VASOACTIVE INTESTINAL PEPTIDE STIMULATES THE RELEASE OF INOSITOL PHOSPHATES IN SQUALUS ACANTHIAS RECTAL GLAND TUBULES

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Chloride secretion by the shark rectal gland can be activated by a variety of agents including vasoactive intestinal peptide (VIP), forskolin and membrane permeant analogues of cyclic AMP (i.e. dibutyryl cAMP, 8-bromo cAMP). Since VIP increases cAMP levels in rectal gland cells (Stoff et al., Am. J. Physiol. 237:F138, 1979) this suggests that VIP stimulates Cl^- secretion by elevating cAMP. However, the Cl^- secretory status of the rectal gland is not a simple function of cellular cAMP levels. 2-Chloro adenosine (2Clado) inhibits forskolin-stimulated Cl^- secretion, but only partially inhibits forskolin-stimulated cAMP accumulation (Kelley et al., Bull. MDIBL, 26:177, 1986). Perfusion of the rectal gland with 0.1 µM 2Clado, a concentration which does not alter Cl^- secretion, results in a significant elevation of cAMP. Somatostatin inhibits both forskolin stimulated Cl^- secretion and cAMP accumulation (Barron et al., Bull. MDIBL, 27:136, 1987) but somatostatin also inhibits dbcAMP stimulated Cl^- secretion (Silva et al., Am. J. Physiol. 249:R329, 1985). These observations illustrate the complex nature of the regulation of rectal gland secretory behavior.

To account for these observations the modulation of rectal gland Cl^- secretion must involve second messenger systems in addition to cAMP generation. Since VIP receptors in other tissues are coupled to phospholipase C and the liberation of phosphatidylinositol (PI) based second messengers (Audigier et al., Brain Res. 376:363, 1986), it is possible that the PI second messenger system is involved in regulating rectal gland secretory activity.

As a first step towards defining the role of PI derived second messengers in the regulation of rectal gland Cl^- secretion, we used the "batch elution" method of Berridge et al (Biochem. J. 206:587, 1982) to identify agents which evoke the release of inositol phosphates (IPs) from cellular PI. This method is based on the assumption that the terminal dephosphorylation of IPs is inhibited by lithium ions. Since the existence of a lithium-sensitive inositol-1-phosphatase in shark tissue has not been previously demonstrated, our initial experiments were designed to measure the activity and lithium sensitivity of this enzyme in the rectal gland. ^{3}H-inositol-1-phosphate was added to fractions of rectal gland homogenate with or without 10 mM LiCl and the mixture incubated at 20°C for various times. Tritiated inositol was separated from tritiated inositol-1-phosphate by anion exchange (Dowex 1-X8) column chromatography. As illustrated in Figure 1, the rectal gland does contain inositol-1-phosphatase activity and this enzyme is inhibited by lithium.

To measure the release of IPs stimulated by VIP and other secretagogues, rectal gland tubules were isolated under sterile conditions as described by Valentich and Forrest (Bull. MDIBL 26:91, 1986). Tubules were maintained in suspension culture in hydrophobic plastic dishes to inhibit tubule attachment and outgrowth. Tubules can be maintained in this way for at least five days with a viability of >90% (based on trypan blue exclusion). Experiments were performed on the third or fourth day following isolation when viability was >95%. Tubules

were labeled for 48 hr with 2-4 μCi/ml ^{3}H-inositol. Tubules were then equilibrated in shark Ringer containing 10 mM LiCl for 30 min. The tubules were aliquoted to microcentrifuge tubes in a volume of 0.9 ml (approx 1 mg protein). Test compounds were added as a 10X concentrate in Ringer (0.1 ml) and the micro tubes placed on a rocking platform at 20°C for 30 - 60 min. Tubules were pelleted and extracted in 10% TCA at 4°C. Insoluble proteins were pelleted and removed. TCA was removed by ether extraction. A 50% (w/v) slurry of Dowex 1-X8 was added to bind all IPs. The resin was pelleted and washed 4X with 5 mM inositol. IPs were then eluted with 1 ml of 1M ammonium formate/0.1M formic acid. Radioactivity was determined by liquid scintillation counting. This method measures total IPs and does not resolve the many possible polyphosphorylated forms of IP produced during hormone stimulated PI breakdown.

As illustrated in Figure 2, a 30 min exposure to 10^{-7}M VIP resulted in a 25-30% increase in cellular levels of IPs relative to unstimulated tubules ($p \leq 0.005$). In contrast, 10^{-6}M forskolin caused a small increase in IPs which was not significantly different from the controls ($p \geq 0.375$). Similar results were obtained when tubules were exposed to VIP or forskolin for 60 min.

Figure 1

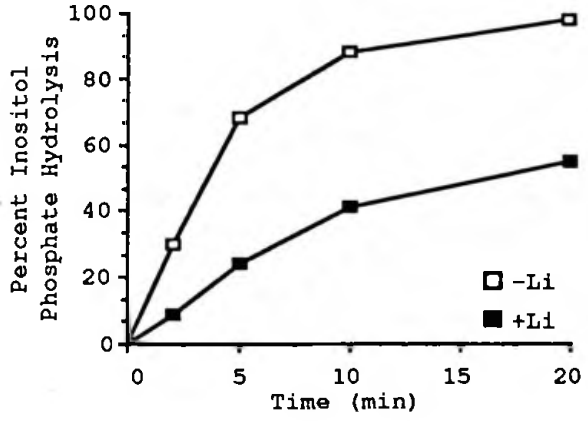
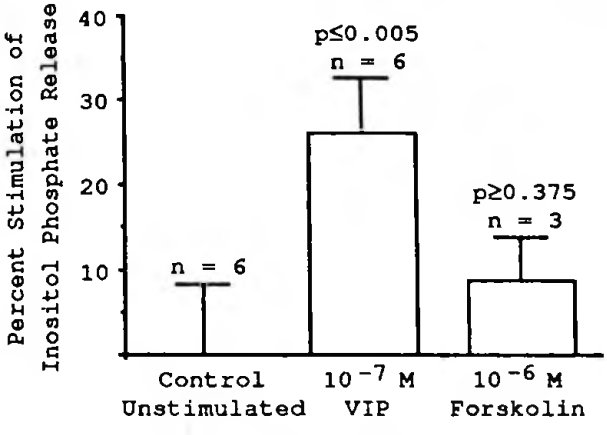


Figure 2



Further experimentation is required to determine the identity of the IP isomers released during VIP stimulated PI breakdown. Since VIP rapidly elevates intracellular calcium concentrations in rectal gland slices (as measured by aequorin luminescence; Kelley et al., Bull. MDIBL 27:129, 1987) we predict that the calcium mobilizing inositol trisphosphate and/or inositol tetrakisphosphate are produced during VIP stimulated PI metabolism. In a preliminary experiment we have detected elevations in inositol monophosphate and inositol bisphosphate as early as 30 sec after VIP addition. We are currently optimizing our labeling and detection protocols so that we can measure changes in the levels of other inositol phosphate isomers during the initial period of VIP stimulated Cl^- secretion.

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