P2Y receptors mediate Ca\(^{2+}\) signaling in duodenocytes and contribute to duodenal mucosal bicarbonate secretion

Xiao Dong,1*, Eric James Smoll,1* Kwang Hyun Ko,1 Jonathan Lee,1 Jimmy Yip Chow,1 Ho Dong Kim,1 Paul A. Insel,1,2 and Hui Dong1

Departments of 1Medicine and 2Pharmacology, University of California, San Diego, La Jolla, California

Submitted 30 April 2008; accepted in final form 8 December 2008

Dong X, Smoll EJ, Ko KH, Lee J, Chow JY, Kim HD, Insel PA, Dong H. P2Y receptors mediate Ca\(^{2+}\) signaling in duodenocytes and contribute to duodenal mucosal bicarbonate secretion. Am J Physiol Gastrointest Liver Physiol 296: G424–G432, 2009. First published December 12, 2008; doi:10.1152/ajpgi.90314.2008.—Since little is known about the role of P2Y receptors (purinoceptors) in duodenal mucosal bicarbonate secretion (DMBS), we sought to investigate the expression and function of these receptors in duodenal epithelium. Expression of P2Y\(_1\) receptors was detected by RT-PCR in mouse duodenal epithelium and SCBN cells, a duodenal epithelial cell line. UTP, a P2Y\(_2\)-receptor agonist, but not ADP (10 \(\mu\)M), significantly induced murine duodenal short-circuit current and DMBS in vitro; these responses were abolished by suramin (300 \(\mu\)M), a P2Y-receptor antagonist, or 2-aminoethoxydiphenyl borate (2-APB; 100 \(\mu\)M), a store-operated channel blocker. Mucosal or serosal addition of UTP induced a comparable DMBS in wild-type mice, but markedly impaired response occurred in P2Y\(_2\) knockout mice. Acid-stimulated DMBS in vivo was significantly inhibited by suramin (1 mM) or PPADS (30 \(\mu\)M). Both ATP and UTP, but not ADP (1 \(\mu\)M), raised cytoplasmic-free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{cyt}\)) with similar potencies in SCBN cells. ATP-induced [Ca\(^{2+}\)]\(_{cyt}\) was attenuated by U-73122 (10 \(\mu\)M), La\(^{3+}\) (30 \(\mu\)M), or 2-APB (10 \(\mu\)M), but was not significantly affected by nifedipine (10 \(\mu\)M). UTP (1 \(\mu\)M) induced a [Ca\(^{2+}\)]\(_{cyt}\) transient in Ca\(^{2+}\)-free solutions, and restoration of external Ca\(^{2+}\) (2 mM) raised [Ca\(^{2+}\)]\(_{cyt}\) due to capacitative Ca\(^{2+}\) entry. La\(^{3+}\) (30 \(\mu\)M), SK&F96365 (30 \(\mu\)M), and 2-APB (10 \(\mu\)M) inhibited UTP-induced Ca\(^{2+}\) entry by 92, 87, and 94%, respectively. Taken together, our results imply that activation of P2Y\(_2\) receptors enhances DMBS via elevation of [Ca\(^{2+}\)]\(_{cyt}\) that likely results from an initial increase in intracellular Ca\(^{2+}\) release followed by extracellular Ca\(^{2+}\) entry via store-operated channel.

P2Y\(_2\) receptor; cytoplasmic-free Ca\(^{2+}\); capacitative Ca\(^{2+}\) entry; store-operated channels; duodenal ion transport

PURINERGIC RECEPTORS (purinoceptors) are a family of widely expressed transmembrane receptors and are composed of two major classes based on their relative responses to adenosine and nucleotides (i.e., ATP, ADP, UTP, and UDP) (1). P1 receptors are activated by adenosine, while nucleotides activate P2 receptors. P2 receptors are subdivided into ionotropic P2X receptors, which are ligand-gated channels, and metabotropic (G protein-coupled) P2Y receptors (39). Activation of P2X receptors (P2X\(_{1,7}\) and P2XM) increases the plasma membrane permeability to Na\(^{+}\) or Ca\(^{2+}\). P2Y receptors (P2Y\(_1\), P2Y\(_2\), P2Y\(_4\), P2Y\(_6\), P2Y\(_{11}\), P2Y\(_{12}\), P2Y\(_{13}\), and P2Y\(_{14}\)) classically signal mainly through G protein-dependent pathways, most commonly via G\(_{q/11}\)-dependent pathways, activating phospholipase C (PLC) and mobilizing intracellular Ca\(^{2+}\) (1, 39). Purinergic receptors have been well characterized in many epithelial tissues, including the gastrointestinal tract, airway, and kidney (6, 10, 39).

Both ATP and UTP are known to modulate electrolyte transport in the intestinal tract, pancreatic duct, and gallbladder (14, 24, 32, 40, 55). The effects of ATP or UTP on epithelial Cl\(^{-}\), bicarbonate (HCO\(_3\)\(^{-}\)), K\(^{+}\) secretion, and Na\(^{+}\) reabsorption have been studied extensively in mouse, rat, or guinea pig tissues and in cultured human cells (Caco-2, T84), mostly by the use of Ussing chambers (24, 28, 37, 39–42, 54). It has been demonstrated that P2Y receptors play an important role in regulating Cl\(^{-}\) secretion in colon (24, 54) and HCO\(_3\)\(^{-}\) secretion in pancreatic duct and gallbladder epithelia (14, 32). However, little is known about the expression and function of purinergic receptors in duodenum, in particular, their role in duodenal mucosal bicarbonate secretion (DMBS). Recently, duodenal brush-border intestinal alkaline phosphatase activity was reported to affect HCO\(_3\)\(^{-}\) secretion in rats (2), presumably by hydrolysis of endogenous luminal ATP. This finding implies that purinergic receptors are involved in the regulation of DMBS. Moreover, an increase in cytoplasmic-free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{cyt}\)) by P2 receptors is associated with intestinal epithelial ion and fluid secretion (39, 40). An increase in [Ca\(^{2+}\)]\(_{cyt}\) in duodenal epithelial cells elicits DMBS, which is a predominant physiological mechanism to defend gastric acid-induced duodenal injury (17, 20, 62). Surprisingly, although [Ca\(^{2+}\)]\(_{cyt}\) is a critical second messenger that regulates duodenal epithelial ion transport (17, 18, 62), the regulatory mechanisms of [Ca\(^{2+}\)]\(_{cyt}\) homeostasis in duodenal epithelial cells and P2Y receptor-mediated [Ca\(^{2+}\)]\(_{cyt}\) in these cells are largely unknown.

Therefore, in the present study, we used duodenal epithelial cells, murine duodenal tissues, and intact mice to ask the following. 1) Are P2Y receptors expressed in duodenal epithelial cells, and, if so, which subtypes are expressed? 2) What mechanisms are involved in P2Y receptor-mediated [Ca\(^{2+}\)]\(_{cyt}\) homeostasis in duodenal epithelial cells? Also, 3) do P2Y receptors regulate DMBS via Ca\(^{2+}\) signaling pathways? Our findings reveal that the P2Y\(_2\)-receptor subtype is expressed in duodenal epithelial cells, and that this receptor subtype appears to play an important role in Ca\(^{2+}\)-mediated DMBS.

MATERIALS AND METHODS

Animals. This study was approved by the University of California, San Diego Animal Subjects Committee. Female adult Harlan C-57

* X. Dong and E. J. Smoll contributed equally to this work.

Address for reprint requests and other correspondence: H. Dong, MD, Division of Gastroenterology, Dept. of Medicine, 9500 Gilman Dr., La Jolla, CA 92039 (e-mail: h2dong@ucsd.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
black mice, wild-type C57BL/6, and P2Y₂ receptor knockout (backcrossed and inbred onto a C57BL/6 background) mice were housed in an animal care room with a 12:12-h light-dark cycle and were allowed free access to food and water. Our laboratory’s use of P2Y₂-receptor knockout mice has been described previously (53). Before each experiment, mice were deprived of food and water for at least 1 h, but for no longer than 90 min.

**Ussing chamber experiments.** Ussing chamber experiments were performed as described previously (19). Briefly, after mice were anesthetized with halothane and euthanized by cervical dislocation, the abdomen was opened by a midline incision. The proximal duodenum was removed and immediately placed in ice-cold isoosmolar CO₂-sensitive electrode. Mice were anesthetized by intraperitoneal injection (10 mg/kg) of the anesthetic agent midazolam (10 mg/kg), and allowed a 5-min washout period. HCO₃⁻ secretion was then recorded for an additional 60 min.

 Acid-stimulated HCO₃⁻ secretion in vivo. In vivo experiments were performed using a well-validated technique, as described previously (27, 62), with the HCO₃⁻ concentration of samples measured via a CO₂-sensitive electrode. Mice were anesthetized by intraperitoneal injection of a Hypnorm/Midazolam cocktail (25% Hypnorm plus 25% Midazolam, 10 mg/kg). Respiratory rate and response to toe-pinch of the animals were carefully monitored. After initiation of anesthesia, the abdomen was opened, and the duodenum accessed through two small incisions: one just below the ribcage on the left side, and the other just below the sternum. The stomach was located through the incision below the pyloric sphincter, and tied firmly into position with silk suture thread around the pyloric sphincter. A soft polyethylene catheter was inserted into the duodenum (5–10 mm) from the stomach. The junction of the pancreatic duct and the duodenum was located through the incision below the sternum. A small incision was made in the duodenum, and a second polyethylene catheter was inserted and tied into place just proximal to the pyloric sphincter, as described previously (19). Briefly, SCBN cells, grown on coverslips, were loaded with 5 μM fura 2-acetoxyethyl ester (AM) (dissolved in 0.01% Pluronic F-127 plus 0.1% DMSO in physiological salt solution) for 20 min. After this initial washout and recovery period, the coverslips were transferred to a temperature-controlled incubator for 10 min. SCBN, a canine duodenal epithelial cell line, was grown according to published methods (11, 12). It grows as polarized confluent monolayers and expresses Ca²⁺-dependent Cl⁻ secretion. Cells of SCBN were grown to confluence (10–14 days) in 75-cm² flasks. Cells were fed with fresh Dulbecco’s modified Eagle medium supplemented with 10% FBS, L-glutamine, and streptomycin every 2–3 days. After the cells had grown to confluence, they were replated onto 12-mm round coverslips (Warner Instruments, Hamden, CT) and incubated for at least 24 h before use.

### SCBN cell culture.** SCBN, a canine duodenal epithelial cell line, was grown according to published methods (11, 12). It grows as polarized confluent monolayers and expresses Ca²⁺-dependent Cl⁻ secretion. Cells of SCBN were grown on coverslips, which were loaded with 5 μM fura 2-acetoxyethyl ester (AM) (dissolved in 0.01% Pluronic F-127 plus 0.1% DMSO in physiological salt solution) for 20 min. After this initial washout and recovery period, the coverslips were transferred to an IBM-compatible computer for further analysis. |Ca²⁺|<sub>T</sub> was calculated from fura 2 fluorescence emission excited at 340 and 380 nm using the ratio method based on the equation |Ca²⁺|<sub>T</sub> = K<sub>D</sub> |SF<sub>T</sub>|/(R<sub>max</sub> − R<sub>min</sub>) |SF<sub>T</sub>|. The dissociation constant for Ca²⁺, R<sub>min</sub>, and R<sub>max</sub> are minimal and maximal ratios, respectively. (25). St<sub>F</sub>/St<sub>B</sub> is the fluorescence ratio for Ca²⁺-free and -bound indicators measured at 380 nm. **RT-PCR analysis.** Total RNA from SCBN cells or mouse duodenal mucosa was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. Five micrograms of total RNA were converted into cDNA with reverse transcriptase. After inactivation at 70°C for 10 min, 1 μl of the reaction mixture was incubated in buffer containing 0.2 mM dATP, dCTP, dGTP, and dTTP, 0.2 μM oligonucleotide primers, as shown below, 3 mM MgCl₂, 500 mM KCl, and a 10× buffer consisting of 200 mM Tris·HCl (pH 8.0), together with 1 unit of Taq polymerase (Invitrogen). Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Mouse P2Y₂-specific sense and antisense primers (GenBank accession number is NM_008773) were 5'-AGCCCAT-TACGTGACTGTC-3' and 5'-CTGAGGCAGAAACAGGAAGG-3', respectively. Mouse P2Y₄-specific sense and antisense primers (GenBank accession number is NM_020621) were 5'-AACAG-GAAGCTGGGGTACT-3' and 5'-GGAGGTCTTCCTAGGTCG-CAGC-3', respectively. Canine P2Y₂-specific sense and antisense primers (GenBank accession number is XM_542321) were 5'-CGTCAACCTGGCTCAAGAAG-3' and 5'-AATCCTACCTGCT-
P2Y MEDIATES Ca\(^{2+}\) SIGNALING AND DMBS

**RESULTS**

mRNA expression of P2Y receptors in SCBN cells and mouse duodenal epithelia. There are eight subtypes of P2Y receptors, but P2Y\(_2\) and P2Y\(_4\) are the major subtypes identified in intestinal epithelia (10, 39). We, therefore, attempted to identify these two subtypes of P2Y receptors in duodenal epithelial cells by using RT-PCR analysis. Figure 1 shows that transcripts for P2Y\(_2\) were readily detected in both SCBN cells and mouse duodenal epithelium. P2Y\(_4\) products were also detected in mouse duodenal epithelium at low levels. However, P2Y\(_4\) products were undetectable in SCBN cells. Therefore, P2Y\(_2\) is the main subtype of P2Y receptors expressed in duodenal epithelial cells.

**Fig. 1.** Expression of P2Y receptors in mouse duodenal epithelium and the SCBN cell line. Total RNA was isolated from mouse duodenal epithelium and SCBN cells. P2Y-receptor transcripts were detected by RT-PCR analysis. These data are representative of 3 separate experiments with identical results.

GGTGGAC-3', respectively. Canine P2Y\(_4\)-specific sense and antisense primers (GenBank accession number is XM_845543) were 5'-GTGCTACTCGCTGATGGTGA-3' and 5'-AAGCGGAGCATGAGGTagAAG-3', respectively. GAPDH sense and antisense primers, as described by Ijichi et al. (29), were 5'-ACCACAGTTCTGCATCAC-3' and 5'-TCCACACCCTCTGTGCTGTA-3', respectively. The samples were amplified in an automated thermal cycler (GeneAmp 2400; Applied Biosystems). DNA amplification conditions included an initial 3-min denaturation step at 94°C, 35 cycles of 30 s at 94°C, 30 s at 57°C, 40 s at 72°C, and a final elongation step of 10 min at 72°C. The products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide (0.5 μg/ml), and then photographed under UV light.

**Chemicals and solutions.** UTP, ATP, ADP, SK&F96365, U-73122, nifedipine, suramin, and pyridoxal-phosphate-6-azophenyl-2,4-dihydrochloride tetrasodium salt (PPADS) were purchased from Sigma. 2-Aminoethoxydiphenylborate (2-APB) was purchased from Tocris Bioscience (Ellisville, MO). Fura 2-AM was from Molecular Probes (Eugene, OR). The other chemicals were obtained from Fisher Scientific (Santa Clara, CA). The mucosal solution used in Ussing chamber experiments contained the following (in mM) and was bubbled with 100% O\(_2\): 140 Na\(^+\), 5.4 K\(^+\), 1.2 Ca\(^{2+}\), 1.2 Mg\(^2+\), 120 Cl\(^-\), 25 gluconate, and 10 mannitol. The serosal solution contained the following (in mM) and was bubbled with 95% O\(_2\) + 5% CO\(_2\): 140 Na\(^+\), 5.4 K\(^+\), 1.2 Ca\(^{2+}\), 1.2 Mg\(^2+\), 120 Cl\(^-\), 25 HCO\(_3^-\), 2.4 H\(_2\)PO\(_4^-\), 10 glucose, and 0.01 indomethacin.

The physiological salt solution used in digital Ca\(^{2+}\) measurement contained the following (in mM): 140 Na\(^+\), 5.0 K\(^+\), 2 Ca\(^{2+}\), 147 Cl\(^-\), 10 HEPES, and 10 glucose. For the Ca\(^{2+}\)-free solution, Ca\(^{2+}\) was omitted, and 0.5 mM EGTA was added to prevent possible Ca\(^{2+}\) contamination. The osmolalities for all solutions were ~284 mosmol/kgH\(_2\)O.

**Statistical analysis.** Results are expressed as means ± SE. Differences between means were considered to be statistically significant at *P* < 0.05 using Student’s *t*-test or one-way ANOVA followed by Newman-Keuls post hoc test, as appropriate.

**Fig. 2.** Effects of nucleotides on murine duodenal epithelial ion transport in vitro. A: time course of nucleotide-induced murine duodenal short-circuit current (*I\(_sc\)*). After baseline was measured for 30 min, solvent or UTP (10 μM) was added to the mucosal side of Ussing chambers, but ADP (10 μM) was added to both sides, as indicated by an arrow. **B:** net peak duodenal bicarbonate (HCO\(_3^-\)) secretion calculated from the difference between the baseline and the peak value at 10 min after addition of UTP (10 μM) or ADP (10 μM). In some experiments, the duodenal tissues were pretreated with suramin (300 μM) or 2-aminoethoxydiphenylborate (2-APB; 100 μM) for 10 min before addition of UTP. Con, control. Values are mean ± SE; *n* = 5–6 for each group. **P < 0.01 vs. control. ##P < 0.01 vs. UTP alone.

AJP-Gastrointest Liver Physiol • VOL 296 • FEBRUARY 2009 • www.ajpgi.org
P2Y-receptor antagonist (1), or 2-APB (100 μM), a cellpermeable antagonist of inositol 1,4,5-trisphosphate (IP3) receptor and store-operated channels (SOC) (9), abolished UTP-induced duodenal \( I_{ec} \) and HCO\(_3\)\(^-\) secretion. However, addition of ADP (10 μM) did not significantly induce duodenal \( I_{ec} \) or HCO\(_3\)\(^-\) secretion (Fig. 2). Thus activation of the P2Y\(_2\) receptors likely increases [Ca\(^{2+}\)]\(_{cyt}\) via SOC and stimulates duodenal Cl\(^-\) and HCO\(_3\)\(^-\) secretion.

To test the polarized function of P2Y\(_2\) receptors, we added UTP (10 μM) mucosally or serosally and then assessed UTP-stimulated HCO\(_3\)\(^-\) secretion: mucosal or serosal addition of UTP induced comparable duodenal HCO\(_3\)\(^-\) secretion (Fig. 3A). In a second set of experiments, we assessed the role of P2Y\(_2\) receptors in mediating duodenal HCO\(_3\)\(^-\) secretion using a genetic approach. As Fig. 3B illustrates, UTP-stimulated HCO\(_3\)\(^-\) secretion was markedly impaired in P2Y\(_2\) knockout mice compared with that in wild-type mice, even though carbachol-stimulated HCO\(_3\)\(^-\) secretion was similar in P2Y\(_2\) knockout and wild-type mice. The latter results indicate that the capacity to respond to another G protein-coupled receptor, the muscarinic cholinergic receptor, is unaltered in the duodenal epithelium of P2Y\(_2\) knockout animals. These data thus indicate that P2Y\(_2\) receptors in mediating duodenal HCO\(_3\)\(^-\) secretion.

**Involvement of P2Y receptors in acid-stimulated DMBS in vivo.** Our experiments conducted with Ussing chambers showed that the P2Y receptors are functionally expressed in murine duodenal epithelia and are involved in duodenal epithelial ion transport. To further investigate whether the P2Y receptors in duodenal epithelia have physiological roles, we measured acid-stimulated duodenal HCO\(_3\)\(^-\) secretion in whole animals. Figure 4A shows a time course study of HCl-stimulated murine DMBS in vivo. Duodenal luminal perfusion of HCl (10 mM) resulted in a robust increase in DMBS in control mice, which reached a maximal level at 30 min after acid stimulation and declined thereafter to the baseline level. Net peak HCO\(_3\)\(^-\) secretion, calculated from the difference between the baseline and the peak value at 30 min, was used to assess acid-stimulated HCO\(_3\)\(^-\) secretion (Fig. 4B). Luminal perfusion of suramin (1 mM) significantly attenuated acid-stimulated DMBS; net peak HCO\(_3\)\(^-\) secretion was inhibited by 67% (Fig. 4).

**Nucleotide-induced Ca\(^{2+}\) mobilization in duodenocytes.** ATP and UTP induce Ca\(^{2+}\) mobilization via activation of the P2Y receptors in many types of mammalian cells (32), but their roles in Ca\(^{2+}\) mobilization in duodenal epithelial cells have not been fully investigated. Therefore, we measured the kinetics of [Ca\(^{2+}\)]\(_{cyt}\) in SCBN cells by using a digital Ca\(^{2+}\) imaging system. ATP (1 μM) treatment in Ca\(^{2+}\)-containing solutions markedly raised [Ca\(^{2+}\)]\(_{cyt}\), which consisted of a rapid rise to a peak, followed by rapid decline to a plateau phase that was relatively sustained until ATP was withdrawn (Fig. 5A). In some instances, we observed oscillation in [Ca\(^{2+}\)]\(_{cyt}\) during ATP treatment. The ATP-induced increase in [Ca\(^{2+}\)]\(_{cyt}\) was reproducible (Fig. 5A) and inhibited by pretreatment with suramin (100 μM, data not shown). The response to UTP (1 μM) was qualitatively and quantitatively indistinguishable from that to ATP (1 μM) (Fig. 5B). However, ADP (1 μM) failed to elicit discernible changes in [Ca\(^{2+}\)]\(_{cyt}\), although higher concentration of ADP (10 μM) induced a slight increase in [Ca\(^{2+}\)]\(_{cyt}\) (Fig. 5C). We found that 1 μM UTP or ATP induced similar increases in [Ca\(^{2+}\)]\(_{cyt}\) measured at 1 min after superfusion of nucleotides in Ca\(^{2+}\)-containing solutions, but re-
response to ADP was much smaller (Fig. 5D). These data are consistent with our RT-PCR and duodenal epithelial ion transport data described above, indicating that P2Y2 receptor is a major subtype of P2Y receptors functionally expressed in duodenal epithelial cells.

ATP-induced intracellular Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} entry via SOC. Activation of the G protein-coupled receptors in many types of mammalian epithelial cells can induce Ca\textsuperscript{2+} release from intracellular stores, which, in turn, promotes opening of SOC in the plasma membrane that leads to further Ca\textsuperscript{2+} entry (47, 48, 50). However, the regulatory mechanisms of [Ca\textsuperscript{2+}]\textsubscript{cyt} in duodenal epithelial cells are largely unknown. Therefore, we tested whether ATP induced an intracellular Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} entry via SOC in duodenal epithelial cells. We found that ATP (1 μM) in Ca\textsuperscript{2+}-containing solutions reproducibly increased [Ca\textsuperscript{2+}]\textsubscript{cyt} (Fig. 6A). Nifedipine (10 μM), a voltage-
 gated Ca\(^{2+}\) channel (VGCC) blocker, did not significantly affect ATP-induced [Ca\(^{2+}\)]\(_{\text{cyt}}\) (Fig. 6B). However, U-73122 (10 \(\mu M\)), a PLC inhibitor, partially inhibited ATP-induced [Ca\(^{2+}\)]\(_{\text{cyt}}\) (Fig. 6C). La\(^{3+}\) (30 \(\mu M\)) and 2-APB (10 \(\mu M\)), two SOC blockers (9, 65), abolished ATP-induced [Ca\(^{2+}\)]\(_{\text{cyt}}\) (Fig. 6, D and E). Figure 6F summarizes the effects of ATP on [Ca\(^{2+}\)]\(_{\text{cyt}}\) measured at 1 min after the cells were exposed to ATP (1 \(\mu M\)) in the absence or the presence of different inhibitors in Ca\(^{2+}\)-containing solutions. In the case of Ca\(^{2+}\)-free solutions, although ATP (1 \(\mu M\)) also increased [Ca\(^{2+}\)]\(_{\text{cyt}}\), the response was transient and followed by rapid decline to the baseline level before withdrawal of ATP (n = 50 cells, data for ATP not shown here, but similar data for UTP are shown in Fig. 7). Unlike in Ca\(^{2+}\)-containing solutions, ATP could not reproducibly induced Ca\(^{2+}\) signaling events in Ca\(^{2+}\)-free solutions. These results suggest that stimulation of P2Y receptors in duodenal epithelial cells induces Ca\(^{2+}\) release from the intracellular stores and extracellular Ca\(^{2+}\) entry, likely via SOC rather than VGCC.

**Fig. 7.** UTP-induced capacitative Ca\(^{2+}\) entry (CCE) via SOCs in SCBN cells. In the absence of extracellular Ca\(^{2+}\) and presence of nifedipine (10 \(\mu M\)), extracellular application of UTP (1 \(\mu M\)) induced a transient increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\). A: approximately 5 min later, when the [Ca\(^{2+}\)]\(_{\text{cyt}}\) transients declined back to the basal level, restoration of 2 mM external Ca\(^{2+}\) induced a large increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) due to Ca\(^{2+}\) entry. Pretreatment of cells with 10 \(\mu M\) 2-APB (B), 30 \(\mu M\) La\(^{3+}\) (C), or 30 \(\mu M\) SK&F96365 (D) abolished CCE via SOCs. Effects of the compounds on UTP-induced intracellular Ca\(^{2+}\) release (E) and their effects on CCE (F) are summarized. Values are mean ± SE; n = 50 cells for each tracing. **\(P < 0.01\) vs. control.

Therefore, CCE through SOC is considered an important mechanism to control [Ca\(^{2+}\)]\(_{\text{cyt}}\) homeostasis and regulation of Ca\(^{2+}\)-dependent biological processes in epithelial cells (7, 21, 48). We thus examined whether SOC is functionally expressed and whether CCE is involved in P2Y-mediated [Ca\(^{2+}\)]\(_{\text{cyt}}\) in duodenal epithelial cells by assessing response to UTP.

UTP (1 \(\mu M\)) induced a marked [Ca\(^{2+}\)]\(_{\text{cyt}}\) transient in Ca\(^{2+}\)-free solutions (Fig. 7A). Restoration of external Ca\(^{2+}\) (2 mM) −5 min after [Ca\(^{2+}\)]\(_{\text{cyt}}\) transients declined back to the basal level (i.e., when the store was depleted) increased [Ca\(^{2+}\)]\(_{\text{cyt}}\) due to Ca\(^{2+}\) entry (Fig. 7A). Nifedipine (10 \(\mu M\)) was applied throughout the experiments to prevent the involvement of VGCC. Pretreatment of cells with 2-APB (10 \(\mu M\)), a cell-permeable antagonist of IP\(_3\) receptor and SOC blocker, partially inhibited UTP-induced intracellular Ca\(^{2+}\) release and abolished CCE through SOC (Fig. 7B). La\(^{3+}\) (30 \(\mu M\)), a nonselective SOC blocker that does not alter UTP-induced intracellular Ca\(^{2+}\) release, abolished UTP-induced CCE (Fig. 7C). Similarly, SK&F96365 (30 \(\mu M\)), a selective SOC blocker, abolished UTP-induced CCE in duodenocytes (Fig. 7D). Figure 7E summarizes the effects of La\(^{3+}\), SK&F96365, and 2-APB on UTP-induced intracellular Ca\(^{2+}\) release, which was significantly inhibited only by 2-APB (59%). However, La\(^{3+}\), SK&F96365, and 2-APB significantly inhibited CCE by 92, 87, and 94%, respectively (Fig. 7F). Thus SOC likely plays an
important role in P2Y2-receptor-mediated \([Ca^{2+}]_{\text{cyt}}\) homeostasis in duodenal epithelial cells.

**DISCUSSION**

Epithelial cells can release nucleotides (such as ATP/UTP) in response to physiological stimuli, including changes in cell volume, membrane stress, and receptor stimulation (8, 33, 36, 38, 43, 55). In the intestinal tract, stimuli, such as ingestion of food and water, can readily evoke ATP/UTP release from epithelial cells (23, 55). Purinergic receptors are expressed in epithelial cells and induce a variety of biological effects (1). Three rodent P2Y receptors are sensitive to UTP: P2Y2, P2Y4, and P2Y6 (52); however, P2Y2 and P2Y4, but not P2Y6, are sensitive to suramin (1, 52). P2Y2 and P2Y4 receptors can couple to \(G_{q/11}\) proteins and activate PLC-\(\beta\), resulting in production of IP3 and mobilization of intracellular Ca\(^{2+}\). P2Y2 receptors, a prominent subtype of luminal P2Y receptors in many epithelia, respond to agonists with the order of potency UTP = ATP > ADP and are inhibited by suramin and PPADS (1, 6, 10).

Purinergic signaling pathways contribute to the regulation of ion transport in intestinal, biliary, and pancreatic duct epithelium (14, 24, 32, 39, 40). However, little is known regarding the expression and function of the P2Y receptors in duodenal epithelium; only one report has implied that endogenous luminal expression and function of the P2Y receptors in duodenal epithelium (14, 24, 32, 39, 40). However, little is known regarding (1, 6, 10).

Epithelial cells can release nucleotides (such as ATP/UTP) in response to physiological stimuli, including changes in cell volume, membrane stress, and receptor stimulation (8, 33, 36, 38, 43, 55). In the intestinal tract, stimuli, such as ingestion of food and water, can readily evoke ATP/UTP release from epithelial cells (23, 55). Purinergic receptors are expressed in epithelial cells and induce a variety of biological effects (1). Three rodent P2Y receptors are sensitive to UTP: P2Y2, P2Y4, and P2Y6 (52); however, P2Y2 and P2Y4, but not P2Y6, are sensitive to suramin (1, 52). P2Y2 and P2Y4 receptors can couple to \(G_{q/11}\) proteins and activate PLC-\(\beta\), resulting in production of IP3 and mobilization of intracellular Ca\(^{2+}\). P2Y2 receptors, a prominent subtype of luminal P2Y receptors in many epithelia, respond to agonists with the order of potency UTP = ATP > ADP and are inhibited by suramin and PPADS (1, 6, 10).

Purinergic signaling pathways contribute to the regulation of ion transport in intestinal, biliary, and pancreatic duct epithelium (14, 24, 32, 39, 40). However, little is known regarding the expression and function of the P2Y receptors in duodenal epithelium; only one report has implied that endogenous luminal expression and function of the P2Y receptors in duodenal epithelium (14, 24, 32, 39, 40). However, little is known regarding (1, 6, 10).
mediate the signaling and permeation mechanisms of SOC (57, 68). Stim1, an ER Ca2+-sensor, and Orai are plasma membrane proteins that constitute pore-forming subunits of SOC. Depletion of ER Ca2+-store induced by activation of plasma membrane receptors results in Stim1 without Ca2+-bound, which causes Stim1 to redistribute from the ER to plasma membrane regions near Orai. Stim1 then activates Ca2+-selective Orai channels (51, 57, 68).

Our findings strongly suggest that [Ca2+]cyt is important in the signaling events in response to activation of the membrane P2Y receptors of duodenocytes and plays a critical role in regulating duodenal ion transport and DMBS in particular. How does activation of the P2Y receptors stimulate DMBS via Ca2+ signaling? An increase in [Ca2+]cyt can activate apical CFTR channels and Cl-/HCO3- exchange in human pancreatic duct cells (44) and murine duodenal epithelium (61, 62) and inhibit the ileal brush-border Na+/H+ exchanger (15, 16) and [Ca2+]cyt, can also increase basolateral Na+/HCO3- co-transport activity in murine colonic crypts (5) and activate basolateral Ca2+-activated K+ channels in murine duodenal epithelium to provide a driving force for HCO3- secretion (19). All of these actions of [Ca2+]cyt may contribute to the molecular mechanisms underlying P2Y2-Ca2+-mediated DMBS observed in the present study. However, further investigation is required to reveal which mechanism plays a major role in P2Y2-Ca2+-mediated DMBS.

In conclusion, the present data show that activation of the P2Y2 receptors in duodenal epithelium increases [Ca2+]cyt without Ca2+- release and extracellular Ca2+ entry via SOCE (47, 48), thereby stimulating duodenal epithelial Cl- and HCO3- secretion. Luminal acid or swelling can induce ATP release, and released ATP can function as an autocrine/paracrine regulator of human intestinal epithelial cells (26, 63). Although defining the precise role of purinergic pathways in regulating intestinal epithelial ion transport will require further investigation, the present findings imply that studies of P2Y, in particular P2Y2 receptor, pathways are a potentially promising target for the treatment of pathological conditions in the intestinal tract, including perhaps protection from acid-induced duodenal injury (3, 4, 31). Increased understanding of the cellular and molecular mechanisms of Ca2+-mediated DMBS via activation of P2Y (e.g., P2Y2) receptors thus has physiological and potential clinical significance.

GRANTS
This work was supported by American Heart Association Beginning Grant-in-Aid Award (0556025Y) and Cystic Fibrosis Foundation (DONG0610) to H. Dong, and by the University of California San Diego Digestive Diseases Research Development Center Grant (DK-080506), in which H. Dong serves as the director of cell imaging core. It was also partially supported by grants from the National Institutes of Health (NIH) (DK073090) and the University of California San Diego Academic Senate Research Grant (RH154H) to J.-Y. Chow and a NIH Grant (GM 66232) to P. A. Insel.

REFERENCES


P2Y mediates Ca\(^{2+}\) signaling and DMBS