Purinergic and cholinergic agonists induce exocytosis from the same granule pool in HT29-Cl.16E monolayers

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Purinergic and cholinergic agonists induce exocytosis from the same granule pool in HT29-Cl.16E monolayers. Am. J. Physiol. 276 (Cell Physiol. 45): C907–C914, 1999.—Several secretagogues induce mucin secretion in epithelial monolayers, as determined by measuring released granule contents. To assess whether different agonists act on the same granule pool, capacitance changes in intact monolayers of the goblet cell line HT29-Cl.16E were measured by a novel impedance method. Apical ATP (purinergic agonist) and basolateral carbachol (cholinergic agonist) induce rapid exocytosis with maximal capacitance changes within 3 min. The maximal levels of exocytosis that can be induced by optimal concentrations of either agonist are the same and produce a 30–40% increase in total monolayer capacitance. When ATP and carbachol are applied simultaneously, the magnitude of exocytosis is unchanged from the single-agonist level. The recovery of capacitance to baseline (endocytosis) is significantly faster after ATP stimulation than after carbachol stimulation. When ATP and carbachol are applied sequentially at doses that give maximal exocytosis, the magnitude of the capacitance increase produced by the second secretagogue is less than or equal to that of the capacitance decrease during the recovery period. Together, these data suggest that purinergic and cholinergic agonists act on the same granule pool.

Mucin secretion; electrophysiology; chloride secretion; membrane capacitance; epithelium

THE FORMATION OF A PROTECTIVE MUCUS SURFACE OVER EPITHELIA results from the exocytosis of mucin-containing granules coupled with the dispersion of the granule contents. Exocytosis of mucin occurs constitutively and as a regulated response to several secretagogues (13). Many of these secretagogues activate phospholipase C, leading to the elevation of cytosolic Ca2+, long recognized as a primary effector of exocytosis (2). Ca2+-independent exocytosis of mucin in response to protein kinase C-e (20) and extracellular nucleotides has also been identified (1, 22, 25). Whereas P2X purinergic receptors can also activate phospholipase C (19), Ca2+-independent exocytosis may be controlled by various products of the phosphoinositide signaling cascade implicated in the control of granule fusion (9). Many epithelial cells can respond to several of these mechanisms (13), enabling them to secrete mucin in response to luminal as well as serosal signaling. Several reports indicate different levels of mucin secretion and synergy in response to multiple secretagogues (1, 12, 22, 24). It is not clear, however, whether these different signaling pathways operate on a common granule pool and with the same patterns of exocytosis and endocytosis.

The Cl.16E subclone of the human colonic cancer cell line HT-29 forms confluent monolayers in culture and differentiates to a goblet cell-like epithelium with large numbers of mucin granules in the apical cytoplasm (3). Different rates of mucin secretion, measured as the release of radiolabeled mucin, are stimulated with the cholinergic agonist carbachol (28) and the purinergic agonist ATP (25). Electron micrographs of ATP-stimulated monolayers indicate that compound exocytosis occurs (25), whereas capacitance measurements for isolated cells (17) and intact monolayers (5) indicate substantial increases in membrane area (exocytosis) in response to ATP. Cl.16E is also a Cl−-secreting cell possessing multiple Cl− conductances under the control of different cellular signaling systems. Purinergically stimulated Cl− secretion has been shown to depend in part on granule fusion (26) and is independent of cytosolic Ca2+ increases and protein kinases A and C (15, 16). These properties of Cl.16E monolayers make them an excellent tool for probing the dynamics of mucin exocytosis and the interactions of the Cl− secretory current.

Mucin secretion is typically measured by quantifying the release of granule contents, e.g., as the release of mucin labeled with a radioactive precursor, or by detection of MUC2, a predominant secreted mucin, by an antibody reaction (13). These methods can identify the contents of granules once they have diffused into the extracellular medium; however, they provide little information on the rate of exocytosis or membrane retrieval. In addition, the rate and extent of secretion can depend on the type of mucin label selected (12), and constitutive secretion typically occurs in parallel with regulated secretion (13). Alternative measurements of exocytosis have been performed by morphological analysis or capacitance measurements. Although they provide detailed information on the state of individual cells at the moment of sectioning (13), morphological methods are difficult to implement for dynamic measurements, and fixation can damage or alter granule structure. Capacitance measurements have traditionally been limited to patch-clamp techniques, which are applied to single cells. Whole cell patch-clamp measurements of capacitance in subconfluent, isolated Cl.16E cells after purinergic stimulation indicate massive granule fusion followed by complete endocytosis in <30 s (17). In contrast, monolayer impedance analysis using ATP-stimulated Cl.16E indicates that the peak of exocy-
MATERIALS AND METHODS

Cell culture. HT29-Cl.16E cells were propagated in Falcon culture flasks (25 cm²) in a humidified atmosphere of 95% air-5% CO₂ at 37°C. The cells were fed daily with DMEM supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine. Cultures were periodically tested for mycoplasma (7) and were found free of contamination. The passage numbers for the reported experiments were between 23 and 45.

Cell monolayers for electrophysiology were grown on Vitrogen-coated, Millicell-CM (size 12 mm) filters. Cl.16E cells were seeded at a density of 0.8–1.2 × 10⁴/filter (0.6 cm²/filter). Cells became visually confluent after 7 days and were used for electrophysiological studies between days 9 and 23. To determine the number of cells per filter at confluency, filters were trypsinized to remove cells and the cells were counted with a hemocytometer.

Electrophysiology. Transepithelial electrophysiology measurements were performed with an Ussing-type chamber modified to accept the Millicell-CM filters (Analytical Bioinstrumentation, Cleveland, OH). The chamber was equipped with a conventional four-electrode system connected to a voltage clamp (model 558-C-5; Univ. of Iowa Bioengineering, Iowa City, IA). The Ussing chamber and all solutions were maintained in an incubator at 37°C and 5% CO₂. Apical and basolateral chambers were perfused separately with slightly hypertonic (110% of normal) Ringer solution (in mM: 114 NaCl, 4 KCl, 1 MgCl₂, 1.25 CaCl₂, 22.98 NaHCO₃, 31.7 sucrose, and 25 d-glucose at pH 7.4). Voltage reference electrode offset and fluid resistance were measured at the start of each experiment with a blank Millicell-CM filter perfused with the Ringer solution, and voltage offset was compensated.

Measurements consisted of simultaneous pulse or trigger generation and acquisition of transepithelial potential and current, performed with an AT-MIO-16F-5 analog-to-digital card (National Instruments, Austin, TX) installed in a personal computer (486DX at 60 MHz). Pulse and trigger signals were applied to the external (command) signal input of the 558-C-5 voltage clamp and the external-trigger input of the 558-C-5 voltage clamp pulse generator, respectively. The voltage clamp was maintained in the short-circuit mode (transepithelial potential was clamped to 0 V). To probe impedance, a short pulse (40.7 µs, 7.5 mV, low-pass filtered with a cutoff frequency of 11 kHz) was applied and signals were acquired at a rate of 24,600 samples/s, until 2,250 samples were collected. This process was repeated eight times consecutively, and the results were averaged. To measure the total direct current (DC) resistance, the voltage-clamp pulse generator was triggered to generate a long pulse (1 s, ± 2.0 mV), and signals were acquired at a rate of 20 samples/s, yielding 100 samples total. Positive currents correspond to anion secretion and cation absorption (lumen at negative potential under open-circuit conditions).

Impedance estimation. Total monolayer capacitance was estimated by a recently developed method of impedance measurement and analysis (5). Briefly, the epithelial monolayer is described by a model having a resistance and capacitance for both the apical and basolateral membranes and a resistance for the paracellular pathway, resulting in five membrane parameters. The impedance of this model is described by an equation having three unique coefficients, one of which is the inverse of total membrane capacitance. The actual monolayer impedance is calculated as the quotient of the fast-Fourier-transformed transepithelial potential and current, measured as described above. The coefficients are estimated by minimizing the error between the actual and model impedances by using a weighted, nonlinear least-squares algorithm. The estimation process provides total membrane capacitance, and total resistance is known from the DC measurement.

Total membrane capacitance and total DC resistance, as well as the acquired transepithelial short-circuit current (I_sc), characterize the dynamic monolayer response without a requirement for auxiliary measurements or other monolayer perturbations (5). The individual membrane parameters themselves cannot be uniquely determined because there are four membrane parameters and only three impedance coefficients (the original 5 membrane parameters can be reduced to 4 by substituting the value of total DC resistance in terms of the individual membrane resistances). The range of values within which the actual membrane parameters must exist can be calculated from the impedance coefficients, however. This is accomplished by determining the physically realizable limits on a single parameter and calculating the limits of all other parameters for each set of impedance coefficients. For this cell line, the limits on basolateral capacitance variation have been established as 5 to 25 µF/cm² (5).

Data analysis. Our impedance method has been shown to estimate total membrane capacitance with ±1% accuracy at the 99% confidence interval (5). The statistical tests were applied to the residuals of each estimation to determine an acceptable fit (5). These same tests were then carried out in accepting estimated coefficients for impedance measurements in the present study. All data are normalized to the macroscopic filter area (0.6 cm²). Data plots give the estimated values for total capacitance and the measured values for total DC resistance, normalized to the average of 10 baseline measurements, and the measured values of I_sc adjusted by subtracting the average of 10 baseline measurements. Tabulated data are shown as means ± SD of n experiments, and each experiment represents an individual filter and n ≥ 4 for all cases. Statistical comparisons were carried out by using Student’s t-test, with P < 0.05 considered significant.

Stimulating exocytosis. Exocytosis in Cl.16E monolayers was stimulated with the purinergic agonist ATP (25) or the cholinergic agonist carbachol (28). Effective dose levels for maximal release of labeled mucin as well as the impact of unstirred layers were established in these earlier studies. The protocol was designed to monitor the time course of exocytosis and endocytosis. After a 20-min incubation in slightly hypertonic Ringer solution, measurements of basal impedance and total DC resistance were performed. After 7 min, the desired secretagogue was applied. For ATP, a bolus was added directly to the apical chamber. Carbachol was added directly to the basolateral perfusate and was continu-
ously perfused. Measurements of impedance and total DC resistance were continued for at least 20 min after each stimulation. These data were then analyzed to estimate the impedance coefficients.

Materials. DMEM, fetal bovine serum, and l-glutamine were bought from GIBCO-BRL (Grand Island, NY). Vitrogen was purchased from Collagen (Palo Alto, CA), and Milli-cell-CM filters were purchased from Millipore (Bedford, MA). All other chemicals were purchased from Sigma (St. Louis, MO).

RESULTS
Measurement of cell parameters. Baseline impedance measurements were performed at the start of each experiment to determine the noise characteristics for use in a weighted estimation (5). For each experiment, 10 baseline values were averaged and the average values were used to normalize total capacitance and total DC resistance in all presented data. Average baseline total capacitance was $2.4 \pm 0.4 \mu F/cm^2$, and average baseline total DC resistance was $269 \pm 79 \Omega \cdot cm^2$ ($n = 22$). At rest, Cl.16E monolayers exhibit Cl$^-$ secretion; the average baseline $I_{sc}$ was $1.7 \pm 1.1 \mu A/cm^2$. The average number of cells at confluency was $1.67 \pm 0.18 \times 10^6/cm^2$ ($n = 3$).

Luminal purinergic stimulation results in exocytosis and a secretory current. Figure 1 demonstrates that the apical addition of ATP results in rapid exocytosis, as indicated by a 38% increase in total capacitance (at 3 mM ATP) and a rapid increase in Cl$^-$ secretion quantified as $I_{sc}$. The rapid exocytosis is followed by a more gradual recovery to baseline. As the concentration of ATP is increased, the time course and amplitude of exocytosis change. The magnitude of exocytosis is maximal with 3 mM ATP, which was used for all additional tests. At concentrations $>3$ mM, two phases of exocytosis are observed. Interestingly, $I_{sc}$ increases proportionally to ATP concentration and does not parallel the exocytosis pattern. The total DC resistance briefly increases immediately after the addition of ATP, then drops back toward baseline.

Serosal cholinergic stimulation results in exocytosis and a secretory current. Figure 2 demonstrates that the basolateral addition of carbachol also results in rapid exocytosis (35% increase), similar in peak magnitude to that observed with ATP. The rate of recovery (endocytosis) is significantly decreased, however, with respect to the ATP response (Table 1). Tests of different carbachol concentrations indicate a plateau in peak capacitance change at $\approx 500 \mu M$; at $20 \mu M$, the peak capacitance change is $\approx 20\%$. The total DC resistance and $I_{sc}$ responses induced by carbachol exhibit some interesting differences from those induced by ATP. Total resistance immediately drops after carbachol addition, then increases above baseline several minutes later. $I_{sc}$ exhibits a brief reversal before peaking and then returning to baseline.

Simultaneous stimulation with purinergic and cholinergic agonists does not increase the magnitude of exocytosis. In Fig. 3, carbachol was added to the basolateral perfusate and then a bolus of ATP was added to the apical compartment at the peak of the carbachol-induced $I_{sc}$. The peak capacitance is similar to that seen with individual secretagogues (38% increase), demonstrating that the effects of the secretagogues are not additive. Interestingly, the total DC resistance and $I_{sc}$ responses change at the point of ATP addition from those typically observed with carbachol to those typically observed with ATP, i.e., total resistance briefly
increases and $I_{sc}$ peaks again. In addition, the rate of recovery is dramatically increased from the typical carbachol response, appearing similar to that after the addition of ATP alone.

To assess whether carbachol was interfering with ATP-induced exocytosis, a submaximal (20 µM) dose of carbachol was added to the basolateral perfusate and then a standard (3 mM) bolus of ATP was added to the apical compartment at the peak of the carbachol-induced $I_{sc}$. From the plot in Fig. 4, it is evident that ATP could stimulate additional exocytosis, but not in excess of the single-agonist response. As with the results in Fig. 3, the total DC resistance and $I_{sc}$ responses change at the point of ATP addition from those typically observed with carbachol to those typically observed with ATP.

Sequential stimulation with purinergic and cholinergic agonists produces multiple exocytosis peaks. Although simultaneous stimulation does not increase the peak exocytosis, additional exocytosis can be stimulated after a recovery period. Figure 5 demonstrates that the addition of ATP 18 min after the addition of carbachol results in a second exocytosis peak. The converse sequence has the same result; the addition of carbachol 20 min after the addition of ATP produces a similar second peak in exocytosis (Table 1). The second peak does not exceed the maximum recovered or the single-dose maximum. Total resistance and $I_{sc}$ responses are typical for each secretagogue applied independently.

Table 1 summarizes the responses to the two secretagogues. Independent ATP or carbachol stimulation or

<table>
<thead>
<tr>
<th>Agent</th>
<th>Peak $\Delta C_T$</th>
<th>$\Delta C_T$ at +12 min, %</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>ATP (1st)</td>
<td>$+35 \pm 15$</td>
<td>$-73 \pm 14$</td>
<td>7</td>
</tr>
<tr>
<td>Carbachol (1st)</td>
<td>$+33 \pm 4$</td>
<td>$-27 \pm 10^*$</td>
<td>9</td>
</tr>
<tr>
<td>ATP + carbachol</td>
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<td>$-77 \pm 13$</td>
<td>4</td>
</tr>
<tr>
<td>Carbachol (2nd)</td>
<td>$+17 \pm 5^*$</td>
<td>$-38 \pm 24^*$</td>
<td>7</td>
</tr>
<tr>
<td>ATP (2nd)</td>
<td>$+20 \pm 10^*$</td>
<td>$-53 \pm 29$</td>
<td>4</td>
</tr>
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Values are means ± $SD$; n = no. of experiments. ATP (3 mM) was added directly to apical chamber as a bolus; carbachol (0.5 mM) was added to basolateral solution and continuously perfused. Peak capacitance change (peak $\Delta C_T$) occurs <4 min from initial application of agents. Return of capacitance ($\Delta C_T$ at +12 min) is measured 12 min after peak capacitance as percentage of difference between (peak – baseline) and (+12 min – baseline) $C_T$ values. *Significantly different from ATP (1st) or ATP plus carbachol (P < 0.005). †Significantly different from ATP (1st), carbachol (1st), or ATP plus carbachol (P < 0.05).
simultaneous ATP and carbachol stimulation resulted in a 30–40% peak increase in total capacitance above baseline. Recovery was significantly delayed after stimulation with carbachol alone. When the two agents were applied sequentially, the effective increase in capacitance stimulated with the second agent was significantly less than that stimulated with the first agent, and always less than or equal to the membrane area recovered following stimulation with the first agent. Whereas ATP applied at the peak of the carbachol response dominates the recovery time, the recovery from the ATP stimulus is significantly delayed when ATP addition is made 20 min after carbachol addition.

DISCUSSION

Cl.16E monolayers have previously been shown, by the measurement of labeled granule contents, to secrete mucin at different rates after stimulation with the cholinergic agonist carbachol (28) or the purinergic agonist ATP (25). We demonstrate here that either agent induces the same maximal exocytosis at the optimal concentration of agonist. Furthermore, monolayers stimulated independently with the two agonists reach peak capacitance in very similar time courses, and in <3 min. The simultaneous addition of both agents does not produce an additive effect on exocytosis beyond that observed with a single agonist, and the use of a submaximal concentration of carbachol indicated that both agents are active in stimulating exocytosis when applied simultaneously. Although the peak capacitances induced by either independently and both simul-
taneously were the same, changes in total resistance, \( I_{sc} \), and rates of endocytosis indicated that both agents retained these other signaling effects. These results suggest that both agents are acting on the same granule pool.

It is important to consider the number of granules that correspond to the measured capacitance changes. A minimal estimate for the peak change in apical capacitance \( C_A \) can be obtained from the baseline total capacitance \( C_T \) of 2.36 \( \mu \)F/cm\(^2\) and a maximal basolateral capacitance \( C_B \) of 25 \( \mu \)F/cm\(^2\) by using the relationship \( C_A = C_T - C_B \). The minimal baseline \( C_A \) is therefore 2.6 \( \mu \)F/cm\(^2\), and a 35% increase in \( C_T \) corresponds to a minimal increase of 1.0 \( \mu \)F/cm\(^2\). Assuming 1-\( \mu \)m-diameter spherical granules and a specific capacitance of 1 \( \mu \)F/microscopic cm\(^2\), the capacitance of a granule is 31 fF. Therefore, the peak change in total capacitance corresponds to at least 33 million fused granules/cm\(^2\) of monolayer. It has been noted that only 48% of the cells in an HT29-Cl.16E monolayer contain mucin granules (3), i.e., the goblet cell density at confluence is \( \sim 800,000/cm^2 \), given the measured total cell density of 1.67 million/cm\(^2\). Thus the measured total capacitance increase 3 min after the stimulus corresponds to an average of at least 42 granules fused per mucin-secreting cell. Corresponding calculations for the upper limit assuming a basolateral capacitance of 5 \( \mu \)F/cm\(^2\) indicate 172 fused granules per mucin-secreting cell. The minimal number of 42 fused granules/cell is consistent with the morphological observations of compound exocytosis in colonic goblet cells (30) and the HT29-Cl.16E cell line (25, 26), which was derived from a colonic tumor. Previous electron-microscopic studies of ATP-stimulated HT29-Cl.16E monolayers (25) have noted that extensive compound exocytosis was evident 5 min after stimulation. In these same electron micrographs, it is evident that the apical membranes of mucin-secreting cells are small, harboring 9–16 granules in direct proximity with the outermost surface. The fusion of 42 granules/cell would require granule fusion in tandem. We have considered alternative explanations for the observation of a ceiling for the capacitance increase observed with optimal concentrations of purinergic and cholinergic agonists. It is possible that a physical limit, such as the number of docking sites, is responsible for a maximal capacitance increase. However, this explanation seems unlikely in light of the large number of fused granules per cell. Furthermore, the demonstration of t-SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein target receptors) on granules in other cells (14, 18, 27) that undergo compound exocytosis provides a ready explanation for granule-to-granule fusion and a lack of limiting docking sites.

As previously noted, the timing of exocytosis and endocytosis in single, patch-clamped cells (17) is significantly faster than that observed in measurements on monolayers. In single cells, both exocytosis and endocytosis are completed within 30 s, whereas in monolayers exocytosis requires at least 2 min and the return to baseline capacitance takes more than 20 min. It is noteworthy that previous electron microscopic (25) and mucin release studies (25, 28) of monolayers are in line with the current capacitance measurements but at variance with the patch-clamp data for single cells. Morphological studies indicated substantial compound exocytosis 5 min after apical ATP stimulation (25, 26), and release of labeled mucin was maximal 15 min after ATP stimulation and after 30 min of carbachol stimulation (28). Nevertheless, these considerations point out that endocytosis could occur in parallel with exocytosis in epithelial cells, as in neuroendocrine cells (29), and
that the measured net increase in total capacitance could be an underestimate of the granule membrane that has undergone fusion.

Although parallel endocytosis may mask the absolute peak of capacitance change, it is not responsible for the lack of additivity when the two agonists are added simultaneously. Carbachol addition alone results in a significantly slower rate of endocytosis than ATP addition alone. The addition of ATP immediately after carbachol overcomes this slower recovery, whereas the addition of ATP 20 min later only marginally accelerates the recovery (Table 1). It is possible that the continued presence of carbachol in the basolateral perfusate is responsible for this slower recovery; however, it is of interest that the simultaneous addition of ATP overcomes this slowed rate. It has been unclear whether cells that have undergone compound exocytosis can recover their granule membranes. Studies have indicated that after compound exocytosis substantial portions of the intracellular membrane are sloughed off into the apical medium (30). The ability of either secretagogue to induce a second round of exocytosis after a recovery period suggests that Cl.16E monolayers are able to recover at least a portion of their exocytosed granules. Furthermore, the magnitude of exocytosis induced by a second application is always found to be less than or equal to the magnitude of the recovered membrane when testing with recovery times of 5–20 min is performed. These results suggest that different mechanisms are involved in the recovery from exocytosis and that cross talk between the mechanisms occurs.

Additional factors suggest that ATP and carbachol act through different signaling mechanisms to modulate exocytosis and Cl\(^{−}\) secretion. ATP induces a large (35%), transient increase in total capacitance when applied to the apical membrane; when applied to the basolateral membrane, ATP induces a small (5%) increase in total cell capacitance, which is maintained until ATP is removed (Bertrand, unpublished observations). The ATP-induced Cl\(^{−}\) secretory current is independent of cellular Ca\(^{2+}\) concentrations (15, 16). In contrast, previous tests of carbachol-induced mucin secretion in Cl.16E monolayers found that the carbachol effect was mediated by muscarinic receptors (28) and that the intestinal muscarinic receptor signals were mediated by Ca\(^{2+}\) increases (8). Purinergic and cholinergic secretagogues thus differ in their receptor types and membrane locations, as well as in their effects on endocytosis.

Interestingly, significant differences in the total resistance changes and I\(_{sc}\) values between the two types of secretagogues have been noted. Immediately after agonist addition, ATP induces a short secretory current spike followed by a longer secretory current phase, whereas carbachol induces an I\(_{sc}\) consistent with luminal K\(^{+}\) secretion followed by a longer Cl\(^{−}\) secretion phase. When the two agents are applied simultaneously, ATP is able to activate an additional I\(_{sc}\) above that induced with carbachol. Previous work with Cl.16E monolayers has demonstrated that the ATP-stimulated Cl\(^{−}\) channels are recruited in part from granules (26); these channels do not appear to be activated to the same extent by carbachol. Immediately after the addition of ATP, monolayer total resistance briefly increases, a phenomenon absent from the carbachol response. Range analyses of the membrane parameters (Fig. 6) suggest that this behavior is the result of increases in the paracellular resistance of the monolayer.

It is possible and interesting to relate differences in the patterns of changes in paracellular resistance, initial secretory currents, and endocytosis rates after purinergic and cholinergic stimulation to previously noted differences in secretion patterns, although these relationships must remain speculative at this point. For example, measurements of labeled granule contents have shown that ATP-induced mucin release was maximal after 15 min (25), whereas carbachol-induced release was maximal after 30 min (28). The slower endocytosis rate after carbachol stimulation noted in this study could actually reflect the slower release of granule contents. In addition, it has long been noted that a driving force should be required to push the contents of the granules out into the extracellular medium, especially during compound exocytosis when long intracellular canaliculi form (11, 13). Given the new information that both ATP and carbachol maximally increase the capacitance with similar time courses and within 3 min, the difference in release rates of granule contents needs to be explained by factors other than the granule fusion rate. Differences in driving forces for expulsion between the two agonists are clearly one possibility. The noted differences in time courses of the current and the resistance may be related to the development of a greater driving force and faster expulsion of contents for ATP. The paracellular resistance in our model (5) is composed of the tight junction in series with the lateral intracellular space (LIS). An increase in the resistance of either or both elements may be responsible for the measured brief increase after ATP stimulation. One possibility is constriction of the LIS due to cell and/or granule swelling (23, 33), i.e., reflecting an increasing driving force. Differences in I\(_{sc}\) are particularly interesting, because ion secretion would support active fluid secretion to hydrate granule contents and flush them out (11, 13). Thus there are several potential mechanisms, as with neuroendocrine cells (10), for the regulation of the rate of release of granule contents by epithelial cells after fusion has occurred. More detailed studies to sort out these potential mechanisms are required.

Substantial progress has been made in identifying protein interactions and the kinetics of exocytosis and endocytosis in neurosecretory cells, especially at the single-cell and even single-fusion-event levels (6, 32) It is now apparent that granule fusion and the release of contents can be temporally distinct (10, 32), that there are multiple endocytosis pathways (i.e., fast and slow; Ref. 6), and that exocytosis and endocytosis can occur simultaneously (29). Numerous interactions involving the SNARE family of proteins control the targeting, docking, and fusion of granules with the plasma mem-
brane (4, 21, 31). It is surprising that progress in epithelial exocytosis has been lagging behind that in the neurosecretory field despite the fact that epithelial cells are surface cells and therefore should be more accessible. One explanation may be that robust dynamic methods for capacitance measurements to describe in detail the kinetics of the processes have been missing for epithelial monolayers. Dynamic capacitance measurements have been crucial for advances in defining granule fusion in the neurosecretory field. Although there are many similarities or analogies at the cellular level between neurosecretion and epithelial secretion, the electrophysiological approach to determining the kinetics of granule fusion by measuring capacitance needs to be different. Neurosecretory cells normally function as single cells, whereas epithelial cells are normally associated with neighboring cells to form polarized cell layers (epithelium). As shown by the example of HT29-C1.16E, substantial differences in the kinetics of granule fusion can be observed when the process of exocytosis is measured by patch-clamping in single cells (17) vs. an impedance analysis of monolayers (this study). This study is the first application of a new method for dynamic impedance measurements in epithelial monolayers (5). As demonstrated in this report, the new method allows a much more detailed kinetic description of granule fusion and thereby the discovery of new information about cellular behavior.

We thank Dr. Calvin U. Cotton and Dr. Robert D. Harvey for helpful discussions during the preparation of this manuscript. This work was supported by National Institutes of Health Grants DK-39658 (to U. Hopfer) and T32-HL-07415 (to C. A. Bertrand). We thank Dr. Calvin U. Cotton and Dr. Robert D. Harvey for helpful discussions during the preparation of this manuscript.

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