ATRIOPEPTIN AND OTHER CHLORIDE SECRETAGOGUES STIMULATE INOSITOL PHOSPHATE RELEASE IN SQUALUS ACANTHIAS RECTAL GLAND TUBULES

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Previously we have shown that vasoactive intestinal peptide (VIP), which activates Cl^- secretion by the perfused shark rectal gland (Stoff et al., Am. J. Physiol. 237:F138, 1979) and monolayer cultures of rectal gland epithelia (Valentich and Forrest, Bull. MDIBL 26:91, 1986), also increases phosphatidylinositol (PI) turnover in rectal gland tubules maintained in suspension culture (Ecay and Valentich, Bull. MDIBL 28:72, 1989). We report here on the effects of other Cl^- secretagogues on PI turnover in rectal gland tubule culture.

Inositol is a major intracellular osmolyte in rectal gland cells. Therefore, cellular uptake of inositol is very slow and intracellular concentrations are 40 fold higher than in serum (MacGregor and Kleinzeller, Bull. MDIBL 26:168, 1986). We have found that the incorporation of ^3H-inositol into phospholipids increases linearly for at least seven days. Because of these factors it has not been possible to label phospholipids with ^3H-inositol to sufficiently high specific activity to facilitate the measurement of inositol trisphosphate (IP_3). Methods to increase the specific activity of labeling, such as high K^+ depletion of intracellular inositol (Kleinzeller et al., Bull. MDIBL 25:64, 1985) prior to the addition of ^3H-inositol, are currently under development.

It has been possible to measure significant increases in inositol monophosphate (IP) and inositol bisphosphate (IP_2) levels following hormonal stimulation of ^3H-inositol labeled rectal gland tubules. Rectal gland tubules were isolated, cultured and labeled with ^3H-inositol as described (Ecay and Valentich, MDIBL Bull. 28:72, 1989). Tubules cultured in suspension for up to seven days were shown to be viable by trypan blue exclusion and ultrastructural analysis. Tritiated inositol phosphates were extracted from tubules after a 30 min exposure to test compounds and were analyzed by ion exchange chromatography (Berridge et al., Biochem. J. 206:587, 1982). Aluminum fluoride (5 μM), which directly activates G-proteins (Blackmore et al., J. Biol. Chem. 260:14477, 1985), increased IP and IP_2 levels (50 ± 8 and 67 ± 18 respectively; mean ± SEM, n=3; $p \leq 0.03$ vs unstimulated tubules) demonstrating that rectal gland cells contain the G-proteins which couple hormone receptors to phospholipase C activation. Atriopeptin I (AP I; rat, synthetic; 10^{-7} M) significantly increased IP and IP_2 levels over unstimulated tubules (40 ± 2% and 96 ± 7% respectively; mean ± SEM, n=3; $p \leq 0.02$). AP III had similar effects. The calcium ionophore, ionomycin (10^{-6} M), increased total inositol phosphate levels (IP and IP_2) by 152 ± 33% (n=6; $p \leq 0.001$) relative to unstimulated tubules.

Based on these results we conclude that rectal gland cells contain VIP and AP receptors which can be coupled to phospholipase C via G-proteins. Ionomycin stimulation of PI turnover suggests that calcium regulates the activity of phospholipase C in these cells.

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