Ca\textsuperscript{2+} and protein kinase C activation of mucin granule exocytosis in permeabilized SPOC1 cells

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Ca\textsuperscript{2+} and protein kinase C activation of mucin granule exocytosis in permeabilized SPOC1 cells. Am. J. Physiol. 275 (Cell Physiol. 44): C285–C292, 1998.—Mucin secretion by airway goblet cells is under the control of apical P2Y\textsubscript{2} phospholipase C-coupled purinergic receptors. In SPOC1 cells, the mobilization of intracellular Ca\textsuperscript{2+} by ionomycin or the activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA) stimulates mucin secretion in a fully additive fashion [L. H. Abdullah, J. D. Conway, J. A. Cohn, and C. W. Davis. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L203–L210, 1997]. This apparent independence between PKC and Ca\textsuperscript{2+} in the stimulation of mucin secretion was tested in streptolysin O-permeabilized SPOC1 cells. These cells were fully competent to secrete mucin when Ca\textsuperscript{2+} was elevated from 100 nM to 3.1 µM for 2 min following permeabilization; the Ca\textsuperscript{2+} EC\textsubscript{50} was 2.29 ± 0.07 µM. Permeabilized SPOC1 cells were exposed to PMA or 4α-phorbol at Ca\textsuperscript{2+} activities ranging from 10 nM to 10 µM. PMA, but not 4α-phorbol, increased mucin release at all Ca\textsuperscript{2+} activities tested: at 10 nM Ca\textsuperscript{2+} mucin release was 2.1-fold greater than control and at 4.7 µM Ca\textsuperscript{2+} mucin release was maximal (3.6-fold increase). PMA stimulated 27% more mucin release at 4.7 µM than at 10 nM Ca\textsuperscript{2+}. Hence, SPOC1 cells possess Ca\textsuperscript{2+}-insensitive, PKC-dependent, and Ca\textsuperscript{2+}-dependent PKC-potentiated pathways for mucin granule exocytosis.

lungs; airways; mucus; goblet cells; cellular regulation

CALCIUM AND protein kinase C (PKC), the active effectors of the phospholipase C (PLC) signal transduction system, have been implicated widely in the control of regulated exocytosis. In contrast to the apparently universal activation of the exocytotic mechanism by Ca\textsuperscript{2+}, the effects of PKC vary by cell type: stimulatory, inhibitory, and modulatory effects on exocytosis have been reported (see Ref. 31). For mucin-secreting cells of the gastrointestinal tract (18–20) and the airways (1, 16), exocytosis is reported to be activated independently by Ca\textsuperscript{2+} and PKC; we have tested this apparent independence in a permeabilized cell model using SPOC1 cells, a mucin-secreting cell line from the airways (2, 46).

Native airway goblet cells secrete mucin in response to the interaction of purinergic agonists [ATP, UTP, adenosine 5'-O-(3-thiotriphosphate) (ATP\textsubscript{S})] with P2Y\textsubscript{2} receptors (– P2\textsubscript{Y\textsubscript{2}}) as do primary cultures of airway epithelial cells and SPOC1 cells (for review, see Ref. 15). Consistent with the known coupling of this receptor class through heterotrimeric G proteins to PLC (10), primary cultures of airway epithelial cells comprised predominantly of mucin-secreting cells release inositol phosphates on stimulation (28). Although intracellular Ca\textsuperscript{2+} levels have yet to be determined in goblet cells, in SPOC1 cells agents known to elevate intracellular Ca\textsuperscript{2+} (ionomycin, thapsigargin) also stimulate mucin secretion (1). Similarly, activation of PKC by phorbol 12-myristate 13-acetate (PMA) elicits mucin secretion in several cultured airway cell models (24, 27, 35, 52, 53) and in SPOC1 cells (Ref. 1; see also Refs. 15, 16). Notably, the mucin secretory response to ionomycin and PMA was fully additive at maximal doses in SPOC1 cells. Downregulation of PKC by overnight exposure to a half-maximal dose of PMA abolished the ability of SPOC1 cells to respond to maximal doses of either PMA or UTP, but they responded maximally to ionomycin (1). These results suggest that Ca\textsuperscript{2+} and PKC are independent in their actions, and, in the experiments reported here, we test whether PMA is effective in promoting mucin secretion in permeabilized cells where Ca\textsuperscript{2+} is controlled by an exogenous Ca\textsuperscript{2+} buffer system.

MATERIALS AND METHODS

Materials. Culture medium was purchased from GIBCO BRL (Gaithersburg, MD), and the supplements were from Collaborative Research (Bedford, MA). Nucleotides were purchased from Boehringer Mannheim (Indianapolis, IN), streptolysin O (SLO) was from Murex Diagnostics (Norcross, GA), and TO-PRO was from Molecular Probes (Eugene, OR). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

SPOC1 cell culture and mucin secretion. SPOC1 cells, passages 7–15, were seeded at densities of 18,000 cells/well in 48-well cluster plates (Costar, Cambridge, MA) and were grown in a DMEM-F12-based culture medium described previously (26, 46). Briefly, the medium was supplemented with 30 mM HEPES, 6.5 mM L-glutamine, 10 µg/ml insulin, 0.1 µg/ml hydrocortisone, 0.1 µg/ml cholera toxin, 5 µM transferrin, 50 µM phosphoethanolamine, 80 µM ethanolamine, 25 ng/ml epidermal growth factor, 1% vol/vol bovine pituitary extract, 1 mg/ml bovine serum albumin (essentially globulin-free, Sigma no. A7638), 50 U/ml penicillin, and 50 µg/ml streptomycin. Except for cells grown solely for passaging, the medium also contained 10 nM retinoic acid. Culture media were changed daily, and the cultures were used for experiments 6–12 days postconfluence.

SPOC1 cell mucin secretion and enzyme-linked lectin assay. Before all experiments, SPOC1 cells were removed from the incubator, washed twice in DMEM-F12, and incubated at 35°C for 30 min; this procedure was repeated twice for a total equilibration period of 90 min. To study the response of intact cells to nucleotide agonist challenges, SPOC1 cells were subsequently exposed to UTP or ATP\textsubscript{S} over a wide range of concentrations, each dose in triplicate, during a single 30-min incubation. Other details of these experiments are given in RESULTS.

Samples of media removed from the wells of the cluster plate were assessed for mucin content by enzyme-linked lectin assay (2): 100-µl samples were bound to 96-well high-
binding microtiter plates (Costar no. 3590) with an overnight or a 2 h incubation at 4 or 37°C, respectively. The plates were washed (Dynatech MR5000; Chantilly, VA) with PBS containing 0.05% Tween 20 and 0.02% thimerosal, blocked with gelatin, and incubated with 1.5 µg/ml horseradish peroxidase-conjugated soybean agglutinin for 1 h at 37°C. After plates were washed and developed (incubation in 0.04% wt/vol of the substrate, O-phenylenediamine in 0.0175 M citrate-phosphate buffer, pH 5.0, containing 0.01% hydrogen peroxide), the reaction was stopped by the addition of 4 M sulfuric acid and optical density was determined at 490 nm (Dynatech model MR5000 microtiter plate reader). Optical density was converted to nanograms mucin from a standard curve using known amounts of purified SPOC1 mucin (2); mucin standards were applied to each microtiter plate assessed.

Cell permeabilization and Ca2+ buffering system. Differentiated SPOC1 cells were permeabilized, as epithelial sheets in the bottom of 48-well cluster plates, by SLO (22). SLO was resuspended at 3 U/ml in intracellular buffer (Buif), which had a final concentration (in mM) of 130 potassium glutamate, 20 PIPES, 1.0 MgATP, and 3.0 total EGTA; the pH was adjusted to 6.8, and pCa was adjusted to a desired activity by the addition of CaEGTA. Free Ca2+ levels were calculated with the aid of the computer program Chelator (49); although these activities were calculated in log units of pCa, for convenience, they are expressed in nanomolar or micromolar in the results and discussion. Stock solutions of EGTA and CaEGTA, nominally 50 mM, were made according to the Ca2+-buffering system of Gomperts and Tatham (22). EGTA concentrations were determined by titration (38), using a Ca2+-solution made from a freshly opened bottle of CaCl2·2H2O; aliquots of these stocks were stored at −20°C. Rapid solution changes during the cell permeabilization procedure were facilitated with a Finnpipette multistepper pipetter (Needham Heights, MA; using 6 of the 8 tips) and a six-tip vacuum manifold fabricated from Delrin and disposable, plastic 100-µl pipette tips. With these tools, the medium in a 48-well cluster plate could be removed and replaced, consistently, in <10 s.

After equilibration (described above), the cells were washed in rapid succession, twice in PBS (400 µl) and twice in pCa 7.0 Buif (400 µl). Unless stated otherwise, the cells were then permeabilized by a 30-s incubation in pCa 7.0 Buif, containing 1 U/ml SLO (150 µl), subsequently washed twice in pCa 7.0 Buif (150 µl), and then incubated in a solution for a time appropriate to the experiment (described in results). In most experiments, this medium was then removed and assessed for mucin content. Last, the degree of cell permeabilization following each experiment was assessed using the membrane-impermeant, DNA-staining dye TO-PRO: the cells were washed with PBS before and after a 10 min incubation in TO-PRO (10 µM in PBS), fixed in 3% formaldehyde in PBS for 10 min, washed, and covered with 200 µl PBS-glycerol (1:1). Nuclear fluorescence was viewed by video microscopy (Hamamatsu C5985 charge-coupled device camera mounted on a Leica IM/DRB microscope), using a ×5 objective to visualize the central portion of each well. In experiments characterizing the permeabilization procedure, fluorescence intensity was quantified by acquiring an image of each well at constant camera gain and integration period (determined for the brightest well on the plate) and determining the full-frame integrated pixel intensity of each image using a MetaMorph image analysis workstation (Universal Imaging, West Chester, PA). In all other experiments, each well in a plate was checked by fluorescence microscopy to confirm that the cells had been permeabilized, as expected.

RESULTS

SPOC1 cells grown in 48-well cluster plates. The spatial variation in mucin secretion and production by intact SPOC1 cells grown on 48-well plates was tested by determining the quantity of mucin released during 40-min basal and agonist-stimulated periods (100 µM ATP·S) and that in the remaining intracellular pool (lysis in hypotonic buffer: 1 mM CaCl2, 1 mM MgCl2, 20 mM TES, pH 7.4). The mucins released during the basal and stimulated periods and the total cellular mucin (= basal + stimulated + lysis) are shown for three SPOC1 cell passages in Table 1. Consistent with previous results (2), the cells responded to ATP·S with a 3.2-fold increase in mucin secretion; this mucin represented 31.1% of the total cellular mucin pool.

Well-to-well variation in mucin release and content, determined as the “coefficient of variation,” was low for total mucin production (7.1%), moderately higher for the mucins secreted during purinergic stimulation (12.1%), and highest for basal secretion (18%).

The behavior of SPOC1 cells grown in 48-well cluster plates was also tested by determining the effects of two purinergic agonists on mucin secretion. Dose-response curves for UTP and ATP·S were constructed from single plates of SPOC1 cells, with each dose tested in triplicate. As shown in Fig. 1, these curves were sigmoidal; the EC50 was 3–4 µM, and the mucin secretory response saturated above 30 µM, consistent with previous results (2). Together, these two studies show that the cells grown in 48-well cluster plates possess reasonably uniform well-to-well cellular mucin pools and agonist responses, making them good candidates for permeabilization experiments.

SLO permeabilization. In extensive experiments not shown, various SLO permeabilization protocols were tested on SPOC1 cells. These attempts included a SLO exposure and wash at 4°C, followed by the cells being rewarmed such that the permeabilization step occurred after unbound SLO and potential contaminants in the material provided by the manufacturer were removed from the medium (34). The only procedure attempted that produced a consistent activation of mucin release by high Ca2+; however, was a brief exposure to SLO in pCa 7.0 Buif (100 mM Ca2+) at 35°C followed by an immediate wash. Figure 2 depicts the 35°C dose-permeabilization effects of SLO on SPOC1 cells, including videomicrographs of TO-PRO-stained cells at selected doses. The low molecular weight (MW) dye, TO-PRO (MW 645), was chosen as a marker of permeabilization because it is similar to EGTA (MW 380) in

Table 1. Variation in mucin production and secretion by SPOC1 cells grown in 48-well cluster plates

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<thead>
<tr>
<th>Agonist and Stimulation Period</th>
<th>Mucin, ng/well</th>
<th>Coefficient of Variation</th>
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<tr>
<td>Basal (40 min)</td>
<td>602 ± 88</td>
<td>18.0 ± 3.4</td>
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<tr>
<td>ATP·S (40 min)</td>
<td>1,946 ± 38</td>
<td>12.1 ± 1.8</td>
</tr>
<tr>
<td>Total mucin pool</td>
<td>6,255 ± 107</td>
<td>7.1 ± 0.2</td>
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Values are means ± SE; n = 3 SPOC1 cell passages. Coefficient of variation is the well-to-well variation in mucin release and content.
size and its fluorescence excitation and emission spectra are similar to fluorescein. As shown in Fig. 2, SLO effectively permeabilized the cells to TO-PRO at doses above 0.1 U/ml. The clustering pattern of nuclear fluorescence that is observed at 0.3 and 1 U/ml SLO is consistent with the pattern of SPOC1 cell differentiation that occurs in culture (2, 17, 46). That is, the cells grow as extensive patches of multilayered cells, with cells in the outermost layers containing mucin secretory granules. The more uniform staining observed at 3 U/ml reflects the permeabilization of cells in areas of the well occupied by those cells growing as a single layer in contact with the plastic substrate. These cells, and their adjacent counterparts in the multilayered areas, resemble the basal cells of pseudostratified airway epithelia.

The time courses of SLO permeabilization to TO-PRO at 1 and 3 U/ml are shown in Fig. 3. TO-PRO fluorescence increased rapidly with increasing SLO exposure time at both doses to a pseudo-plateau between 30 and 60 s at 70–80% of maximal. Longer exposures resulted in only moderately higher levels of TO-PRO fluorescence.

Mucin release in permeabilized SPOC1 cells. In preliminary experiments, Ca \(^2+\)-dependent mucin release was maximal following a 30-s exposure of SPOC1 cells to 1 U/ml SLO; a higher SLO dose (3 U/ml) or a longer exposure time (60 s) resulted in a reduced amount of mucin released to the bath (data not shown). Figure 4 shows the results of experiments designed to establish two important parameters related to permeabilization with a 30-s, 1 U/ml SLO protocol, namely, the postpermeabilization period of time necessary to achieve maximal mucin release and recovery and the period of time SPOC1 cells were capable of supporting Ca \(^2+\)-dependent mucin release following permeabilization. The data show first that a 10- to 15-min incubation in high Ca \(^2+\) (3.2 µM) was necessary to achieve a maximal release of mucin to, and recovery from, the medium (Fig. 4A). Second, the permeabilized cells were fully competent to secrete mucin in response to a high Ca \(^2+\) stimulus for a minimum of 2 min. When the cells were held at 100 nM Ca\(^2+\) for longer periods, they exhibited a reduced response to the subsequent high Ca\(^2+\) stimulus such that after 10 min the cells released ~50% of the amount of mucin released by cells stimulated by high Ca\(^2+\) immediately following permeabilization. This 2-min window of full secretory competency for permeabilized SPOC1 cells is narrower than that observed in other permeabilized cell models (see DISCUSSION); however, it was sufficiently broad for the purposes of the experiments reported herein. That the cells were ca-

Fig. 1. Purinergic agonist activation of mucin secretion in intact SPOC1 cells. Cells were grown on two 48-well cluster plates and after equilibration were exposed to the indicated doses of agonist for 40 min, with each dose tested in triplicate. Quantity of mucin released into the medium was determined by enzyme-linked lectin assay; mean values for each dose are presented.

Fig. 2. Streptolysin O (SLO) permeabilization of SPOC1 cells. Cells were exposed to indicated doses of SLO in pCa 7.0 intracellular buffer (Bufi; 100 nM) at 35°C for 10 min and then stained with TO-PRO. Fluorescence from the central portion of each well in 48-well cluster plates was imaged by a ×5 objective, acquired by a cooled charge-coupled device video camera, and quantified as the full-frame, integrated pixel intensity. Insets: sample images for selected SLO doses. Each dose was tested in triplicate on each plate, and data are presented as means ± SE (n = 3 SPOC1 cell passages).

Fig. 3. Time course of SLO permeabilization of SPOC1 cells. Cells were incubated at 1 or 3 U/ml SLO in pCa 7.0 Bufi (100 nM), in triplicate, at 35°C for time periods indicated and then washed and incubated for 10 min in TO-PRO in the same buffer. After fixation, cellular fluorescence was quantified as described in Fig. 2. Data are presented, normalized to the maximal fluorescence on each plate, as means ± SE (n = 3 SPOC1 cell passages).
Mucin release was greatly diminished, and at high Ca\(^{2+}\), the mucins were almost completely recovered, whether released at 100 nM Ca\(^{2+}\) or at 3.2 µM Ca\(^{2+}\). Note that mucin release was strongly inhibited at 3.2 µM Ca\(^{2+}\), with a maximal decrease of 2.49 ± 0.55-fold increase over control. The Ca\(^{2+}\) EC\(_{50}\) for the mucin secretory response was 2.29 ± 0.07 µM.

To test whether PKC activation of mucin granule exocytosis is dependent on or independent of Ca\(^{2+}\), SPOC1 cells grown in pairs of 48-well plates were permeabilized and incubated at Ca\(^{2+}\) activities ranging from 10 nM to 10 µM. One-half of the cells on each plate was exposed to a maximal dose of PMA (300 nM; Ref. 1), whereas one-half of the cells on the other plate was exposed to the same concentration of 4α-phorbol, an inactive phorbol ester. PMA stimulated mucin release in these studies was slightly increased at Ca\(^{2+}\) activities between 100 nM and 1 µM Ca\(^{2+}\), strongly stimulated above 1 µM Ca\(^{2+}\), and saturated at higher Ca\(^{2+}\), with a maximal 2.49 ± 0.55-fold increase over control. The Ca\(^{2+}\) EC\(_{50}\) for the mucin secretory response was 2.29 ± 0.07 µM.
in permeabilized SPOC1 cells over the entire range of Ca\(^{2+}\) activities (Fig. 7). At 10 nM Ca\(^{2+}\), which is approximately one-tenth of basal intracellular Ca\(^{2+}\) levels in most cells, mucin release was stimulated 2.1-fold over the 100 nM Ca\(^{2+}\) control. This stimulation by PMA, in fact, was as strong as the maximal Ca\(^{2+}\)-dependent response, a 2.1-fold stimulation at 10 µM Ca\(^{2+}\). As Ca\(^{2+}\)-stimulated mucin granule exocytosis at activities >1 µM, the PMA response was potentiated. At 4.7 µM Ca\(^{2+}\), PMA-stimulated cells released 27% more mucin relative to their paired controls than at 10 nM Ca\(^{2+}\) (P < 0.05, paired t-test); the Ca\(^{2+}\)-EC\(_{50}\) for cells exposed to PMA was 1.0 µM compared with 2.2 µM for the paired control. The PMA response at 2.1 µM Ca\(^{2+}\) appeared to be diminished relative to the maximal levels recorded at 4.7 and 10 µM, but it was still significantly higher than its paired control. Because 4α-phorbol had no discernible effect at any Ca\(^{2+}\) activity, the PMA effects appeared to be specific and were presumably due to PKC activation.

DISCUSSION

Regulated exocytosis by neurons and secretory cells has been the focus of intense investigation over the past 20 years (recent reviews in Refs. 4, 41, 57). These efforts were aided substantially by the development of permeabilization techniques pioneered by Baker et al. (7, 30) and Gomperts et al. (8, 21, 25), which allowed access to and control of the intracellular milieu (see also Refs. 3, 9, 39). Many permeabilized secretory cell models have been subsequently described for study of the control of exocytosis by intracellular Ca\(^{2+}\), PKC, nucleotides, and specific proteins (e.g., see Refs. 11, 22, 23, 34). Pancreatic acini, however, represent the only other permeabilized epithelial cell model so developed, and this study with SPOC1 cells represents the first model developed for an epithelium studied in a polarized configuration. The bacterial toxin SLO was chosen as the permeabilization agent for these studies to ensure efficient intracellular Ca\(^{2+}\) buffering by EGTA. This toxin binds plasma membrane cholesterol and then polymerizes to form ring-shaped 24- to 30-nm pores that render the plasma membrane permeable to organic molecules as large as lactate dehydrogenase (MW 140,000; Refs. 3, 50). Use of ionophores to control intracellular Ca\(^{2+}\) is impractical because of the difficulties in controlling the intracellular quantities of permeant EGTA or related buffers. Use of α-toxin for this purpose is also questionable because of restricted EGTA diffusivity through its 2- to 3-nm pores. In α-toxin-permeabilized gonadotropes, for instance, 30 mM CaEGTA buffers, or a 20-min preequilibration with 10 mM CaEGTA buffers at 0°C, were required to effectively demonstrate Ca\(^{2+}\)-activated luteinizing hormone release (6). Because efficient Ca\(^{2+}\) buffering was required in these studies with SPOC1 cells to test the effects of PKC activation at very low levels of intracellular Ca\(^{2+}\), we chose to use SLO.

Permeabilization was most efficient with short luminal SLO exposures of SPOC1 cells at 35°C, followed by a rapid wash in Buf. These cells were fully competent to secrete mucin in response to an elevation in Ca\(^{2+}\) for 2 min following permeabilization (Fig. 4). In other SLO-permeabilized cells, the period of secretory competency is tens of minutes in duration (e.g., see Ref. 29, 55), and secretory activity in rundown cells can be restored through the addition to the medium of cytosol (14, 47) or purified proteins (37, 43, 44). Under optimal conditions (30-s exposure to 1 U/ml SLO; Figs. 3 and 5), and following a maximal activation by Ca\(^{2+}\), permeabilized SPOC1 cells released a quantity of mucin only ~20% less than that secreted by intact cells following
purinergic stimulation (Fig. 5). Thus, although brief, the window of secretary competency for permeabilized SPOC1 cells was sufficiently broad and the secretory response was sufficiently robust to allow the experiments necessary to test for Ca$^{2+}$- and PKC-activated exocytotic release of mucin.

Permeabilized SPOC1 cells exhibited a graded mucin secretory response to increases in Ca$^{2+}$- activity (Figs. 5–7), with the initial responses occurring above 320 nM. In preliminary measurements with intact SPOC1 cells, we have determined basal Ca$^{2+}$ activities to be 80–100 nM (data not shown). Consequently, these cells are similar to virtually every other secretary cell studied in possessing a secretory pathway activated by Ca$^{2+}$ at suprabasal levels. The Ca$^{2+}$ EC$_{50}$ of this response in SPOC1 cells, 2.29 ± 0.07 µM, was in the same low micromolar range described for most other permeabilized cells (e.g., see Refs. 13, 29, 33, 36, 40, 48). These data are consistent with the positive effects of ionomycin and thapsigargin on intact SPOC1 cells (1), and together they lend strong support for a role of Ca$^{2+}$ in mediating agonist responses in airways mucin secreting cells (see also Ref. 16; cf. Ref. 32).

The effects of PKC activation in secretory cells are more varied than the effects of Ca$^{2+}$. At Ca$^{2+}$ activities below the nominal 100 nM basal intracellular Ca$^{2+}$ levels, some cells are not affected by PMA or other PKC-activating reagents (i.e., pancreatic acini, Ref. 29; chromaffin cells, Ref. 30); however, most secretory cells exhibit some degree of PKC-activated exocytosis (e.g., gonadotropes, Ref. 36; PC-12 cells, Ref. 48). At 10 nM Ca$^{2+}$, SPOC1 cells were powerfully stimulated by PMA; the amount of mucin released in response to PMA under these conditions was the same as that released maximally by micromolar levels of Ca$^{2+}$ (Fig. 7). Indeed, in the robustness of this PMA response at subbasal Ca$^{2+}$ levels, SPOC1 cells stand out from all other secretary cells studied.

In most other secretary cells, PMA has been shown to potentiate Ca$^{2+}$-dependent responses (e.g., PC-12 cells, Ref. 48; mast cells, Ref. 40). In SPOC1 cells, PMA had slight synergism with Ca$^{2+}$, with the PMA-related increase in Ca$^{2+}$-dependent secretion being 27% greater than the effects of PMA at subbasal Ca$^{2+}$ (Fig. 7). Given the apparent additivity of ionomycin and PMA in intact SPOC1 cells (1), this minor degree of synergism between PKC and Ca$^{2+}$ is not surprising.

PKC has been shown in recent years to be a family of at least 11 isoforms that may be categorized into 3 or 4 subfamilies (for review, see Ref. 42). Pertinent to this discussion are those isoforms activated by phosphatidylinositol and DAG or PMA, that is, the conventional or cPKC isoforms (which are Ca$^{2+}$-dependent) and the novel or nPKC isoforms (which are Ca$^{2+}$-insensitive) (PMA does not activate the atypical isoforms or PKCγ). Because secretion in SPOC1 and other cells is activated by PMA at subbasal Ca$^{2+}$ levels, a likely possibility is that nPKC isoforms will prove to be responsible for this effect. Recent data in fact support this notion: nPKC isoforms have been implicated in the agonist regulation of secretion for colon cell lines (24), pancreatic acini (45), and lachrymal glands (56), and overexpression of nPKCε in GH4 cells leads to a selective increase in basal prolactin secretion rates (5). In other secretary cells, however, cPKC isoforms have been implicated in modulating secretion (e.g., RBL cells, Ref. 11). Hence, the PKC isoforms active in activating and/or modulating secretion are likely cell-type dependent.

In conclusion, the purinergic regulation of mucin secretion in SPOC1 cells appears to possess Ca$^{2+}$- independent, PKC-activated, and PKC-potentiated Ca$^{2+}$-dependent pathways; in this regard, they are similar to many other nonepithelial secretory cells but not to pancreatic acini. The identities of the PKC isoforms responsible for Ca$^{2+}$-independent mucin secretion and the degree of independence between these two pathways at the molecular level require further investigation. For the latter topic, a major question is whether multiple exocytotic mechanisms exist in a given secretory cell type or, alternately, whether the apparent independence between Ca$^{2+}$ and PKC lies with one or more rate-limiting steps (e.g., cortical microfilaments; Ref. 54) situated proximal to exocytotic docking sites.

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