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Intracellular Mediators of Goblet Cell Degranulation in Isolated Pancreatic Ducts

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The involvement of particular intracellular signalling pathways in agonist-evoked degranulation of guinea pig pancreatic duct goblet cells was investigated. Carbachol, vasoactive intestinal peptide (VIP), calcium ionophore A23187, phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA), cyclic AMP analogue Sp-5,6-DCl-cBIMPS and forskolin each caused degranulation of goblet cells in isolated ducts. Degranulation induced by carbachol was not inhibited by okadaic acid, cytochalasin-D or nocodazole. These results indicate that at least two major signalling pathways are involved in pancreatic duct goblet cell secretion.

Goblet cells in the interlobular and main ducts of the mammalian pancreas secrete mucus, which coats and protects the lumenal surfaces of duct epithelial cells (1). Until recently, nothing was known of the physiological control of pancreatic mucus secretion. However, studies in our laboratory using isolated segments of the guinea pig main pancreatic duct have now demonstrated that bombesin, VIP, and the cholinergic agonist carbachol each cause degranulation of goblet cells in the duct epithelium (2). In many cell types, including pancreatic acinar cells, bombesin and carbachol stimulate secretion through increased hydrolysis of membrane inositol-containing phospholipids, while VIP activates adenylyl cyclase, leading to an increase in cellular cyclic AMP (3). The current studies were therefore undertaken to determine whether direct activation of either of these two intracellular signalling pathways could

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elicit degranulation of pancreatic duct goblet cells and to identify some of the intracellular mediators involved.

MATERIALS AND METHODS

Duct isolation and incubation: Main ducts were isolated from pancreata of fasted male guinea pigs by digestion using chromatographically-purified collagenase (Worthington) as described previously (4). Segments 2 mm in length were cut from the proximal half of the duct and transferred to glass vials containing 5 ml of Hepes-buffered Ringers solution (pH 7.4). Selected potential agonists of goblet cell secretion were added to the suspensions and the duct segments were incubated with gentle shaking for up to 4 h at 37°C under 100% O_2.

Fixation and embedding: Incubations were terminated by replacement of the Ringers solution with 5 ml of 0.1 M sodium cacodylate (pH 7.4) containing 1.0% formaldehyde and 1.6% glutaraldehyde. Duct segments were fixed for a minimum of 90 min at 23°C before postfixation in 1% osmium tetroxide, dehydration through a series of ethanol solutions, and embedding in Spurr low viscosity embedding medium (Polysciences). As described previously (2), duct segments were oriented in latex molds during embedding so that sections could be obtained through the longitudinal axis of each duct segment.

Morphometric analysis: For morphometric analysis of the effects of potential secretagogues on duct goblet cells, 1 µm thick sections through the central lumena of the embedded duct segments were obtained using a Sorvall MT-2B ultramicrotome. These were transferred to glass slides, stained with 1% toluidine blue, and observed with a Reichart light microscope. The total number of cells and of quiescent (granule-replete) goblet cells in the simple columnar epithelium lining both sides of the lumen in each section of main pancreatic duct were counted. The contribution of quiescent goblet cells to the epithelium was then expressed as a percentage of the total cell population. Comparison of the means of samples from groups of duct segments exposed to different potential secretagogues or inhibitors was carried out by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Earlier studies in our laboratory demonstrated that exposure of isolated segments of guinea pig main pancreatic duct to carbachol resulted in degranulation of goblet cells in the epithelium lining the duct lumen (2). This degranulation occurred through the slow release of a bolus of granules (t_{50} = 2.5 h) rather than by rapid compound exocytosis, as has been observed in intestinal goblet cells exposed to cholinergic agonists (5). Carbachol-evoked degranulation of duct goblet cells could be blocked by atropine, indicating that the response is mediated by activation

of muscarinic receptors, which have been shown to be abundantly expressed in guinea pig pancreatic ducts (6,7).

Activation of muscarinic receptors in other cell types leads to elevation of intracellular free calcium levels and activation of protein kinase C. To determine whether these events might constitute parts of the signalling pathway in pancreatic duct goblet cell secretion, isolated main duct segments were incubated for 4 h with 1 μM A23187 and 1 μM TPA separately and in combination (Fig. 1). In control duct segments, 18.5 ± 1.1% of the epithelial cell population consisted of quiescent goblet cells. This percentage was decreased by 69% in duct segments incubated with A23187 and by 66% in segments exposed to TPA. In duct segments incubated with both compounds simultaneously, the percentage of quiescent goblet cells in the epithelium was reduced by 94% from control values. When duct segments were incubated with TPA in the presence of 10 μM chelerythrine, a potent inhibitor of protein kinase C (8), degranulation was blocked. These studies suggest the involvement of calcium-mediated signalling events and activation of protein kinase C in the intracellular pathways that result in pancreatic duct goblet cell secretion.

Our previous studies also demonstrated pancreatic duct goblet cell degranulation in response to VIP, an agonist that increases cyclic AMP levels in guinea pig pancreatic acinar cells (3). To assess the possible role of cyclic AMP in pancreatic duct goblet cell degranulation, isolated duct segments were incubated for 4 h with 0.1 μM VIP, 0.1 mM forskolin, and 0.5 mM Sp-5,6-DCl-cBIMPS, a membrane permeant and specific activator of protein kinase A (9). These compounds caused decreases in the percentage of granule-replete goblet cells in the duct epithelium of 61%, 47%, and 53%, respectively (Fig. 2), suggesting that elevation of cyclic AMP, with subsequent activation of protein kinase A, may mediate the stimulatory effects of VIP on goblet cell secretion.

Regulation of digestive enzyme release from pancreatic acinar cells by protein phosphatase activity recently has been demonstrated (10). To determine whether phosphatase activity plays a role in the observed goblet cell degranulation, isolated duct segments were incubated for 4 h with 0.1 mM carbachol in the

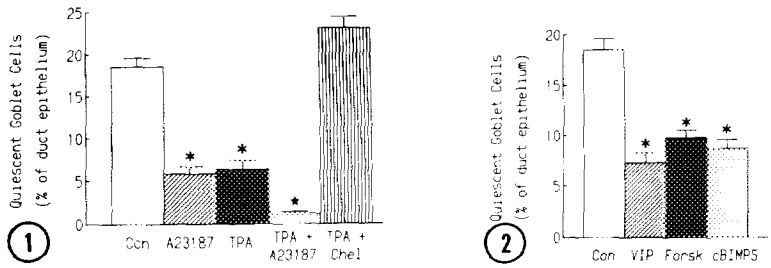

Figure 1. Effects of A23187, TPA, and chelerythrine on degranulation of goblet cells in isolated segments of the guinea pig main pancreatic duct. Isolated duct segments were incubated for 4 h at 37°C in Hepes-buffered Ringers solution (pH 7.4) containing no drugs (Con), 1 μM A23187, 1 μM TPA, 1 μM A23187 and 1 μM TPA, and 1 μM TPA plus 10 μM chelerythrine (Chel). Results represent means ± S.E. of determinations from 15 individual duct segments in each group. * significantly different from control value ($P < 0.05$). ★ significantly different than values for A23187 and TPA ($P < 0.05$).

Figure 2. Effects of VIP, forskolin, and Sp-5,6-DCl-cBIMPS on goblet cell degranulation in isolated segments of the guinea pig main pancreatic duct. Isolated duct segments were incubated at 37°C for 4 h in Hepes-buffered Ringers solution (pH 7.4) containing no drugs (Con), 0.1 μM VIP, 0.1 mM forskolin (Forsk) and 0.5 mM Sp-5,6-DCl-cBIMPS (cBIMPS). Results represent means ± S.E. of determinations from 11-15 individual duct segments in each group. * significantly different ($P < 0.05$) from control value.

absence and presence of 0.1 μM okadaic acid, a potent inhibitor of protein serine-threonine phosphatases (11). Incubation of duct segments with okadaic acid caused small, non-significant decreases in the numbers of quiescent goblet cells in the epithelium, but did not inhibit degranulation induced by the cholinergic agonist (Fig. 3). Duct segments also were incubated with 1 μM okadaic acid, but this higher concentration caused morphological disruption of the duct epithelium. These observations indicate that protein phosphatase activity does not play a central role in carbachol-evoked pancreatic duct goblet cell degranulation.

In the current studies, we also investigated the possible involvement of the goblet cell cytoskeleton in the degranulation response. Duct segments were incubated for 4 h in the absence and presence of 0.1 mM carbachol and 10 μg/ml of nocodazole and cytochalasin-D (Fig. 4). We earlier confirmed that these concentrations of cytochalasin-D and nocodazole, respectively, disrupt microfilament and microtubule arrays in pancreatic duct epithelial cells (12). Neither compound caused a change in the percentage of granule-replete goblet cells in the duct epithelium

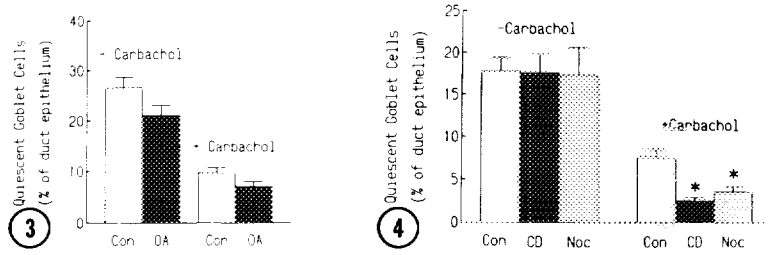

Figure 3. Effect of okadaic acid on carbachol-induced goblet cell degranulation in isolated segments of the guinea pig main pancreatic duct. Isolated duct segments were incubated for 4 h at 37°C in Hepes-buffered Ringers solution (pH 7.4) containing no drugs (Con) and 0.1 μM okadaic acid (OA). Paired samples also were incubated in the presence of 0.1 mM carbachol. Results represent means ± S.E. of determinations from 12-13 individual duct segments in each group. Differences between control and okadaic acid-exposed values were not significant ($P > 0.05$) in either the presence or absence of carbachol.

Figure 4. Effects of nocodazole and cytochalasin-D on carbachol-induced degranulation of goblet cells in isolated segments of the guinea pig main pancreatic duct. Isolated duct segments were preincubated for 1 h at 37°C in Hepes-buffered Ringers solution (pH 7.4) containing no drugs (Con), 10 μg/ml of nocodazole (Noc), and 10 μg/ml of cytochalasin-D (CD) and then for a further 4 h in the absence and presence of 0.1 mM carbachol. The cytoskeletal-disrupting drugs were present throughout the 4 h incubation. Results represent means ± S.E. of determinations from 8-12 individual duct segments in each group. * significantly different from carbachol group control value ($P < 0.05$).

in the absence of carbachol, nor did they inhibit the secretory response to the agonist. Instead, each significantly increased the extent of degranulation induced by carbachol during the incubation. These observations suggest that microtubules and microfilaments in pancreatic duct goblet cells may passively restrain extrusion of granules.

In conclusion, our results indicate that activation of either protein kinase A or protein kinase C is sufficient to elicit goblet cell degranulation in guinea pig pancreatic duct goblet cells, as has recently been shown to be the case for mucus-secreting T84 adenocarcinoma cells (13). By contrast, protein phosphatase activity and the integrity of microtubules and microfilaments are not essential to degranulation of goblet cells induced by muscarinic receptor activation.

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