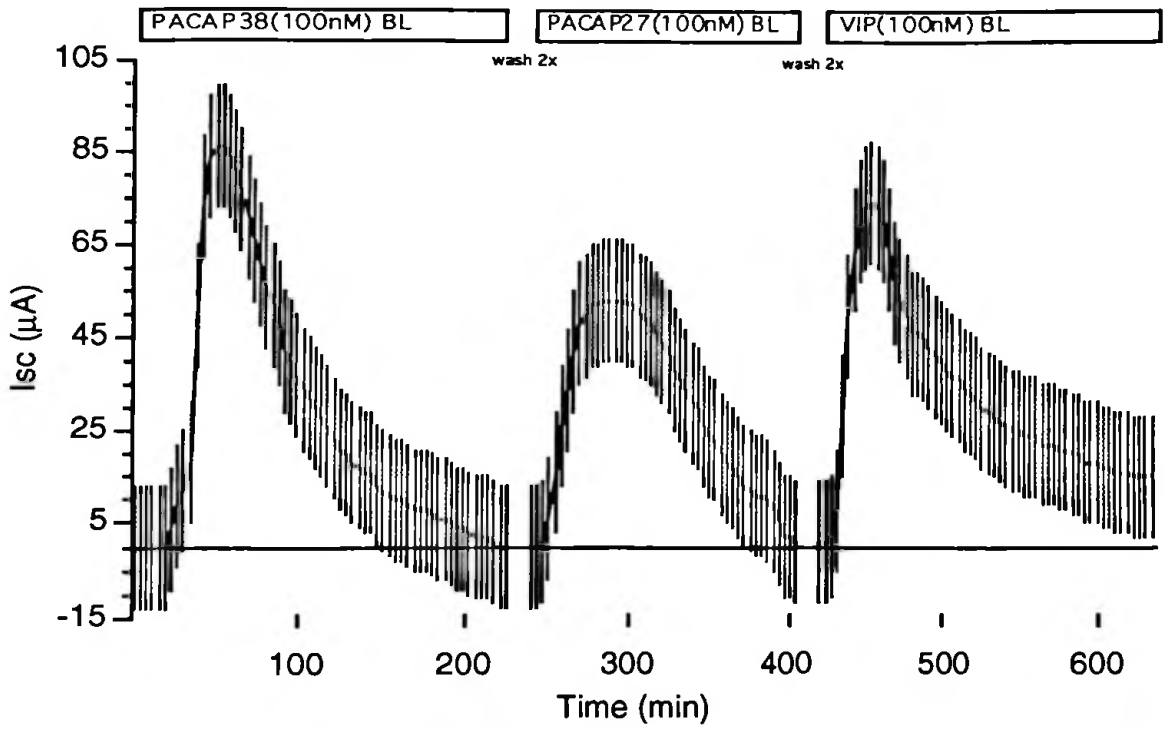

Figure 2. Effects of PACAP-27, PACAP-38 and VIP on short circuit current in cultured monolayers of rectal gland cells (I_{sc}=μA/cm^2).

These data indicate that the novel neuropeptides PACAP-38 and -27 are potent activators of chloride secretion in the rectal gland. Receptors responsive to PACAP are present on rectal gland cells per se since the effects are observed on cultured cells devoid of neural elements. The specific receptor in the rectal gland that is responsive to PACAP and VIP is likely the PACAP-2 receptor. Specific binding studies and cloning and expression of these receptors will be required to determine if more than one receptor type is present in the rectal gland.

Work supported by NIH grant DK 34208-09, American Heart Association Grant 92011310, NSF EPSCoR grant EHR 9108766, and NIEHS P30-ES3828 Center for Membrane Toxicology Studies to Dr Forrest.