FUNCTIONAL EXPRESSION OF THE SHARK VIP RECEPTOR IN *XENOPUS* OOCYTES: ACTIVATION OF CFTR CHLORIDE CHANNELS

Marie Bewley [1,3], Gerhard J Weber, [1,2,3], and John N. Forrest, Jr. [1,3]
[1]Mount Desert Island Biological Laboratory, Salisbury Cove, Maine 06472
[2]Medical University Hannover, Germany
[3]Department of Medicine, Yale University School of Medicine, New Haven, CT 06520

The neuropeptide vasoactive intestinal peptide (VIP), has been shown to stimulate chloride secretion in the shark rectal gland of *Squalus acanthias* in perfused glands, perfused tubules and cultured cells. Two years ago, our lab reported the cloning and molecular characterization of the shark VIP-like receptor (Pena, J. et al., Bull. MDIBL. 40:133-135, 2001). Phylogenic analysis showed this receptor to be similar to other seven transmembrane G protein coupled receptors in the VIP/secretin family. Perfusion studies of the rectal gland in our lab have examined the potency order of four related agonists stimulating chloride secretion. These experiments show an order of potency of secretin <<< PACAP < PHI < VIP. We report here the functional expression and characterization of the cloned shark VIP (sVIP-R) receptor that is the likely target for VIP and related hormones that stimulate salt secretion in the rectal gland.

To functionally characterize shark VIP-R, we determined dose response curves for the targeted agonist, VIP, and an agonist affinity potency for members of the VIP/secretin peptide family. Oocytes from *Xenopus laevis* frogs were harvested and injected with water or hCFTR or co-injected with 5ng hCFTR + 5ngVIP-R cRNA. Two electrode voltage clamping (TEVC) was used under constant perifusion with frog Ringer's solution and added agonists. Oocytes injected with water or CFTR only showed no response to VIP. CFTR only injected oocytes did respond to perifusion with forskolin + IBMX. To determine a dose response curve for VIP, co-injected oocytes (sVIP-R and hCFTR) were first perifused with frog Ringer's solution. Then seven concentrations of VIP (0.1 nM to 0.5 μM) were added sequentially to the perifusate in increasing order. When activation levels plateaued at the highest concentration, the oocytes were washed out with frog Ringer's solution. In some experiments, glibenclamide (100 μM), a known inhibitor of hCFTR activity, was added in conjunction with VIP to show that activation was hCFTR induced. To establish the potency order, an additional series of oocytes were again bathed in frog Ringer'solution. Each peptide was added to the perifusate at a concentration of 50 nM. The peptides were added from least to greatest affinity as predicted by previous perfused gland experiments.

When hCFTR and sVIPR were co-expressed in *Xenopus* oocytes, chloride conductance increased logarithmically after perifusion with VIP. Figure 1 shows an IV plot of chloride conductance under basal, VIP and washout conditions. Figure 2 shows mean chloride conductance as a function of concentration of the agonist, VIP, added to the perifusate. The EC_{50} for activation of the expressed receptor was 9.4 nM VIP.

A unique and unexpected potency order was established for the affinity of similar peptide hormones. Both secretin and PACAP minimally stimulated chloride secretion while PHI and VIP stimulated markedly. Figure 3 shows the mean stimulation of the four peptides demonstrating a potency order of PACAP < Secretin <<< PHI < VIP.

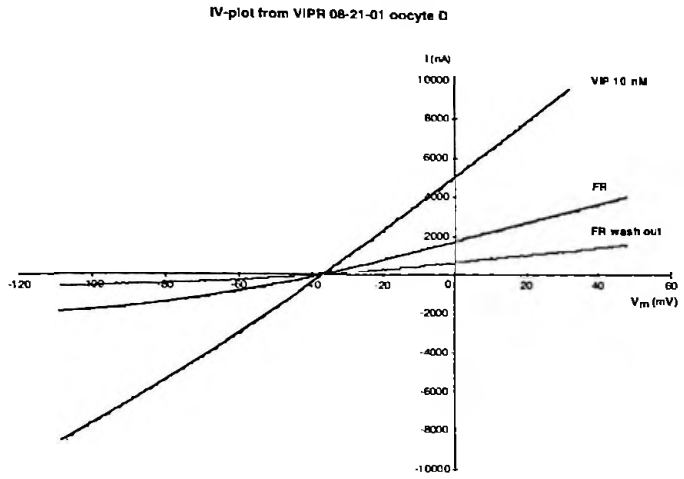

Figure 1. I/V-plot of an oocyte co-expressing sVIPR and hCFTR stimulated with 10nM VIP (FR=Frog Ringers solution)

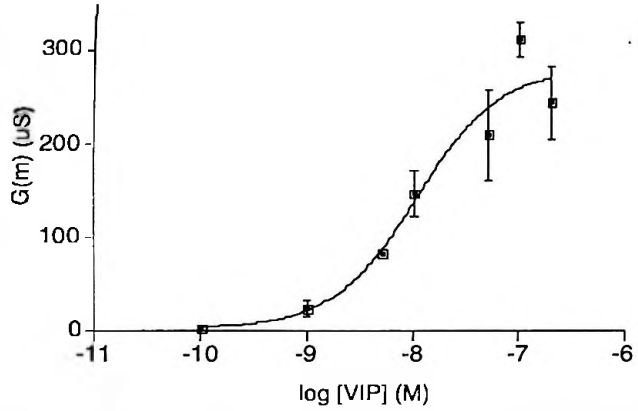

Figure 2: Dose response curve of VIP-R activation. Values are mean ± SEM of maximum Cl conductance after stimulation with seven concentrations. EC_{50} for activation of the expressed receptor = 9.4 nM VIP.

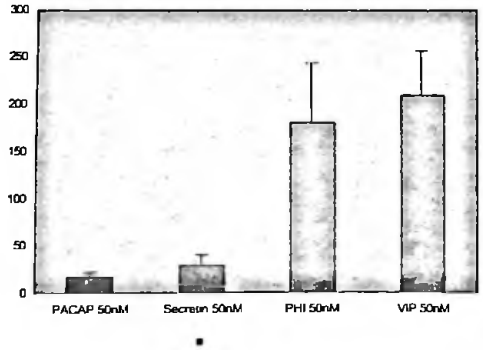

Figure 3: Stimulation of Cl conductance by different G protein receptor agonists. Values are mean ± SEM conductance after stimulation with 50nM PACAP, Secretin, PHI and VIP (n=4 each) (FR=Frog Ringer's solution).

In summary, we report the functional characterization of the shark rectal gland VIP receptor. Shark VIP-R co-expressed with human CFTR in *Xenopus* oocytes shows a dose response curve with the VIP hormone giving an EC_{50} of 9.4 nM. Chloride conductance is increased by other peptides in the secretin/PACAP/VIP family with an order of potency wherein PACAP and secretin show much less stimulation than PHI and VIP respectively. These findings differ from experiments in the perfused rectal gland where PACAP shows similar activation to that of PHI and VIP. This difference between perfusion experiments and expression studies with sVIP-R suggests that there may be an additional endogenous receptor in the shark rectal gland that has affinity for PACAP. The findings of this study suggest that the cloned receptor is the target for VIP stimulation of chloride secretion in the intact shark rectal gland.

This work was supported by NIH grants DK 34208 and NIEHS P30-ES 3828 (Center for Membrane Toxicology Studies)