HORMONAL EFFECTS ON PHOSPHOLIPASE C IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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The effect of hormones on their target cells often involves more than one intracellular second messenger system. These different intracellular systems mediate agonistic or antagonistic responses and provide a means for the regulation of cellular activity. In the rectal gland at least three different intracellular messengers pathways have been identified: cyclic AMP, cyclic GMP and the phosphatidyl inositol pathway. Recently, Ecay and Valentich (Bull. MDIBL 28:72, 1989) showed that VIP stimulated the release of inositol phosphates in cultured rectal gland tubules suggesting that stimulation of chloride secretion by this hormone involves the activation not only of adenyl cyclase but also of phospholipase C, the initial step in the phosphatidyl inositol pathway. In the present report we examined the effect on phospholipase C of several hormones known to have a stimulatory effect on chloride secretion.

Rectal gland plasma membranes were prepared by homogenization and differential centrifugation.

Phospholipase C (PLC) was measured by following the generation of inositolphosphates from ^{3}H-phosphatidylinositol 4,5-biphosphate (^{3}H-PIP2). ^{3}H-PIP_2 was evaporated under nitrogen and dissolved in 10% sodium cholate overnight. Water was then added to yield a final sodium cholate concentration of 3.8%. The PLC assay contained: 0.1 M Tris-HCl, pH 7.4; 1 µM $CaCl_2$; 8-40 µg membrane protein; hormones or controls; 9 mM sodium cholate; and 10,000 cpm ^{3}H-PIP2 in a final volume of 50 µl, at a temperature of 25°C. The reaction was started by addition of the plasma membranes, incubated for 10 sec and stopped by rapid cooling on ice. Phosphoinositides and inositol phosphates were separated using a two-phase system. The 50 µl reaction mixture was mixed with 150 µl of chloroform/methanol/concentrated HCl (1/2/0.2, v/v) followed by 50 µl of chloroform and 50 µl of 2M KCl. The sample was vortex mixed and centrifuged for 5 min in a microcentrifuge to separate the phases. Two aliquots of the aqueous phase were counted to determine the amount of ^{3}H-PIP_2 hydrolyzed. Results are expressed as pmoles of PIP_2 hydrolyzed per mg of protein.

Table I shows the effect of VIP on phospholipase C from the shark rectal gland.

	Phosphatidyl inositol 4,5-biphosphate hydrolyzed (pmol/mg)	
	VIP	2-chloro adenosine
Concentration (Molar)		
0	2.1±0.4 (8)	2.3 (2)
10^{-9}	2.7±0.5 (4)	2.7 (2)
10^{-8}	3.0±0.8 (8)	3.2 (2)
10^{-7}	3.4±0.8 (8)	3.1 (2)
10^{-5}	3.9±0.6 (8)	2.9 (2)
10^{-3}		3.5 (2)

Figures are mean ± SE, (n).

These results show that VIP has a dose related stimulatory effect on the activity of plasma membrane associated phospholipase C. The effect was significant, $p < 0.01$, as determined by analysis of variance. To test for the effect of adenosine on phospholipase C we used 2-chloroadenosine a non-hydrolyzable analog of adenosine. Table I shows two preliminary experiments that indicate that 2-chloroadenosine also stimulates phospholipase activity in the rectal gland. We next tested for the effect of atrial natriuretic peptide (ANP) on phospholipase C and found that in two experiments it had neither a stimulatory nor an inhibitory effect.

These experiments show that the phospholipase C of the rectal gland is stimulated by both VIP and adenosine. The effect of VIP increased in direct relation to the concentration of the hormone. However, no maximal effect was observed at the concentrations tested. The effect was small and not significant at a 10^{-9} M, a concentration that regularly stimulates chloride secretion in the perfused gland. The functional significance of the effect of VIP on phospholipase C is yet to be determined.

Although the experiments with 2-chloro adenosine are too few to provide unequivocal information, it appears that it has a maximal effect on phospholipase C at a concentration of 10^{-8} M. This concentration is within the range of the inhibitory adenosine receptor suggesting that the activation of phospholipase C by adenosine may complement its inhibitory effect on adenyl cyclase.

The absence of an effect of ANP on phospholipase C is not surprising in light of our previous observation that it has no direct effect on the rectal gland, rather its stimulatory effect on chloride secretion is mediated by VIP. However, ANP appears to have a direct stimulatory effect on cultured rectal gland tubules that cannot be evoked in fresh tubule preparations (Lear et al., Bull MDIBL, submitted). It is possible that in cultured rectal gland tubules, that may express de novo receptors to ANP, this hormone might have an effect on phospholipase C.

In summary, these experiments show that phospholipase C present in rectal gland cells is stimulated by VIP and adenosine. Although the functional effects of phospholipase C in the rectal gland were not addressed by these experiments, it is probable that this pathway participates in the intracellular regulation of chloride secretion.

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