FUNCTIONAL EXPRESION OF SHARK VIP RECEPTOR IN XENOPUS OOCYTES: ACTIVATION OF CFTR CHLORIDE CHANNELS

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We previously reported the cloning, full length sequencing and molecular characterization of a VIP-like receptor from the shark rectal gland (Pena et al., Bull. MDIBL 40:133-135,2001). The deduced shark VIP receptor (sVIP-R) protein had only 57% identity with mouse VIP-1 receptor and 56% identity with human and rat VIP-1 receptor. This receptor is the likely target for VIP and related hormones that stimulate salt secretion in the rectal gland.

We have now prepared a full-length expression clone of the shark VIP receptor and report here the functional expression of this receptor in Xenopus oocytes using the co-expression of human CFTR as the reporting element. In control experiments, oocytes injected with water or with CFTR cRNA only did not respond to exogenous VIP (100nM). Figure 1 below demonstrates that oocytes co-injected with sVIP-R and hCFTR cRNA respond to 10 nM VIP with an increase in chloride conductance that is reversible following removal of VIP from the bath.

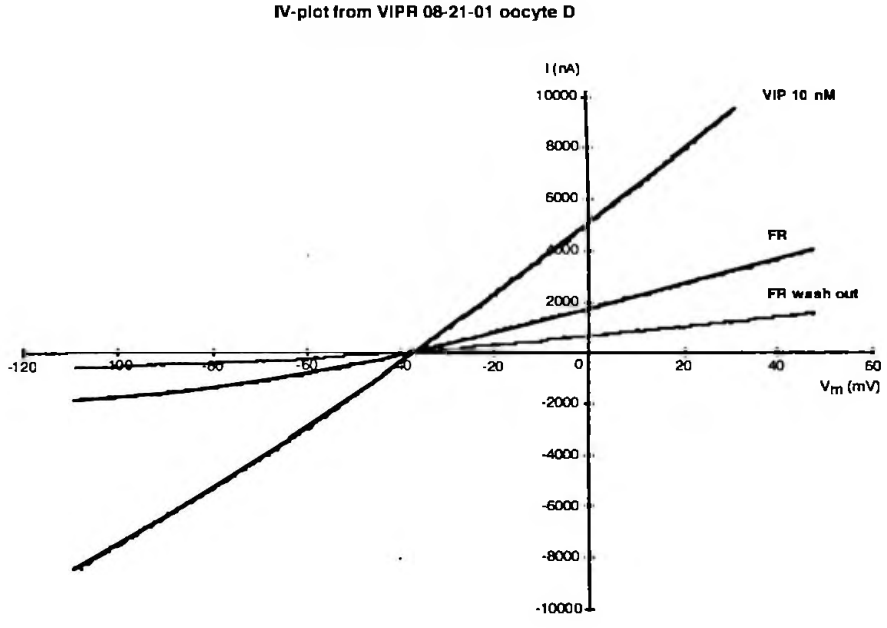

Figure 1. Current voltage plot during a 2-s voltage ramp from –120 mV to +60 mV on a representative oocyte expressing shark sVIP-R and hCFTR, before and after exposure to 10 nM VIP.

A dose response to mammalian VIP in sVIR-R and CFTR cRNA co-injected oocytes is shown in figure 2. VIP (1nM) elicited a detectable increase in chloride conductance and the EC_{50} for VIP was approximately 8 nM. Maximal activation was observed at 80-100 nM VIP. The expressed shark VIP receptor also responds to mammalian pituitary adenylate cyclase activating

peptide (PACAP), peptide histidine isoleucineamide (PHI) and secretin. Further dose response studies are needed to determine the relative potency of these agonists.

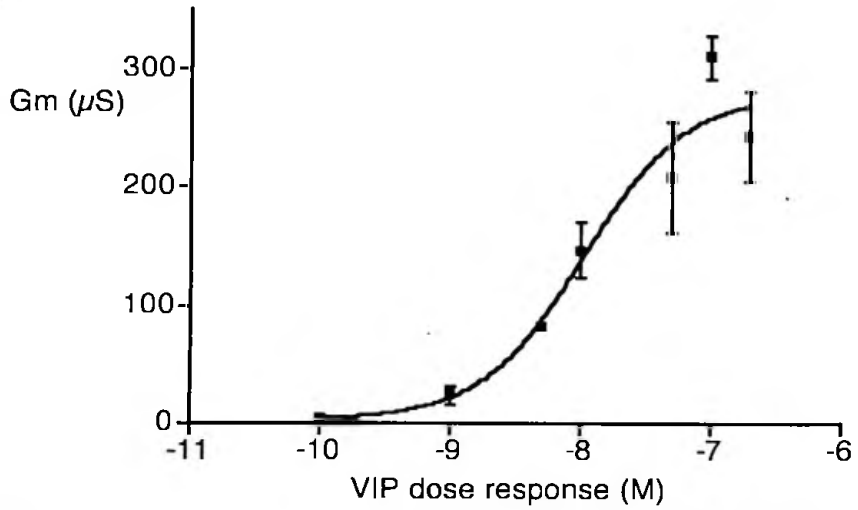

Figure 2. Dose response to VIP in Xenopus oocytes expressing shark VIP-R and hCFTR (n=3-5 oocytes each point).

In summary, we have successfully co-expressed the shark rectal gland VIP-like receptor with hCFTR in Xenopus oocytes and show that this receptor is responsive to mammalian VIP, PHI, PACAP and secretin. The cloned receptor is therefore the likely membrane protein mediating VIP and related secretagogue stimulated chloride secretion in the intact rectal gland.

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