EFFECT OF PROTEIN KINASE C ACTIVATION ON CHLORIDE SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS

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In previously unreported experiments we identified inositol mono-, di- and triphosphate in the shark rectal gland indicating that this pathway is present in that epithelium. Recently, Ecay et al., showed that vasoactive intestinal peptide (VIP) stimulates inositol phosphate release in cultured rectal gland cells grown in suspension (Bull MDIBL 28:72-73, 1989). Brand et al., confirmed that VIP activates the inositol phosphate pathway showing that it activates phospholipase C in plasma membranes of shark rectal gland. These authors also showed that 2-chloro adenosine also activated phospholipase C in the same preparation but that human alpha-atrial natriuretic peptide (ANP) failed to do so (Bull MDIBL 29:96-97, 1990). Simultaneously, Ecay et al., found that rat ANP I and III increased the levels of inositol mono- and diphosphates in cultured rectal gland tubules (J Cell Physiol 146:407-16, 1991).These authors also found that ionomycin 10^{-6}M increased total inositol phosphates.More recently, Feero et al., have found that phorbol myristate acetate (PMA) 10^{-7}M increased short circuit current in cultured rectal gland cells grown to confluence, an effect not reproduced by an inactive phorbol ester and decreased by the inhibitor of protein kinase C, staurosporine (Bull MDIBL 30:63-64, 1991). The same group has also reported that ionomycin 10^{-6}M stimulates short circuit current in the same preparation. To date there is no reported data on the role of the inositol phosphate pathway on chloride secretion in the intact rectal gland. The present experiments were performed to investigate its role in isolated perfused, but otherwise intact, rectal glands.

Isolated shark rectal glands were perfused using a technique developed in our laboratory. Dogfish were pithed and the rectal glands removed by an abdominal incision. The rectal gland artery, vein and duct were catheterized and the glands placed in a glass perfusion chamber maintained at a temperature of 15° C with running sea water. The glands were perfused by gravity at a pressure of 40 mm Hg. The composition of the perfusate was (in mM): Na, 280; Cl, 280; K, 5; bicarbonate, 8; phosphate, 1; Ca, 2.5; Mg, 1; sulfate, 0.5; urea, 350; glucose, 5; ph, 7.6 when gassed with 99% O_2/ 1% CO_2. Rectal gland secretion was collected in tared 1.5 ml centrifuge tubes over 10 minute intervals. Chloride concentration in the rectal gland secretion was measured by amperometric titration. Glands were stimulated to secrete chloride with vasoactive intestinal peptide (VIP) 1.5 x 10^{-9}M dibutyryl cyclic AMP 5 x 10^{-5}M and theophylline 2.5 x 10^{-4}M.

In two experiments, TPA at a concentration of 1.6 x 10^{-6}M had no effect on chloride secretion in isolated perfused rectal glands stimulated to secrete chloride with VIP, Figure 1. Oleyl acetyl glycerol at a concentration of 6.3 x 10^{-5}M also had no effect on VIP stimulated chloride secretion as also shown in Figure 1.

Both TPA and oleyl acetyl glycerol activate protein kinase C but do not increase the calcium concentration of the cytoplasm that is also required for the activation of protein kinase C. Therefore, experiments were done in which TPA was used in combination with ionomycin that increases the entry of calcium into the cell. TPA, 1.6 x 10^{-6}M, and ionomycin 10^{-6}M had no effect on chloride secretion, Figure 2.

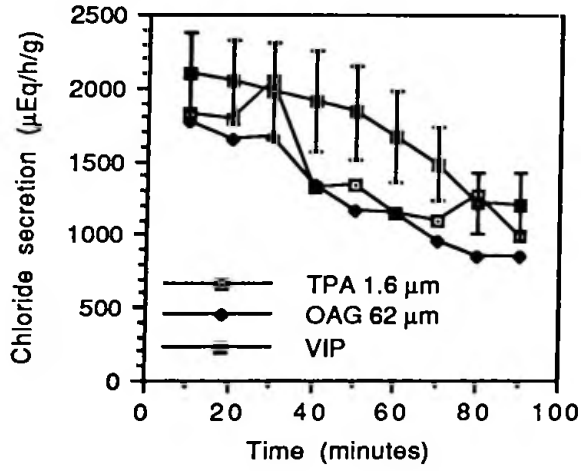

Figure 1. Effect of TPA 1.6 µM and oleyl acetyl glycerol 62 µM on chloride secretion in rectal glands stimulated with VIP. Neither TPA nor OAG had any effect on chloride secretion. Values are mean ± SEM, n: VIP=6, TPA=2, OAG=2.

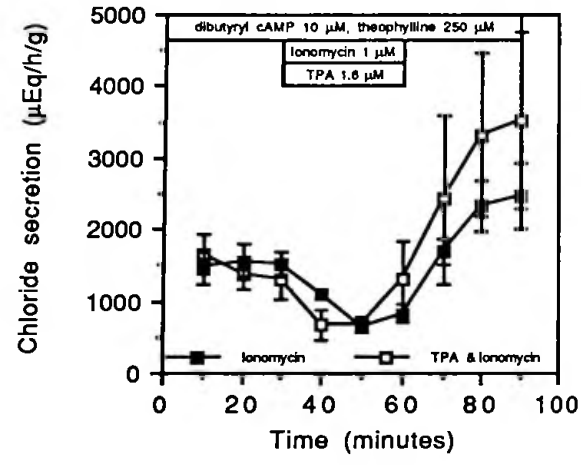

Figure 2. Effect of TPA 1.6 µM combined with ionomycin 1 µM and ionomycin 1 µM alone on chloride secretion in glands stimulated with theophylline 2.5 x 10^{-4}M and dibutyryl cyclic AMP 5 x 10^{-5}M Values are mean ± SEM, n: TPA plus ionmycin=6, ionomycin alone=4.

In glands stimulated with theophylline 2.5 x 10^{-4}M and dibutyryl cyclic AMP 5 x 10^{-5}M the combination of TPA and ionomycin caused a small but tically not significant decrease in chloride secretion followed by a large increase in chloride secretion. The increase in chloride secretion was the result of a large increase in fluidsecretion with a fall in chloride concentration. The increase in chloride secretion started after the TPA perfusion had been stopped and persisted for the remainder of the experiment. Ionomycin 10^{-6}M alone produced the same effect as that seen in combination with TPA.

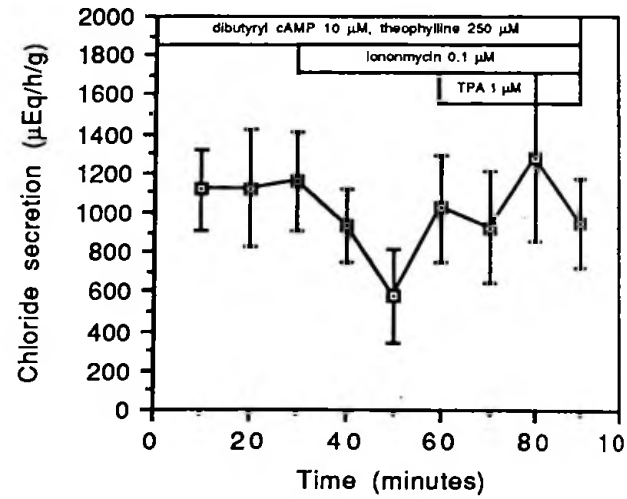

Figure 3. Effect of ionomycin 10^{-7}M followed by TPA 1 µM on chloride secretion in glands stimulated with theophylline 2.5 x 10^{-4}M and dibutyryl cyclic AMP 5 x 10^{-5}M. At this concentration ionomycin had no effect on clhloride secretion nor did TPA. Values are mean ± SEM, n=6.

Lowering the concentration of ionomycin 10 fold to 10^{-7}M prevented all effects of ionomycin. TPA added after the ionomycin, while the latter was still in the perfusate, had no additional effect as can be seen in Figure 3.

Ionomycin 10^{-6}M evoked the same secretory effect associated with a decrease in the concentration of chloride in glands that were not stimulated

to secrete chloride. TPA, however, had no effect on unstimulated glands, Figure 4..

The above experiments show that activation of protein kinase C has no effect on chloride secretion by the rectal gland. Neither TPA nor oleyl acetyl glycerol, that activate protein kinase C, had any effect on chloride secretion in the perfused rectal gland when used alone. When the calcium ionophore, ionomycin was used at a concentration of 10^{-7}M in addition to TPA again there was no effect. Ionomycin used alone at a concentration of 10^{-7}M had no effect, but at a concentration of 10^{-6}M it produced a large increase in fluid

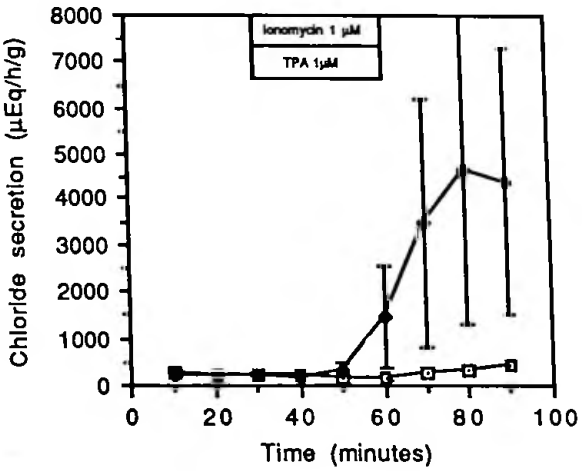

Figure 4. Effect of TPA 1 µM or ionomycin 1 µM on chloride secretion in unstimulated glands. Values are mean ± SEM, n: TPA (open squares)=6, ionomycin (closed diamonds)=4.

secretion associated with a fall in the concentration of chloride in the secretory fluid. The fall in the concentration of chloride suggests that there was ionomycin-induced damage to the rectal gland epithelia. The concentration of chloride in the rectal gland secretion remains constant at a level approximately twice that of the perfusate when chloride secretion is stimulated. This is the result of the luminal membrane of the rectal gland cells impermeability to urea. If the cell membrane becomes permeable to urea, or urea is removed from the perfusate, the concentration of chloride in the perfusate falls and the volume of fluid increases. The finding that ionomycin increases chloride secretion while decreasing the concentration of chloride suggests that it is increasing luminal permeability.

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