Regulation of exocytosis by purinergic receptors in pancreatic duct epithelial cells

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Jung, Seung-Ryoung, Mean-Hwan Kim, Bertil Hille, Toan D. Nguyen, and Duk-Su Koh. Regulation of exocytosis by purinergic receptors in pancreatic duct epithelial cells. Am J Physiol Cell Physiol 286: C573–C579, 2004. First published November 5, 2003; 10.1152/ajpcell.00350.2003.—In epithelial cells, several intracellular signals regulate the secretion of large molecules such as mucin via exocytosis and the transport of ions through channels and transporters. Using carbon fiber amperometry, we previously reported that exocytosis of secretory granules in dog pancreatic duct epithelial cells (PDEC) can be stimulated by pharmacological activation of cAMP-dependent protein kinase (PKA) or protein kinase C (PKC), as well as by an increase of intracellular free Ca2+ concentration ([Ca2+]i). In this study, we examined whether exocytosis in these cells is modulated by activation of endogenous P2Y receptors, which increase cAMP and [Ca2+]i. Low concentrations of ATP (<10 μM) induced intracellular Ca2+ oscillation but no significant exocytosis. In contrast, 100 μM ATP induced a sustained [Ca2+]i rise and increased the exocytosis rate sevenfold. The contribution of Ca2+ or cAMP pathways to exocytosis was tested by using the Ca2+ chelator BAPTA or the PKA inhibitors H-89 or Rp-8-bromoadenosine 3’,5’-cyclic monophosphorothioate. Removal of [Ca2+]i rise or inhibition of PKA each partially reduced exocytosis; when combined, they abolished exocytosis. In conclusion, ATP at concentrations >10 μM stimulates exocytosis from PDEC through both Ca2+ and cAMP pathways.

secretion; amperometry; photometry; calcium, adenosine 3’,5’-cyclic monophosphate

EUKARYOTIC CELLS DISCHARGE secretory products by fusing secretory vesicles with the plasma membrane. This process, called exocytosis, is regulated by several intracellular messengers. In excitable cells such as endocrine cells or neurons, exocytosis is commonly triggered by intracellular free Ca2+ concentration ([Ca2+]i) elevation (4, 9, 13, 31), whereas in several epithelial cells it is also regulated by cAMP and PKC (1, 10, 28).

Using pharmacological approaches, we previously confirmed that exocytosis in pancreatic duct epithelial cells (PDEC) can be stimulated by direct activation of PKA or PKC as well as by [Ca2+]i (16). We now examine whether these signaling pathways can also stimulate exocytosis under physiological conditions, e.g., by activation of endogenous receptors. Dog PDEC are known to express several G protein-coupled receptors including histamine, protease-activated, and purinergic receptors (19–22). We decided to test the effect of purinergic receptor activation on exocytosis because 1) PDEC express P2Y2 receptors coupled to [Ca2+]i and P2Y11 receptors coupled to both [Ca2+]i and cAMP (19, 20) and 2) these cells may be exposed to ATP released along with digestive proteases from acinar cells (29). In addition, mechanical stimulation of polarized epithelial cells may cause release of ATP and UTP from both apical and basolateral sides (11).

Using carbon fiber amperometry to measure exocytosis from a single cell and photometry to measure [Ca2+]i, we demonstrated that only high concentrations (>10 μM) of ATP can efficiently induce exocytosis. The effect was mediated by both cAMP and [Ca2+]i signals, which may interact synergistically. ATP-induced exocytosis developed slowly with a time delay of about 30 s.

MATERIALS AND METHODS

Chemicals. Stock solutions of 10 mM H-89 and 10 mM 1,2-bis(2aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid acetoxymethyl ester (BAPTA-AM) were prepared in dimethyl sulfoxide (DMSO), and 100 mM ATP or UTP was made up in saline used as bath solution. Rp-8-bromoadenosine 3’,5’-cyclic monophosphorothioate (Rp-8-BromoAMPS) was dissolved directly in saline solution at a final concentration of 1 mM. Rp-8-BromoAMPS and H-89 were purchased from Calbiochem, and dopamine-HCl, ATP (magnesium salt), and UTP (sodium salt) were from Sigma-Aldrich.

PDEC cell culture. The derivation and culture of pancreatic epithelial cells from the main pancreatic duct of the dog have been described (24). These nontransformed cells can be subcultured repeatedly, and they form a monolayer with subcellular structure and biochemical properties similar to those of native pancreatic duct epithelial cells. Cells were cultured on Transwell inserts (3-m pore size, 24.5-mm diameter; Corning Costar) coated with Vitrogen (Collagen, Palo Alto, CA) and placed above a confluent feeder layer of human gallbladder myofibroblasts. These myofibroblasts may secrete growth factors necessary for maintaining and propagating these well-differentiated PDEC. The cells were maintained in Eagle’s minimum essential medium with 10% fetal bovine serum, 2 mM l-glutamine, 20 mM HEPES, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml bovine insulin, 5 μg/ml human transferrin, 5 ng/ml sodium selenite, 1% MEM vitamins solution, and 1% MEM nonessential amino acid solution.

For single-cell experiments, cells were plated on small coverglass chips (5 × 5 mm) coated with a thick layer of Vitrogen. The cells were fed with medium conditioned by human gallbladder myofibroblasts. Experiments were performed by using single isolated cells 2–5 days after plating. Cells at this subconfluent stage may not yet be completely differentiated or polarized.

Loading of monoamines and amperometric measurement of exocytosis. Carbon fiber amperometry was used to detect exocytosis from single cells in real time. Amperometry provides the high resolution
necessary to detect the molecules released from single secretory vesicles and the stability necessary for prolonged recording. Because amperometry requires cells that secrete an oxidizable molecule, we incubated PDEC for 40 min at room temperature (22–24°C) in high concentrations of dopamine (in mM: 70 dopamine, 68 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 10 d-glucose, and 10 HEPES, pH 7.3 adjusted with NaOH) to force the exogenous monoamine to distribute passively via the cytoplasm into acidic secretory vesicles (14, 16). In addition, 1.4 mM l-ascorbic acid was added to the loading solution to reduce oxidation of dopamine. Cells were then transferred to a dopamine-free saline solution (in mM: 137.5 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 10 d-glucose, and 10 HEPES, pH 7.3 adjusted with NaOH), and exocytosis was measured as pulses of electric current generated by oxidation of dopamine at the tip of a carbon fiber electrode polarized to +400 mV. A local perfusion system allowed complete exchange of solutions bathing the cells within 1 s.

Carbon fiber microelectrodes were fabricated by using 11-μm carbon fibers and polypropylene 10-μm micropipette tips (15). The amperometric current signals were recorded with an EPC 9 (HEKA Elektronik) patch-clamp amplifier, filtered at 0.1 kHz, and sampled digitally at 0.5 kHz.

**Single-cell photometry.** [Ca²⁺], was measured by using the Ca²⁺-sensitive fluorescent dye indo 1-AM. The cells were incubated for 30 min in saline solution with 2 μM indo 1-AM at room temperature (22–24°C). The excitation wavelength was 365 nm (100-W mercury lamp), and fluorescence signals were recorded at 405 and 500 nm, using a pair of photon-counting photomultiplier tubes. The sampling rate was 0.5 Hz, and background fluorescence measured from a cell-free area was subtracted. The [Ca²⁺], was calculated as [Ca²⁺], = K′(R – Rmin)/(Rmax – R), where R is the fluorescence ratio at 405 to 500 nm (F405/F500), Rmin and Rmax are the ratios for Ca²⁺-free and Ca²⁺-bound dye, respectively, and K′ is a correction factor (8). Rmin, Rmax, and K′ were measured on cells perfused for at least 10 min with Na⁺-rich external solutions containing 20 μM ionomycin plus 20 mM EGTA or 15 mM Ca²⁺ or plus 20 mM EGTA and 15 mM Ca²⁺. All solutions used for calibration contained carbonyl cyanide m-chlorophenylhydrazone, a mitochondrial Ca²⁺ uniporter blocker, and thapsigargin, a sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) pump blocker, for a fast equilibration of cytosplasmic Ca²⁺ with the external Ca²⁺ calibration buffers. Rmin, Rmax, and K′ were 0.38, 4.20, and 3,110 nM, respectively (n = 10–14 cells for each value).

**Data analysis.** Amperometric recordings were semi-automatically analyzed by using software written in Igor (WaveMetrics). Because the peak amplitude of the signals depends on the extent of vesicle filling with dopamine, we considered only the number of the amperometric events as a measure of exocytosis (14). The rate of exocytosis was defined as the number of amperometric spikes per 30-s time bin. To adjust for cell-to-cell variation, the rate of exocytosis of each experiment was normalized to the baseline value before being averaged (normalized rate of exocytosis). Relative exocytosis was calculated as the ratio of the average rate of exocytosis obtained for 6 min during treatment to the rate of exocytosis obtained for 3 min before treatment. All numerical values are given as means ± SE, where n is the number of cells examined. Statistical significance was determined by Student’s t-test, and P ≤ 0.05 was considered significant.

**RESULTS**

**ATP stimulates [Ca²⁺], increase and exocytosis.** Exocytosis in PDEC previously loaded with exogenous dopamine was monitored with a carbon fiber electrode positioned on the cell membrane, and fusion of each single vesicle was detected as an amperometric spike (Fig. 1A). When inspected at a high time resolution as shown in Fig. 1A, inset, each amperometric current spike represents the typical oxidation of dopamine released from single secretory vesicles (12). The frequency of spontaneous amperometric spikes in the control solution was ~2 spikes per 30 s (Fig. 1A). After application of 100 μM ATP, more spikes were observed. The rate of exocytosis (spikes per 30-s time bin) is illustrated in a rate histogram (Fig. 1B). Exocytosis increased within 1 min after ATP addition and slowly decreased even under continuous ATP exposure. The maximal rate of exocytosis evoked by 100 μM ATP was about 70 spikes per 30 s.

To determine the relation between Ca²⁺ and exocytosis, we monitored [Ca²⁺], using the Ca²⁺-sensitive fluorescent dye indo 1 at different ATP concentrations (Fig. 2, A–C). At a low concentration of 2 μM, ATP evoked different patterns of [Ca²⁺]. Of 15 cells tested, 4 cells showed regular [Ca²⁺], oscillations as those shown in Fig. 2A. 2 cells showed irregular oscillations, and 9 cells showed a monotonic Ca²⁺ decrease after a peak up to 1 or 2 μM had been reached. Cells with [Ca²⁺], oscillations showed an average period, duration, and number of peaks of 20 ± 3 s, 264 ± 85 s, and 13 ± 4, respectively (n = 4). In contrast, 10 μM ATP consistently evoked Ca²⁺ oscillations (Fig. 2B). The average period, duration, and number of peaks were 29 ± 8 s, 233 ± 49 s, and 9 ± 1, respectively (n = 4). These values are not significantly different from those at 2 μM ATP. With 100 μM ATP, the [Ca²⁺], oscillations (a few peaks) were masked by a large and slowly decreasing plateau (Fig. 2C).

In parallel, we compared the rate of exocytosis at different concentrations of ATP (Fig. 2, D–F). Low ATP concentrations of 2 or 10 μM were less efficient than 100 μM in inducing exocytosis, with relative rates of 1.1 ± 0.2 (n = 5), 1.9 ± 0.4 (n = 5), and 6.9 ± 1.8 (n = 14) for 2, 10, and 100 μM ATP, respectively. With 100 μM ATP, although [Ca²⁺], peaked within a few seconds after ATP stimulation, exocytosis developed with a time delay of 30–60 s.
Because both exocytosis and mucin secretion in PDEC can be mediated through cAMP as well as Ca$^{2+}$ (16, 19, 20), the contribution of cAMP to the ATP effect was also considered. To examine whether the large [Ca$^{2+}$]i increase observed with 100 μM ATP alone can induce exocytosis, we tested the effects of UTP, since UTP stimulates P2Y$_2$ receptors on PDEC to raise [Ca$^{2+}$]i without stimulating the cAMP pathway (19). When cells were exposed to 100 μM UTP, both [Ca$^{2+}$]i (Fig. 3A) and the average rate of exocytosis (Fig. 3B) increased considerably. The averaged [Ca$^{2+}$]i increase was similar to the averaged [Ca$^{2+}$]i obtained with 100 μM ATP (data not shown), and the relative exocytosis rate was 4.8 ± 1.4 (n = 12), or 65 ± 24% (n = 12) of the rate observed with 100 μM ATP. Exocytosis stimulated by UTP exhibited a time course similar to that stimulated by 100 μM ATP, including a 30-s delay. In conclusion, a Ca$^{2+}$ increase alone without cAMP can stimulate exocytosis to some extent.

**Mediation of ATP effect through both cAMP and Ca$^{2+}$ pathways.** To determine the relative contribution of the cAMP or Ca$^{2+}$ pathways to exocytosis in PDEC, we characterized the effects of an intracellular Ca$^{2+}$ chelator and of PKA inhibitors. We wanted to study exocytosis under conditions with strong Ca$^{2+}$ buffering. Preincubation of cells with the cell-permeant Ca$^{2+}$ chelator BAPTA-AM (10 μM) for 1 h at 37°C lowered the resting [Ca$^{2+}$]i level from 118 ± 22 nM (n = 17) to 78 ± 17 nM (n = 4), indicating effective buffering of [Ca$^{2+}$]i by BAPTA (Fig. 4). The baseline exocytosis rate in BAPTA-treated cells was 80% of that of untreated cells, but the difference was not statistically significant (P = 0.5, Student’s t-test). As expected, Ca$^{2+}$ photometry revealed that BAPTA abolished the [Ca$^{2+}$]i increase stimulated by 100 μM ATP (n = 4, Fig. 4A). However, even in the absence of an increased [Ca$^{2+}$]i, 100 μM ATP still stimulated a moderate increase of exocytosis, with relative exocytosis rate of 2.4 ± 0.4 (n = 6, Fig. 4B), or 25 ± 7% (n = 6) of the corresponding rate obtained with PDEC not treated with BAPTA. Thus, even when Ca$^{2+}$ signaling is abolished, 100 μM ATP can still trigger exocytosis, presumably through a PKA-dependent cAMP signaling pathway. Indeed, we previously observed that 100 μM ATP stimulated an increase in intracellular cAMP in these same PDEC (19).

ATP-stimulated exocytosis developed with a delay after stimulation, reached a peak within ~1 min, and returned to the basal level within ~2 min. In a previous study, when hormone action was mimicked by activating adenyl cyclase directly
with forskolin (20 μM), the activation was first apparent at 30–60 s (10, 16).

The contribution of increased \([Ca^{2+}]_i\) to exocytosis was next determined by using inhibitors of PKA that act downstream from cAMP generation (Fig. 5). We previously demonstrated (16) that the PKA inhibitor H-89 selectively and completely blocks cAMP-induced exocytosis. When PDEC were treated with 5 μM H-89 for 5 min, a modest increase in \([Ca^{2+}]_i\) was observed (Fig. 5A). Further treatment with 100 μM ATP led to an additional large \([Ca^{2+}]_i\) increase observed in all tested cells (n = 5). Treatment with H-89 slightly reduced the baseline exocytosis rate obtained with a control saline perfusion to a relative exocytosis rate of 0.8 ± 0.2, but the change was not statistically significant (P > 0.1, n = 7, Fig. 5B). H-89 reduced the exocytosis stimulated by 100 μM ATP, with a relative exocytosis rate of 2.4 ± 0.5 (n = 7) (vs. H-89 alone), equivalent to only 24 ± 8% (n = 7) of the ATP-stimulated rate observed in the absence of H-89. Again, exocytosis peaked within ~1 min after 100 μM ATP stimulation and returned to baseline within ~2 min. Hence, a \([Ca^{2+}]_i\) rise following physiological stimulation (ATP) suffices to stimulate exocytosis in PDEC. When ionomycin was used to induce \([Ca^{2+}]_i\) flux into the cytoplasm, the resulting artificial \([Ca^{2+}]_i\) rise also stimulated exocytosis in these cells (16).

Figure 6 illustrates the effect of Rp-8-BrcAMPS, a more specific inhibitor of PKA, which at 2 mM completely inhibits the exocytosis induced by 20 μM forskolin (16). Rp-8-BrcAMPS (1 mM) itself did not affect baseline exocytosis but reduced the exocytosis stimulated by 100 μM ATP to a relative exocytosis rate of 2.6 ± 0.8 (n = 4) (vs. Rp-8-BrcAMPS alone), equivalent to 27 ± 14% (n = 4) of the corresponding rate with forskolin (20 μM).
value observed in the absence of the PKA blocker. The similar inhibitions observed with H-89 and Rp-8-BrCAMPs suggest that both blockers mainly acted on PKA and that the Ca$^{2+}$ component of the signal from P2Y receptors could be an effective trigger of exocytosis by itself. To confirm that the combined activation of the Ca$^{2+}$ and cAMP pathways accounted for most of the exocytosis stimulated by ATP, we blocked both pathways with the corresponding inhibitors (Fig. 7). In Fig. 7A, in the presence of BAPTA, [Ca$^{2+}$], remained low in the presence of 5 μM H-89 alone or in combination with 100 μM ATP. In Fig. 7B, the effect of ATP on exocytosis was also abolished when both signaling pathways were blocked, with a relative exocytosis rate of 1.1 ± 0.3 (n = 6), or only 2 ± 5% (n = 6) of the expected ATP response in BAPTA- and H-89-untreated PDEC. This finding indicates that exocytosis induced by ATP was activated by both the Ca$^{2+}$ and the cAMP/PKA pathways. Notably, the rate of exocytosis gradually increased during the washout period with control saline perfusion. This effect may result from the removal of H-89 and consequent activation of PKA by the residual cAMP that had been generated during the ATP challenge.

Figure 8 summarizes exocytosis induced by 100 μM ATP under the different conditions discussed. Interestingly, because UTP would not activate P2Y11 receptors and the cAMP pathway, the relative exocytosis induced by 100 μM UTP should be similar to that observed after sole activation of the Ca$^{2+}$ pathway by 100 μM ATP in PDEC treated with PKA inhibitors. However, more exocytosis was observed in PDEC treated with UTP than in cells pretreated with Rp-8-BrCAMPs or H-89 and then treated with ATP, suggesting that PDEC have additional pyrimidine-prefering receptor(s) or that UTP is more efficient on P2Y2 receptors than ATP at the same concentration. In conclusion, exocytosis from PDEC can be induced by ATP through the Ca$^{2+}$- or cAMP pathways, acting alone or in combination.

**DISCUSSION**

Activation of purinergic receptors regulates exocytosis via Ca$^{2+}$ and cAMP. Extracellular ATP and UTP mediate physiological responses in mammalian cells through the activation of G protein-coupled P2Y receptors. Whereas all cloned P2Y receptor subtypes are functionally coupled to the phospholipase C and the Ca$^{2+}$-pathway, the P2Y11 receptor, which binds to ATP but not UTP, is unique in that it is also coupled to the cAMP/PKA pathway. Dog PDEC, which express both P2Y2 and P2Y11 receptors and are a good model system for exocytosis in nonexcitable cells, were used to study the role of intracellular signals such as Ca$^{2+}$ or cAMP/PKA on ATP-stimulated exocytosis. In these cells, we previously observed (19) that ATP, but not UTP, stimulated an increase in intracellular cAMP.

Only high concentrations of ATP stimulated exocytosis through P2Y receptors. Exocytosis rate measured amperometrically increased up to sevenfold with 100 μM ATP, and this effect was mediated by both Ca$^{2+}$ and cAMP pathways, because it could be inhibited by a Ca$^{2+}$ chelator or by PKA inhibitors. The ATP dependence of mucin secretion from PDEC is similar, with 80 and 260% increases of mucin secretion with 10 and 100 μM ATP (20). Notably, even though
[Ca\(^{2+}\)]\(_i\) increased to several micromolar within 2 s of 100 \(\mu M\) ATP addition, exocytosis was delayed by up to a minute. A similar delay (30–60 s) was also observed after ionomycin-mediated Ca\(^{2+}\) influx into PDEC (16) or UTP treatment. Because UTP activates only Ca\(^{2+}\) signaling via P2Y\(_2\) receptors in these PDEC, this delay does not likely reflect additional time required for cAMP/PKA activation. The considerably slower kinetics of epithelial exocytosis compared with the brief stimulation. These differences may account for the slower and tonic response in nonexcitable cells compared with the brief stimulation. Evidently, some unique requirement for cAMP/PKA activation. The considerably slower kinetics of epithelial exocytosis compared with the brief stimulation. These differences may account for the slower and tonic response in nonexcitable cells compared with the brief stimulation. Evidently, some unique requirement for cAMP/PKA activation.

Although the Ca\(^{2+}\) and cAMP/PKA pathways can function independently, they may also interact synergistically. In our work, activating them simultaneously induced a larger rate of exocytosis than the sum of the individual stimulations. It is worthwhile to note that cAMP increased significantly only with 100 \(\mu M\) ATP (19). In PDEC, low concentrations of ATP (<10 \(\mu M\)) induced Ca\(^{2+}\) oscillation with peak [Ca\(^{2+}\)]\(_i\) =1 \(\mu M\) but stimulated only a small increase in exocytosis. Evidently, exocytosis in epithelial cells requires persistent Ca\(^{2+}\) increase or concerted action with other second messengers, such as cAMP. Similarly, in pancreatic 

Another result indicates that sustained micromolar [Ca\(^{2+}\)]\(_i\), rises are required to induce exocytosis in PDEC. In excitable cells, exocytosis from readily releasable vesicles is mediated by Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels (6), where the local [Ca\(^{2+}\)]\(_i\) rises into the 10–100 \(\mu M\) range near the vesicles. However, in nonexcitable cells such as PDEC, which lack voltage-gated Ca\(^{2+}\) channels, [Ca\(^{2+}\)], does not increase above 10 \(\mu M\) and the rate of exocytosis peaks only 1–2 min after stimulation. These differences may account for the slower and tonic response in nonexcitable cells compared with the brief intense burst typical of excitable cells (23).

**Activation of purinergic receptors under physiological conditions.** The role of ATP as an intercellular messenger is supported by its extracellular presence (5, 17) and by the expression of specific receptors in many cell types. Established sources of ATP include intracellular granules in activated platelets and nerve terminals, which corelease ATP along with norepinephrine or acetylcholine. ATP can also be released from epithelial cells (11, 26) and more specifically from pancreatic acini (29). In the pancreaticobiliary tree, the concentration of ATP is ~9 \(\mu M\) in the vicinity of acini and ~2 \(\mu M\) in human bile (2), enough to stimulate purinergic receptors in bile duct epithelial cells (26). In addition, because there is active degradation of ATP by ectonucleotidase, local concentrations of ATP may exceed the concentrations measured in ductal lumen (18).

The exact mechanism for ATP release from epithelia is still under debate. Whereas initial studies supported direct transport through the cystic fibrosis transmembrane conductance regulator (CFTR) (25, 27), more recent evidence suggests CFTR potentiation of ATP release through a separate channel or no role for CFTR (7, 32).

The effects of ATP released into the extracellular space depend on the specific receptors available. The dog PDEC studied in this report express P2Y\(_2\) receptors on apical and basolateral membranes and express P2Y\(_{11}\) receptors only on the basolateral membranes (19, 20). Thus, on the luminal side, ATP activates only the Ca\(^{2+}\) pathway through P2Y\(_2\) receptors, whereas on the serosal side, it activates both the Ca\(^{2+}\) pathway through P2Y\(_2\) and P2Y\(_{11}\) receptors and the cAMP pathway through P2Y\(_{11}\) receptors and, therefore, presumably induces stronger exocytosis. We previously correlated this differential receptor expression with a differential activation of ion transport pathways such as CFTR (19). Luminal and serosal ATP may be present under different circumstances, because luminal ATP may be released from neighboring acinar or duct cells into the pancreatic juice, whereas serosal ATP may be released from immune cells or nerve terminals during inflammation, or by the epithelial cell itself (11). We are actively investigating the ATP release mechanism from PDEC.

**Role of Ca\(^{2+}\) oscillation.** Activation of purinergic receptors on PDEC by ATP or UTP increases intracellular Ca\(^{2+}\) in a concentration-dependent manner. Low ATP induces [Ca\(^{2+}\)]\(_i\) oscillations, whereas high ATP stimulates a sustained [Ca\(^{2+}\)]\(_i\) increase followed by a slow decay. Low ATP induces Ca\(^{2+}\) oscillations that do not efficiently stimulate exocytosis in PDEC in clear contrast to endocrine cells, where strong exocytosis is closely synchronized with Ca\(^{2+}\) oscillations (31). For these cells, oscillations may reduce the toxic effects of Ca\(^{2+}\) while maintaining a secretory output comparable to that attained by sustained elevation of [Ca\(^{2+}\)]\(_i\). For the target cell, the Ca\(^{2+}\) oscillations in endocrine cells optimally produce a maximal effect without desensitization of the target cell. Because PDEC also mediate the secretion of fluid and electrolytes (mainly bicarbonate), these ATP-induced Ca\(^{2+}\) oscillations may have a major role in regulating ion transport pathways (19, 20). Our patch-clamp measurements reveal that Ca\(^{2+}\) oscillation activates Ca\(^{2+}\)-dependent K\(^+\) channels with a half-maximal conductance at 1 \(\mu M\) (Jung S-R, unpublished observations). The conductance change, synchronous with Ca\(^{2+}\) oscillation, might affect the membrane potential and, consequently, ion transport or secretion.

In conclusion, ATP stimulates exocytosis at high concentrations by using both Ca\(^{2+}\) and cAMP pathways and may modulate the membrane ionic conductance at low concentration. These findings point to a diversified, yet specialized, role for ATP in regulating PDEC function: ATP regulates ion transport pathways and exocytosis, two distinct mechanisms for secretion. Furthermore, the ability and degree of activation of these pathways may vary depending on the site of interaction (apical vs. basolateral membrane), the receptor subtype involved (P2Y\(_2\) vs. P2Y\(_{11}\)), and the concentration of ATP ([Ca\(^{2+}\)]\(_i\) oscillations at low concentrations vs. sustained [Ca\(^{2+}\)]\(_i\) at higher concentrations). These complex interactions support an important regulatory role for ATP in PDEC secretion that warrants additional investigation.

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REFERENCES

3. Chavez-Noriega LE and Stevens CF. Increased transmitter release at Gastrointest Liver Physiol 270: G246, 1996.