Purinoceptors Evoke Different Electrophysiological Responses in Pancreatic Ducts

P2Y INHIBITS K+ CONDUCTANCE, AND P2X STIMULATES CATION CONDUCTANCE*

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In epithelia, extracellular nucleotides are often associated with regulation of ion transporters, especially Cl– channels. In this study, we investigated which purinoceptors are present in native pancreatic ducts and how they regulate ion transport. We applied whole-cell patch-clamp recordings, intracellular Ca2+ and pH measurements, and reverse transcription-polymerase chain reaction (RT-PCR) analysis. The data show two types of purinoceptors and cellular responses. UTP and ATP produced large Ca2+ transients, a decrease in intracellular pH, 8–10-mV depolarization of the membrane voltage, and a decrease in the whole-cell conductance. The membrane effects were due to closure of K+ channels, as confirmed by dependence on extracellular K+. UTP/ATP effects could be associated with P2Y2 purinoceptors, and RT-PCR revealed mRNAs for P2Y2 and P2Y4 receptors. On the other hand, 2′,3′-O-4-benzoylbenzoyl-ATP induced Ca2+ influx and ~20-mV depolarization of the membrane voltage with a concomitant increase in the whole-cell conductance. These effects were dependent on extracellular Na+, not Cl−, indicating opening of cation channels associated with P2X7 purinoceptors. RT-PCR showed mRNAs for P2X7 and P2X4 receptors. In microperfused ducts, luminal (but not basolateral) ATP caused large depolarizations of membrane voltages recorded with microelectrodes, consistent with luminal localization of P2X7 receptors. Thus, P2Y2 (and possibly P2Y4) purinoceptors inhibit K+ channels and may not support secretion in native ducts. P2X7 (and possibly P2X4) receptors are associated with cation channels and may contribute to regulation of secretion.

Extracellular nucleotides are regulators of a wide range of cellular functions in various cells, including regulators of epithelial transport (1). They act through specific P2 receptors, which, according to their molecular structure and signal transduction, have been divided into two distinct receptor families: G protein-coupled receptors (P2Y receptors) and ligand-gated ion channels (P2X receptors) (1). In epithelia cultured from upper respiratory airways, intestine, and exocrine glands, including pancreas, UTP and ATP stimulate Cl− transport through P2Y receptors, which were described pharmacologically as P2U receptors and, in many cases, may correspond to the cloned P2Y2 receptors (2–11). However, similar action of pyrimidines on Cl− transport could also be exerted through P2Y4 or P2Y6 (12, 13). Due to their effects on Cl− transport, nucleotides were proposed as therapeutic agents for treatment of cystic fibrosis, as they could bypass the defective function of the cystic fibrosis transmembrane regulator Cl− conductance and restore secretion by activation of a Ca2+-dependent Cl− conductance (8). Interestingly, recent studies on exocrine glands indicate that the P2Y receptors and associated signal transduction pathways are not static, but dynamic. For example, the P2Y2 receptor expression increases dramatically in response to cystic fibrosis (14). The P2Y, receptor activity changes during the gland development (15). Thus, it is important to consider which P2 receptors are present in freshly prepared cells from exocrine glands. Although there is evidence for some P2Y-type receptors (4, 11, 16, 17), more commonly accounted purinoceptors are those linked directly to cation influx (18–23). Occupation of these purinoceptors leads to stimulation of Cl− transport, volume changes, and secretion of enzymes and peptides (19, 21, 24), but in some cases, interference with the Ca2+ signaling utilized by other receptor pathways has been reported (25, 26). These receptors have been described pharmacologically as P2Z receptors, but it is unclear if these correspond to the pore-forming P2X receptors originally found in immunoreactive cells (1). Interestingly, in situ hybridization analysis shows P2X7 receptors on salivary gland acini, but not on ducts (27). Thus, from pharmacological studies, it seems that exocrine glands can express receptors belonging to P2X and P2Y families, but their identity, localization, and function are unclear.

Pancreatic ducts modify enzyme- and Cl−-secretion originating from acini by adding a bicarbonate-rich fluid. Bicarbonate secretion is achieved by coupling of ion transport through the luminal cystic fibrosis transmembrane regulator Cl− channels and the luminal Cl−/HCO3− exchanger (28–30). The driving force for the Cl−exit is kept by the K+ exit via the basolateral K+ channels (29, 31–33). The main regulators of pancreatic bicarbonate secretion are secretin, vasoactive intestinal peptide, acetylcholine, and noradrenaline (30, 34, 35). In earlier studies, we showed that isolated ducts also respond to ATP by releasing stored Ca2+ and initiating Ca2+ influx through channels different from those used by acetylcholine (36–38). Furthermore, we demonstrated that ATP inhibits secretin-evoked changes in the membrane voltage (37). We used Ca2+ signals and pharmacological profiles to describe two types of receptors: one responding to UTP and ATP and belonging to the P2Y superfamily (P2Y, like receptor) and another responding to BzATP1 and ATP and belonging to the P2X superfamily.

1 The abbreviations used are: BzATP, 2′,3′-O-4-benzoylbenzoyl-ATP; RT-PCR, reverse transcription-polymerase chain reaction; BCECF, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; Vmem, membrane voltage; Gt, total whole-cell conductance; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid.
were held between concentric pipettes at each end, and the lumen was perfused according to methods described earlier (32). Such ducts were used for microelectrode recordings.

During all physiological experiments, the chamber was continuously perfused at a rate of 10–15 ml/min to avoid mechanical disturbances, while the bathing solution was composed of 145 mM NaCl, 3.6 mM KCl, 1.5 mM CaCl2, 1 mM MgCl2, 125 mM Cl−, 25 mM HCO3−, 2 mM phosphate, and 5 mM glucose. The pH was equilibrated to 7.4 with 5% CO2 in O2. The temperature was kept constant at 37 °C during all experiments. In some experiments, K+, Na+, or Cl− concentrations were modified as follows: K+ was increased from 3.6 to 20, 25, or 50 mM by equimolar substitution for Na+; Na+ was decreased from 145 to 5 mM and substituted with N-methyl-D-glucamine; and Cl− was decreased from 125 to 5 mM and substituted with gluconate. The pancreatic ducts were stimulated with ATP, UTP, and BzATP in concentrations of 0.1 mM. In a few experiments, concentrations of agonists were varied from 10−6 to 10−4 M, except for BzATP, which was not used at concentrations higher than 0.1 mM due to the cost.

**Electrophysiological Measurements**—The cell responses to nucleotides were monitored in whole-cell nystatin patch-clamp recordings adopted for pancreatic ducts (39). Patch pipettes had resistances of 3–5 MΩ, and the initial seal was at least 1 gigaohm. The success rate of obtaining seals was ~10%, and stable cell membrane voltage recordings were obtained only in a fraction of these. The cell membrane under the pipette was permeabilized to make direct electrical access to duct cells. Nystatin (0.02–0.1 mM) was dissolved in a pipette solution adjusted to pH 7.2 that had the following composition: 11 mM Na+, 125 mM K+, 32 mM Cl−, 96 mM gluconate, 1 mM Mg2+, 6 mM phosphate, and 5 mM glucose; and Ca2+ was adjusted to 0.1 mM with EGTA. A flowing 1 M KCl electrode was used as a reference. The membrane voltage (Vm) was continuously monitored during experiments in a current-clamp mode (zero current clamp). Periodically, the whole-cell current (I) was measured in a voltage-clamp mode, where the voltage was first clamped to the spontaneous cell voltage and then in increasing and decreasing 10-mV steps of 500-ms duration. In some experiments, Vm was clamped at the spontaneous cell voltage, and continuous whole-cell currents were measured. Where possible, the total conductance (Gt) was corrected for the series resistance to obtain the cell membrane conductance. The series resistance was estimated by the compensation circuit of the patch-clamp amplifier (EPC 9, Heka, Lambrecht, Germany), and in some cases where this was not possible, series resistance and cell membrane conductance estimations were made subsequently by fitting an exponential function to the initial part of the current trace (40). Since duct cells can be variably coupled to each other, Gt changes with the agonists were relative rather than absolute. In some experiments, ducts were micromanipulated and microperfused according to methods described earlier (32). Single duct cells were impaled with 100–200 MΩ Ling-Gerard microelectrodes, and Vm was recorded with a WPI electrometer.

**Measurements of Intracellular Ca2+ and Intracellular pH**—The fura-2 method was used to estimate [Ca2+]i in pancreatic ducts. Briefly, as we have described earlier (37), ducts were loaded with 0.5–1.0 μM fura-2/AM, and the fluorescence intensity of 10–20 cells was measured with a photomultiplier at 510 nm after excitation at 340, 360, and 380 nm. The fluorescence ratio (340/380 nm) was an estimate of [Ca2+]i. Signals were calibrated in situ with ionomycin (1.0–5.0 μM). For pH measurements, ducts were stimulated with BCECF/AM as described earlier (41). Emission intensity of duct cells was measured at 510 nm after excitation at 490 and 440 nm. The fluorescence ratio (490/440 nm) was an estimate of pH. Signals were calibrated in situ with the protophore carbonyl cyanide p-chlorophenylhydrazone or nigericin (1 or 10 μM).

**Statistics**—Physiological data are presented as original recordings, summaries, and means ± S.E. In most experiments, control and test measurements were made within one duct cell or one duct, and n refers to measurements on different ducts. The paired Student’s t test was applied, and p < 0.05 was accepted as significant. The F-test was used to test for the difference between two regression coefficients obtained for the current-voltage relations.

**RNA Isolation, cDNA Synthesis, and Polymerase Chain Reaction Amplification**—Total RNA was isolated from small intralobular and extralobular ducts (prepared from collagenase digests of rat pancreas) (Fig. 1) using total RNA isolation reagent (Advanced Biotechnologies Ltd.) according to the manufacturer’s instructions. First strand cDNA was synthesized utilizing an anchored oligo(dT) primer using Reverse-iT (Advanced Biotechn-
TABLE I
Primer sequences for RT-PCR on total RNA from pancreatic duct cells.

| Primer          | Expected size (bp) |
|-----------------|--------------------|
| Rat carbonic anhydrase |                   |
| Fwd             | 5'-GGGATACGGCAAGGCAAAGG-3' | 541 |
| Rev             | 5'-CAGGGAGAAGGGGACAGAG-3' |     |
| Rat thrombin     |                   |
| Fwd             | 5'-GGCTATTTTGCTCTACTCC-3' | 278 |
| Rev             | 5'-CACTTTCCAACCTTTCTTC-3' |     |
| Rat amylase      |                   |
| Fwd             | 5'-ATATTGGCAAGAATGTGACGGTA-3' | 971 |
| Rev             | 5'-CTGGAAATTCTCGTGCAATGAA-3' |     |
| P2X receptor primers |                     |
| Fwd             | 5'-ATGCTTCTCCAGCAACGAG-3' | 584 |
| Rev             | 5'-GTTCTTCTCCTACAGTACC-3' |     |
| Fwd             | 5'-GTTCAGAGCCTCCTACGC-3' | 405 |
| Rev             | 5'-GATGACTCCAATGACACC-3' |     |
| Fwd             | 5'-TTCTTCACCTACGAGACTACC-3' | 650 |
| Rev             | 5'-TTAACACACATCCCCCTACC-3' |     |
| Fwd             | 5'-AGGCTACCGAGGAGGAGAAT-3' | 550 |
| Rev             | 5'-GATGTGCGCAAGGCGGAT-3' |     |
| Fwd             | 5'-ATCTCTACTGTCCTCTCCC-3' | 595 |
| Rev             | 5'-TTGCTATTCTGCTCTCCC-3' |     |
| Fwd             | 5'-TAACCAACTCTCTTGACACC-3' | 552 |
| Rev             | 5'-TGAAATTGTACCCCCTCTCC-3' |     |
| Fwd             | 5'-ACGAAAGTTAGTACACGGAAT-3' | 360 |
| Rev             | 5'-GGCCGGGGAAGTCGTATGG-3' |     |
| Human P2Y<sub>1</sub> |                     |
| Fwd             | 5'-CATCACCCTTGTAAGGACACC-3' | 406 |
| Rev             | 5'-TCCTCCTCCTGAAGTATCCC-3' |     |
| Rat P2Y<sub>2</sub> |                     |
| Fwd             | 5'-TGCGGCTATGCGCCCGGAG-3' | 632 |
| Rev             | 5'-GAGGATCACTGCTGTACGGA-3' |     |
| Rat P2Y<sub>4</sub> |                     |
| Fwd             | 5'-ATGGCATCACTGCGCCGGA-3' | 401 |
| Rev             | 5'-CTTTGGGAGGCCTGCCACC-3' |     |
| Human P2Y<sub>6</sub> |                     |
| Fwd             | 5'-TGACCGCCAGCGCGGCGTAC-3' | 214 |
| Rev             | 5'-CCGGCAGCAGCGGCGTATG-3' |     |

* bp, base pairs; Fwd, forward; Rev, reversed.

Fig. 2. Effect of UTP, ATP, and BzATP on intracellular Ca<sup>2+</sup>.
A, effect of agonists (0.1 mM) on Ca<sup>2+</sup> transients in a single duct; B, concentration-response curves for ATP, UTP, and BzATP. Responses are given as changes in the fluorescence ratio 340/380 nm. Each point is a mean ± S.E. for 3–11 experiments.

largely due to release of Ca<sup>2+</sup> from intracellular stores, whereas the plateau reflects largely influx of Ca<sup>2+</sup> from extracellular medium (36, 38). UTP evoked a similar response, but a more pronounced Ca<sup>2+</sup> influx component. On the other hand, stimulation with BzATP led to a monophasic Ca<sup>2+</sup> response, which was almost fully dependent on extracellular Ca<sup>2+</sup> and thus due to Ca<sup>2+</sup> influx. On the basis of these and other observations, we postulated that there are at least two types of receptors belonging to both P2Y and P2X families (36). Fig. 2B summarizes the maximal Ca<sup>2+</sup> responses for the three purinergic analogues used in this study. The EC<sub>50</sub> values for UTP, ATP, and BzATP were estimated at 5, 4, and ~20 μM, respectively. UTP, ATP, and BzATP were the best agonists regarding the intracellular Ca<sup>2+</sup> signals compared with ITP, 2-methylthio-ATP, β,γ-methylene-ATP, and adenosine, as shown earlier (36). Since native pancreatic ducts are not suitable for extensive electrophysiological experiments (see “Experimental Procedures”), we focused on using ATP, UTP, and BzATP as pharmacological markers for further patch-clamp experiments aimed at elucidating how purinoceptors couple to ion transport.

Effect of UTP and ATP on Cell Membrane Voltage and Conductance—In contrast to the Ca<sup>2+</sup> responses evoked by UTP and ATP, the V<sub>m</sub> responses measured in whole-cell patch-clamp experiments were relatively small, although there was some variability between cells. On average, UTP and ATP evoked ~10-mV changes in V<sub>m</sub> and in several cells, the effects were very small, as shown in an original recording in Fig. 3A. In contrast, BzATP depolarized V<sub>m</sub> reversibly by ~20 mV in the same cell. Fig. 3B shows a whole-cell current measurement in another duct cell, where again the ATP and UTP responses were small, whereas BzATP stimulated a sustained inward current of ~100 pA at a holding potential of ~60 mV. In this experiment, carried out in a current-clamp mode, ATP depolarized V<sub>m</sub> by 9 mV, and UTP by 4 mV (not shown). Similar
Paired experiments were performed on 14 ducts, and Fig. 4A shows the summary of the \( V_m \) data for the three agonists. The summary shows that UTP and ATP caused small but significant \( V_m \) depolarizations of 8 ± 2 and 10 ± 2 mV, respectively. BzATP depolarized \( V_m \) by 22 ± 3 mV in the same experiments, and this effect was significantly larger compared with the UTP and ATP responses (\( p < 0.005 \)).

In the next series of experiments, we investigated the electrophysiological events underlying the \( V_m \) depolarization caused by UTP and ATP. To illustrate how \( V_m \) changes correlate with the cell conductance changes, Fig. 5A shows an experiment in which the \( V_m \) responses to UTP and ATP were some of the largest obtained. The corresponding current-voltage relations in Fig. 5B show that during the UTP and ATP stimulation, \( V_m \) shifted away from the resting voltage; but notably, \( G_t \) decreased. A summary of the whole-cell conductance data for 14 experiments is given in Fig. 4B. UTP and ATP caused small but significant reductions in \( G_t \) of 17 and 20%, whereas BzATP increased \( G_t \) by 18%. In 10 of these experiments, where it was possible to correct \( G_t \) for series resistance, changes in the cell membrane conductance evoked by UTP, ATP, and BzATP were qualitatively and quantitatively similar.

The depolarization of \( V_m \) and the reduction in whole-cell conductance, triggered by UTP and ATP, could be due to a closure of \( K^+ \) channels. This theory was tested in current-clamp experiments in which the bath \( K^+ \) concentration was changed from 4 to 20 mM (see “Experimental Procedures”). In unstimulated ducts, this \( K^+ \) concentration change resulted in ~20-mV depolarization of \( V_m \), as shown in the original recordings in Fig. 6A. During the ATP stimulation, however, the same \( K^+ \) concentration step had a smaller effect, depolarizing \( V_m \) by ~10 mV. Fig. 6B summarizes seven paired experiments with similar protocols in unstimulated and UTP/ATP-stimulated ducts. Thus, in the stimulated ducts, the \( K^+ \) concentration step depolarized \( V_m \) by 13 ± 3 mV. In the same but unstimulated ducts, the \( K^+ \) step depolarized \( V_m \) significantly more by 19 ± 1 mV (\( p < 0.05 \)). In additional experiments, duct cells were voltage-clamped in bath solution containing 4, 25, or 50 mM \( K^+ \) in the presence or absence of UTP or ATP. An example of such a paired experiment on one duct cell is shown in Fig. 6C and D. At 4 mM \( K^+ \), UTP decreased \( G_t \) from 10.8 to 5.8 nanosiemens (\( p < 0.001 \)) and shifted the reversal potential by 16 mV away from the \( K^+ \) equilibrium potential (Fig. 6D). At 25 and 50 mM \( K^+ \) (only 50 mM \( K^+ \) is shown in Fig. 6D), the control \( G_t \) was higher compared with 4 mM \( K^+ \), as expected for \( K^+ \) currents. UTP decreased \( G_t \) from 25.3 to 16.0 nanosiemens (\( p < 0.001 \)).
but the reversal potential was not affected as the cell voltage lies close to the equilibrium potentials for $K^+$, $Cl^-$, and nonspecific cation conductance. Similar paired observations were obtained for an additional four cells.

Effect of ATP on Intracellular pH—Taken together, UTP/ATP depolarize $V_m$ due to closure of $K^+$ channels. One candidate for the $K^+$ channel, the renal $K^+$ channel (see "Discussion"), is down-regulated by intracellular Ca$^{2+}$ and acidic pH. Therefore, in a separate set of experiments, we monitored pH, in duct cells loaded with BCECF in response to ATP. Fig. 7 shows the reversible effect of ATP (0.1 mM) on the pH, of a pancreatic duct. For comparison, this figure also shows the pH, of another duct stimulated with a classical duct secretagogue, secretin (1 nM). In summary, ATP decreased pH, in similar experiments, i.e. pH, was 7.49 ± 0.05 in resting ducts and 7.50 ± 0.05 in stimulated ducts ($n=16$).

Effect of BzATP on Cell Membrane Voltage and Conductance—In another series of experiments, we investigated the ionic basis for BzATP-evoked depolarization in $V_m$, and increased whole-cell conductance. Fig. 8 shows examples of two protocols carried out in four experiments. Fig. 8A is the original recording of whole-cell currents in a duct stimulated with BzATP in control 145 mM Na$^+$ solution and in a test 5 mM Na$^+$ solution. Fig. 8B shows the results of a voltage-clamp protocol carried out on another cell stimulated with BzATP in control and low Na$^+$ solutions. The current-voltage relation indicates that BzATP increases the whole-cell conductance, but only in the presence of control Na$^+$ concentrations. Hence, these experiments show that BzATP opens cation channels that allow Na$^+$ and also Ca$^{2+}$ influx, as shown earlier (36). In another set of experiments ($n=4$), we tested whether BzATP stimulation affects the Cl$^-$ conductance. Fig. 9 presents an example of one of four experiments in which the bath Cl$^-$ concentration was decreased from 125 to 5 mM. This maneuver seemed to have no effect on BzATP-evoked $V_m$ changes, which indicates that the Cl$^-$ conductance was unaffected. For comparison, in ducts stimulated with secretin or isoproterenol, a similar Cl$^-$ concentration step causes 15–20-mV depolarization, which is due to an increase in the cystic fibrosis transmembrane regulator Cl$^-$ conductance (30, 34).

Microelectrode Recordings on Perfused Ducts—In one series of experiments, the $V_m$ of single duct cells was monitored with microelectrodes on microperfused ducts obtained by microdissection, and one such experiment is shown in Fig. 10A. ATP application from the basolateral side led to a small depolarization of $2 ± 1$ mV ($n=8$), sometimes preceded by a few millivolts of hyperpolarization (also see Ref. 37). These $V_m$ changes are very similar to UTP/ATP responses observed using the whole-cell patch-clamp recordings in duct fragments (Figs. 3–6). In contrast, luminal application of ATP resulted in a significantly larger and reversible depolarization of $17 ± 3$ mV ($n=10$). These $V_m$ changes were similar to the BzATP responses observed in duct fragments (Figs. 3 and 4).

P2Y Purinoceptor-Subtype Expression in Pancreatic Duct Cells Analyzed by RT-PCR—Since the pharmacological data presented above indicated the existence of both P2X and P2Y receptors, we used RT-PCR to investigate which subtypes of the purinergic receptor were expressed in ducts. As shown in Fig. 11A, by use of primers for the P2X family (P2X1 to P2X7), we could detect transcripts of both P2X5 and P2X7. There were two bands for P2X5; the upper band was seen only when total
RNA from pancreatic ducts was used, and not when RT-PCR was performed on total RNA from other tissues. Whether the upper band is due to mispriming or to a yet unknown P2X receptor requires further investigations. Regarding the P2Y family of purinergic receptors, we did not include primers for P2Y3, as the sequence available from GenBank is non-mammalian, or primers for P2Y5 and P2Y7, as their identity as purinoceptors is doubtful (1). In the case of the P2Y receptors, we were able to detect transcripts of P2Y2 and P2Y4, but not of P2Y6. As a control for duct epithelium, we show expression of mRNA for carbonic anhydrase II. The samples were free of RNA contaminants from acini and blood vessels since no products for amylase and thrombin were detected. The primers that gave no product in the ducts (P2X1, P2X2, P2X3, P2X5, P2X6, P2Y1, and P2Y6) were tested on total RNA from testis, blood vessels, and whole pancreas to verify the ability of these primers to give products (Fig. 11B).

**DISCUSSION**

The key observations are that UTP/ATP evoked large Ca\(^{2+}\) signals, a small depolarization of the membrane voltage, and a decrease in the whole-cell conductance, effects due to an inhibition of K\(^{+}\) channels. BzATP/ATP increased cell Ca\(^{2+}\) and caused a large depolarization of the membrane voltage and an increase in the whole-cell membrane conductance, effects due to opening of cation channels and influx of Na\(^{+}\) and Ca\(^{2+}\).

Thus, the two types of electrophysiological and Ca\(^{2+}\) responses are due to at least two types of purinoceptors in pancreatic ducts. RT-PCR analysis showed that native pancreatic ducts possess mRNAs for P2Y2, P2Y4, P2X4, and P2X7 purinoceptors. These are the first molecular biological data on native pancreatic duct epithelium.

**P2Y Receptors**—Recently, it became evident that pyrimidines can exert their action via several P2Y purinoceptors (1). In expression systems, the P2Y2 receptor is stimulated equally well with UTP and ATP; the human P2Y6 receptor has a greater selectivity for UTP over ATP; and the P2Y1 receptor is stimulated preferentially with UDP (42, 44, 45). In pancreatic ducts, we did not find expression of mRNA for P2Y6, which is the luminal receptor on respiratory and intestinal epithelia.
responsible for Ca\(^{2+}\)-mediated Cl\(^{-}\) secretion (12, 13). However, we detected transcripts for both P2Y\(_2\) and P2Y\(_4\) receptors (Fig. 11). Unfortunately, rat P2Y\(_2\) and P2Y\(_4\) receptors show similar pharmacology regarding agonists and accept ATP and UTP equally well (46). Both cell Ca\(^{2+}\) and electrophysiological responses are slightly more pronounced with UTP compared with ATP. However, this may simply be due to the ability of ATP to cross-react with the second type of receptors (P2X) discussed below. Thus, we cannot on this basis conclude which receptor is associated with the physiological response we observed in rat ducts. Nevertheless, stimulation of P2Y\(_2/P2Y_4\) leads to a large Ca\(^{2+}\) transient and inhibition of K\(^{+}\) channels (Figs. 4–6). The inhibition of K\(^{+}\) channels is an unexpected finding, as in many similar epithelia, including cultured human and dog pancreatic duct epithelia (see the Introduction), P2Y\(_2\)-like receptors stimulate K\(^{+}\) fluxes as well as Cl\(^{-}\) fluxes (2, 3, 7, 10, 47). Some differences between our results and these data may be accounted for by different experimental conditions, as mentioned in the Introduction, or by different transport properties of epithelia derived from small intralobular ducts compared with main/large pancreatic ducts used for the tissue culture (48). Nonetheless, there are a few recent reports indicating that P2Y\(_2\) receptors can inhibit M-type K\(^{+}\) currents in neurons (49), IsK/KvLQT1 K\(^{+}\) channels in vestibular dark cells (50), and Ca\(^{2+}\)-activated K\(^{+}\) channels in spermatogenic cells (51). It is not yet clear what cellular mechanisms cause, on one hand, an increase in cell Ca\(^{2+}\) and, on the other hand, an inhibition of K\(^{+}\) channels. Inhibitory mechanisms could be indirect, involving calmodulin II, protein kinase C, or membrane-bound phosphatases; or they could be directly exerted by intracellular Ca\(^{2+}\), e.g. the eag K\(^{+}\) channel and renal K\(^{+}\) channels, or by acidic pH (52–56). Our earlier studies on ducts show that the major cell K\(^{+}\) conductance resides on the basolateral as opposed to the luminal membrane (32, 33); but except for a few patch-clamp studies on pancreatic ducts (31, 57), the identity of K\(^{+}\) channels is unknown. However, from our earlier studies, we know that the basolateral K\(^{+}\) channels are inhibited by acidic pH; and consequently, ducts cannot secrete (33). Interestingly, in this study, we have shown that ATP actually decreases pH (Fig. 7). Thus, a fall in pH or changes in cell Ca\(^{2+}\) and associated messengers could close the K\(^{+}\) channels in pancreatic ducts. Given that P2Y\(_2/P2Y_4\) receptors inhibit K\(^{+}\) channels in pancreatic duct cells, they cannot support secretion, as Cl\(^{-}\)-efflux cannot be followed by K\(^{+}\) efflux (see the Introduction). Perhaps then P2Y\(_2/P2Y_4\) receptors could be involved in a down-regulation of secretion evoked by the true secretagogues.

**P2X Receptors**—In our recent study, we identified the BzATP-responsive receptor pharmacologically as the P2Z (P2Z-like) receptor (36). BzATP or high ATP levels evoked Ca\(^{2+}\) transients resulting from a Ca\(^{2+}\) influx, which was sensitive to DIDS, Mg\(^{2+}\), La\(^{3+}\), and extracellular pH (36, 38). This study shows that BzATP activates an inward Na\(^{+}\) current, supporting the idea that this is a ligand-gated receptor. The P2Z receptor would in many cases correspond to the cloned P2X\(_7\) receptor (58). Since we also found transcripts for the P2X\(_7\) receptor in pancreatic ducts, the simplest interpretation is that the P2Z receptors we have described earlier are also of this type. However, the receptor does not form a pore either in our preparation or in other exocrine glands (4, 11, 17, 19–23, 26), as it does in immunoreactive cells. One possibility is that the P2X\(_7\) receptor expressed in glands does not contain the carboxy-terminal domain necessary for the pore formation (58). Another possibility is that our cells do not express ancillary factors necessary for the P2X\(_7\) pore formation. This possibility is not remote, as the P2X\(_7\)-associated pore formation depends on the expression system (59). Finally, P2X receptors are two transmembrane-spanning proteins that can form heteromers, e.g. P2X\(_2\) and P2X\(_{3}\) (60); and given the expression of P2X\(_7\) in ducts, it is possible to speculate that the P2X\(_2\) and P2X\(_7\) proteins could form heteromers responsible for the physiological properties we observed in glands. Nevertheless, we cannot exclude that the P2X\(_2\) homomer could also be responsible for some of the characteristics we observed. This receptor displays a higher affinity for ATP than P2X\(_7\) (43, 61, 62), consistent with the observed effects on Ca\(^{2+}\) transients (Fig. 2). However, since ATP also cross-reacts with the P2Y receptors described above, one cannot use the EC\(_{50}\) values conclusively. Nevertheless, the lower sensitivity of P2X\(_7\) to DIDS and a,β-methylene-ATP (43, 62) compared with the sensitivity we observed (36) indicates that the P2X\(_7\) response may be dominant.

The present finding that the stimulation of the P2X\(_7\) receptor is associated with increased Na\(^{+}/Ca^{2+}\) conductance is in agreement with earlier findings on salivary gland and lacrimal gland acini, which express the P2X\(_2\)-like receptors (17, 19–23, 26). Moreover, in these glands, ATP also increases protein secretion, cell volume changes, and ion fluxes, all indicative of secretory activity. With respect to pancreatic ducts, if the P2X\(_7\) receptors increase only the Na\(^{+}/Ca^{2+}\) conductance, it is difficult to envisage how this could support secretion. Clearly, there must be an opening of the luminal Cl\(^{-}\) channels and the basolateral K\(^{+}\) channels as well as activation of exchangers, as observed with true secretagogues (30, 33). Therefore, on the basis of the present data, this receptor on its own could probably not evoke secretion; rather, by virtue of its effect on Ca\(^{2+}\), it could regulate secretion evoked by other secretagogues.

Since, in the perfused duct, ATP had the strongest effects on the luminal, reminiscent of those BzATP had on the duct fragments, the most likely localization of the P2X\(_7\) receptor is on the luminal membrane. In another type of ductal epithelium, namely ducts from submandibular glands, the luminal P2X\(_7\)-like receptor seems to have the unusual effect of opening Cl\(^{-}\) channels, without reported effects on the cation conductances (4, 11). One interpretation is that salivary gland ducts do not secrete fluid as do pancreatic ducts; and thus, coupling of receptors and transporters may be different.

In conclusion, our studies demonstrate that native pancreatic ducts express mRNAs for P2Y\(_2\) and P2Y\(_4\) receptors as well as for P2X\(_2\) and P2X\(_7\) receptors. Stimulation of P2Y\(_2\) (and possibly P2Y\(_4\)) receptors leads to an increase in cell Ca\(^{2+}\), but a decrease in cell K\(^{+}\) conductance, a process that is not compatible with secretion. Thus, UTP/ATP acting on these receptors would down-regulate secretion evoked by other secretagogues. In contrast, stimulation of P2X\(_7\) (and possibly P2X\(_2\)) receptors is associated with increased cation conductance, a process that may modulate action of other secretagogues.

**Note Added in Proof**—After acceptance of our paper, Luo and co-workers published an article (Luo, X., Zheng, W., Yan, M., Lee, M. G., and Muallem, S. (1999) Am. J. Physiol. 277, C205–C215) showing a greater variety of P2X and P2Y receptors in pancreatic tissue. Differences between these and our results may be due to purity of duct tissue used for RT-PCR.

**REFERENCES**

1. Ralevic, V., and Burnstock, G. (1998) Pharmacol. Rev. 50, 413–492
2. Chan, H. C., Cheung, W. T., Leung, P. Y., Wu, L. J., Chew, S. B., Ko, W. H., and Wong, P. Y. (1996) Am. J. Physiol. 271, C469–C477
3. Cheung, C. Y., Wang, X. F., and Chan, H. C. (1996) Biol. Signals Recept. 7, 321–327
4. Lee, M. G., Zeng, W., and Muallem, S. (1997) J. Biol. Chem. 272, 32951–32955
5. Leipziger, J., Nitschke, R., and Greger, R. (1991) Cell. Physiol. Biochem. 1, 273–285
6. Mason, S. J., Paradiso, A. M., and Boucher, R. C. (1991) Br. J. Pharmacol. 103, 1649–1656
7. Nguyen, T. D., Moody, M. W., Savard, C. E., and Lee, S. P. (1998) Am. J. Physiol. 275, G104–G113
8. Parr, C. E., Sullivan, D. M., Paradiso, A. M., Lazarowski, E. R., Burch, L. H., Olsen, J. C., Erh, L., Weisman, G., Boucher, R. C., and Turner, J. T. (1994)
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9. Stuits, M. J., Chinet, T. C., Mason, S. J., Fulton, J. M., Clarke, L. L., and Leuchter, R. C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 131–142
10. Novak, I., and Greger, R. (1998) Pfluegers Arch. Eur. J. Physiol. 432, 278–285
11. Novak, I., and Hug, M. J. (1995) Cell. Physiol. Biochem. 5, 344–352
12. Pahl, C., and Novak, I. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5133–5137
13. Novak, I., and Pahl, C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5133–5137
14. Novak, I., and Hug, M. J. (1995) Cell. Physiol. Biochem. 5, 344–352
15. Ogden, D. C., and Stanfield, P. R. (1994) in Microelectrode Techniques (Ogden, D., ed) pp. 63–81, Company of Biologists Ltd., Cambridge, United Kingdom
16. Novak, I., and Greger, R. (1997) Comp. Biochem. Physiol. A Comp. Biochem. Physiol. A 122, 303–308
17. Wang, C. Z., Namba, N., Gono, T., Inagaki, N., and Seino, S. (1996) Biochem. Biophys. Res. Commun. 220, 196–202
18. Novak, I. (1998) Pfluegers Arch. Eur. J. Physiol. 436, 33–39
19. Hug, M., Pahl, C., and Novak, I. (1996) Pfluegers Arch. Eur. J. Physiol. 432, 278–285
20. Nguyen, T., Erb, L., Weisman, G. A., Marchese, A., Heng, H. H. Q., Garrad, R. C., George, S. R., Turner, J. T., and O'Dowd, B. F. (1995) J. Biol. Chem. 270, 30684–30688
21. Bogdanov, Y. D., Wildman, S. S., Clements, M. P., King, B. F., and Burnstock, G. (1998) Br. J. Pharmacol. 124, 428–430
22. Montserrat, C., Merten, M., and Figarella, C. (1996) FEBS Lett. 393, 264–268
23. Novak, I. (1998) in pH Homeostasis: Mechanism and Control (Haussinger, D., ed) pp. 447–470, Academic Press Ltd., London
24. Filippov, A. K., Webb, T. E., Barnard, E. A., and Brown, D. A. (1998) J. Neurosci. 18, 5170–5179
25. Marcus, D. C., Sunose, H., Liu, J., Shen, Z., and Sciofield, M. A. (1997) Am. J. Physiol. 273, C2022–C2029
26. Wu, W. L., So, S. C., Sun, Y. P., Chung, Y. W., Grima, J., Weng, P. Y., Yan, Y. C., and Chan, H. C. (1998) Biochem. Biophys. Res. Commun. 248, 728–732
27. Bleich, M., Schlatter, E., and Greger, R. (1990) Pfluegers Arch. Eur. J. Physiol. 411, 555–563
28. Kubokawa, M., McNicholas, C. M., Higgins, M. A., Wang, W., and Gebisch, G. (1995) Am. J. Physiol. 269, F355–F362
29. Kubokawa, M., Wang, W., McNicholas, C. M., and Gebisch, G. (1995) Am. J. Physiol. 269, F211–F219
30. McNicholas, C. M., Yang, Y., Gebisch, G., and Herbert, S. C. (1996) Am. J. Physiol. 271, F275–F285
31. Stansfeld, C. E., Riper, J., Grundig, J., Wesseloh, R. M., Marsh, S. J., Brown, D. A., and Pongs, O. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9910–9914
32. Novak, I., and Pahl, C. (1993) Pfluegers Arch. Eur. J. Physiol. 422, R71 (abstr.)
33. Surprenant, A., Rassendren, F., Kawashima, E., North, R. A., and Buell, G. (1996) Science 272, 735–738
34. Petrov, S., Ugor, M., Drummond, R. M., Singer, J. J., and Walsh, J. V. J. (1997) FEBS Lett. 411, 339–345
35. Lewis, C., Neihart, S., Holy, C., North, R. A., Buell, G., and Surprenant, A. (1995) Nature 377, 432–435
36. Garcia-Guzman, M., Soto, F., Gomez-Hernandez, J. M., Lund, P. E., and Stuhmer, W. (1997) Mol. Pharmacol. 51, 109–118
37. Soto, F., Garcia-Guzman, M., Gomez-Hernandez, J. M., Pollmann, M., Karschin, C., and Stuhmer, W. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3684–3688