The Lipid Products of Phosphoinositide 3-Kinase Contribute to Regulation of Cholangiocyte ATP and Chloride Transport*

Andrew P. Feranchak‡, Richard M. Roman‡, R. Brian Doctor‡, Kelli D. Salter‡, Alex Toker*, and J. Gregory Fitz‡

From the ‡Departments of Pediatrics and Medicine, Children’s Hospital and the University of Colorado Health Sciences Center, Denver, Colorado 80262 and the *Signal Transduction Group, Boston Biomedical Research Group, Boston, Massachusetts 02114.

ATP stimulates Cl⁻ secretion and bile formation by activation of purinergic receptors in the apical membrane of cholangiocytes. The purpose of these studies was to determine the cellular origin of biliary ATP and to assess the regulatory pathways involved in its release. In Mz-Cha-1 human cholangiocarcinoma cells, increases in cell volume were followed by increases in phosphoinositide (PI) 3-kinase activity, ATP release, and membrane Cl⁻ permeability. PI 3-kinase signaling appears to play a regulatory role because ATP release was inhibited by wortmannin or LY294002 and because volume-sensitive current activation was inhibited by intracellular dialysis with antibodies to the 110 kDa-subunit of PI 3-kinase. Similarly, in intact normal rat cholangiocytes, increases in cell volume stimulated luminal Cl⁻ secretion through a wortmannin-sensitive pathway. To assess the role of PI 3-kinase more directly, cells were dialedyzed with the synthetic lipid products of PI 3-kinase. Intracellular delivery of phosphatidylinositol 3,4-bisphosphate, and phosphatidylinositol 3,4,5-trisphosphate activated Cl⁻ currents analogous to those observed following cell swelling. Taken together, these findings indicate that volume-sensitive activation of PI 3-kinase and the generation of lipid messengers modulate cholangiocyte ATP release, Cl⁻ secretion, and, hence, bile formation.

Intrahepatic biliary epithelial cells, or cholangiocytes, have a major influence on the volume and composition of bile through absorption and secretion of fluid and electrolytes. Opening of chloride channels in the apical membrane has been identified as one important point for regulation of cholangiocyte secretion and involves the cystic fibrosis transmembrane conductance regulator (CFTR), the protein product of the CF gene. Mutations in this gene inhibit channel function and result in cholestatic liver disease in 17–38% of patients (1–4). It is unclear why the prevalence of biliary disease in CF is less than that of pancreatic or pulmonary disease. One potential explanation for these clinical differences is that CFTR-independent pathways may also regulate biliary secretion.

Recently, Cl⁻ channels other than CFTR have been identified in cholangiocytes, and they appear to contribute importantly to modulation of cellular transport to meet changing physiologic demands. In isolated cells, Cl⁻ currents activated by increases in cell volume are 2–3-fold greater than those activated by increases in cAMP (5–8). The resulting solute efflux favors movement of water out of the cell and recovery of volume toward basal values, a general process referred to as regulatory volume decrease (9). The cellular mechanisms mediating channel opening and volume regulation in biliary cells have not been identified. However, cell volume increases appear to stimulate ATP release, and either removal of extracellular ATP or blockade of P2 receptors prevents cell volume recovery (10). Consequently, ATP release may serve as a signal activating Cl⁻ channel opening by binding to apical P2 receptors.

In other cell types, cell volume increases stimulate parallel activation of multiple kinases (11) including phosphoinositide (PI) 3-kinase (12). PI 3-kinase is a heterodimer composed of a 110-kDa catalytic unit and an 85-kDa regulatory unit that are tightly associated (13, 14). Upon activation, PI 3-kinase phosphorylates phosphatidylinositol and is capable of producing three lipid products: phosphatidylinositol 3-phosphate (PtdIns-3-P), phosphatidylinositol 3,4-bisphosphate (PtdIns-3,4-P₂), and phosphatidylinositol 3,4,5-trisphosphate (PtdIns-3,4,5-P₃). In liver cells, physiologic increases in cell volume have been shown to be a potent stimulus for PI 3-kinase activation (12), and PI 3-kinase has been shown to play a role in vesicle trafficking and bile formation in the isolated perfused rat liver (15). Based on the observations that cell volume and extracellular ATP are potent stimulators of cholangiocyte secretion, the purpose of these studies was to assess the cellular origin of biliary ATP and the potential role of PI 3-kinase as a signal modulating cholangiocyte Cl⁻ secretion.

EXPERIMENTAL PROCEDURES

Cell Culture—Studies in isolated cells were performed in Mz-Cha-1 cells, originally isolated from human adenocarcinoma of the gallbladder (16). Studies in polarized monolayers were performed utilizing normal rat cholangiocytes (NRC) in culture (17). Each model system expresses phenotypic features of differentiated biliary epithelium including receptors, signaling pathways, and ion channels similar to those found in primary cells (6, 18, 19). Moreover, increases in Mz-Cha-1 (8) and NRC (data not shown) cell volume are followed by opening of membrane K⁺ and Cl⁻ channels. Mz-Cha-1 cells were passaged at weekly intervals and maintained in culture at 37 °C in a 5% CO₂ incubator in HCO₃⁻-containing CMRL-1066 media (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin (8). NRC cells were cultured as described previously (17).
Cell Size Measurements—Mean cell volume was measured in Mz-Cha-1 cell suspensions by electronic cell sizing (Coulter Multisizer, Accucomp software version 1.19, Hialeah, FL) using an aperture of 100 μm. Cells in subconfluent culture were harvested with 0.05% trypsin, suspended in cell culture media, centrifuged for 1 min at \(\sim 1,000 \times g\), resuspended in 5 ml of isotonic buffer, and incubated with constant agitation for 30–45 min. Aliquots (2 μl for control, 20 μl for hypotonic exposure) were measured using whole cell patch clamp techniques (20, 21) in Mz-Cha-1 cells. Cells on a coverslip were mounted in a chamber (volume, \(~400 \mu l\)) and perfused at 4–5 ml/min with a standard extracellular solution containing 140 mM NaCl, 4 mM KCl, 1 mM CaCl\(_2\), 2 mM MgCl\(_2\), 1 mM KH\(_2\)PO\(_4\), 10 mM glucose, and 10 mM HEPES/NaOH (pH 7.40). The standard intracellular (pipette) solution for whole cell recording contained 130 mM KCl, 4 mM CaCl\(_2\), 2 mM MgCl\(_2\), 1 mM KH\(_2\)PO\(_4\), and 1 mM EGTA (pH 7.32), corresponding to a free \(\left[\text{Ca}^{2+}\right]_0\) of \(~100 \mu M\) (22). Patch pipettes were pulled from Corning 7052 glass and had a resistance of 3–7 MΩ. Recordings were made with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) and were digitized (3 kHz) on a computer and analyzed using pCLAMP version 6.0 programs (Axon Instruments, Burlingame, CA) as described previously (21, 23). Pipette voltages \((V_p)\) are referred to the bath. Current-voltage relationships were measured between \(\sim 120 \text{ mV}\) and \(+100 \text{ mV}\) in 20-mV increments (400-ms duration, 2 s between test potentials). In the whole cell configuration, \(V_m\) corresponds to the membrane potential, and upward deflections of the current trace indicate outward membrane current. Changes in membrane \(K^+\) permeability were assessed at a test potential of \(\sim 80 \text{ mV} (E_K)\) to minimize any contribution of \(K^+\) currents and values were reported as current density \((\text{pA/\mu m}^2)\) to normalize for differences in cell size as recently described (24).

Transepithelial \(V_{oc}\) Transport Measurements—NRC cells were utilized to study vectorial \(Cl^-\) movement across monolayers. Cells were grown to confluence on collagen-treated polycarbonate filters with a pore size of 0.4 μm (Costar, Cambridge, MA) until a resistance of \(>1,000 \Omega \text{ cm}^2\) was achieved as measured by an epithelial tissue voltmeter (EVOHM; World Precision Instruments, Sarasota, FL). Cells were mounted in a Trans-24 mini-perfusion system for tissue culture (24). A-Epipodocalyxin \(\alpha\)-Phosphatidyl-D-myo-inositol-4,5-bisphosphate (PIP-2, Calbiochem) was utilized as a control lipid. ATP and other reagents were synthesized by previously described methods (32) and were delivered to the cell interior by inclusion in the patch pipette. The lipid products of PI 3-kinase (PtdIns-3-P, PtdIns-3,4-P\(_2\), and PtdIns-3,4,5-P\(_3\)) were synthesized by previously described methods (32) and were analyzed by Western blotting for Akt (protein kinase B), a downstream target of PI 3-kinase, and phosphorylated Akt was detected by a phospho-Akt antibody (Thr308 antibody, 1:1,000; New England Biolabs). Samples were analyzed by Western blotting for Akt (protein kinase B), to minimize any potential effects of cell culture plates or repetitions for other assays. Student's paired or unpaired \(t\) test was used to assess statistical significance as indicated, and \(p\) values < 0.05 were considered to be statistically significant. 

RESULTS

Inhibition of PI 3-Kinase Delays Cholangiocyte Volume Recovery from Swelling—Exposure of cholangiocyte cell suspensions to hypotonic buffer (40% decrease in NaCl) resulted in an initial in 3 min prior to hypotonic exposure. In separate patch clamp studies, PI 3-kinase was inhibited by intracellular dialisys with a purified rabbit polyclonal antibody recognizing a sequence corresponding to residues 1054–1068 of the 110-kDa \(\alpha\)-catenin subunit of PI 3-kinase (Upstate Biotechnology, Inc., Lake Placid, NY) (31). Heat-inactivated p110 PI 3-kinase antibodies (30% control and 70% polyclonal rabbit antibodies to \(\beta\)-galactosidase) (5 Prime – Prime Inc., Boulder, CO) were utilized as controls. The lipid products of PI 3-kinase (PtdIns-3-P, PtdIns-3,4-P\(_3\), and PtdIns-3,4,5-P\(_3\)) were synthesized by previously described methods (32) and were delivered to the cell interior by inclusion in the patch pipette. t-n-Phosphatidyl-t-o-myo-inositol-4,5-bisphosphate (PIP-2, Calbiochem) was utilized as a control lipid. ATP and other reagents were obtained from Sigma.

Statistics—Results are presented as the means ± S.E., with \(n\) representing the number of cells for patch clamp studies and the number of culture plates or repetitions for other assays. Student’s paired or unpaired \(t\) test was used to assess statistical significance as indicated, and \(p\) values < 0.05 were considered to be statistically significant.

Inhibition of PI 3-Kinase Delays Cholangiocyte Volume Recovery from Swelling—Exposure of cells to hypotonic buffer (40% decrease in NaCl, \(~205 \mu M\)) caused a rapid initial increase in relative volume to 1.20 \(\pm 0.01 (n = 5, \ p < 0.001)\) within 3 min. The increase was followed by a gradual recovery toward basal values despite the continued exposure to hypotonic buffer (Fig. 1). To evaluate whether PI 3-kinase contributes to cell volume recovery, analogous studies were performed in the presence of wortmannin. Hypotonic exposure after preincubation with wortmannin (50 nM) resulted in a similar initial increase to 1.18 \(± 0.01\) at 3 min. However, volume recovery was significantly delayed. The relative volume of 1.15 \(± 0.01\) at 20 min and 1.14 \(± 0.01\) at 30 min in the presence of wortman-
Inhibited volume-sensitive currents.

In Mz-Cha-1 cells, cell volume recovery from swelling was small (2.02 ± 0.2 pA/pF) and tended to return toward basal values over 10 min despite the continued presence of hypotonic buffer. In the presence of wortmannin (50 nM), the response to hypotonic exposure was inhibited with a maximum Cl− current density of −6.1 ± 2.5 pA/pF (n = 7, p < 0.001; Fig. 2A and C). Similar results were obtained with LY294002 (10 μM), a structurally unrelated PI 3-kinase inhibitor (−9.5 ± 4.8 pA/pF, n = 5, p < 0.001; Fig. 2C).

Fig. 2. Inhibition of PI 3-kinase prevents volume-dependent Cl− currents. Whole cell currents were measured under basal conditions and during increases in cell volume stimulated by hypotonic exposure (20% decrease in NaCl). Wortmannin (50 nM) and LY294002 (10 μM) were utilized as inhibitors of PI 3-kinase. A, representative whole cell recording. Currents at −80 mV (downward deflection of the current tracing) correspond to IC−. In control cells (upper tracing), hypotonic exposure stimulated a reversible increase in currents. Incubation with wortmannin (lower tracing) partially inhibited current activation by hypotonic exposure. B, the average current-voltage relation of whole cell currents. Under isotonic conditions, little current activity is observed. Hypotonic exposure results in a large increase in currents, characterized by outward rectification and reversal near 0 mV (○), and the activation is inhibited by wortmannin (▼). C, average currents at −80 mV were measured under basal conditions and during hypotonic exposure. When compared with control cells, both wortmannin (n = 7, p < 0.001) and LY294002 (n = 5, p < 0.001) inhibited volume-sensitive currents.
rapid activation of PI 3-kinase following hypotonic exposure is correlated with Akt phosphorylation. Additionally, the increase in cholangiocyte cell volume activates PI 3-kinase as observed in all cell types studied (32–34). To determine whether increases in cholangiocyte cell volume activate PI 3-kinase as observed in other cell types (12), an assay measuring Akt phosphorylation was utilized. In Mz-Cha-1 cells, hypotonic exposure (40% decrease in NaCl) increased Akt Thr308 phosphorylation 20–50% by 6–12 min in all trials (n = 4). There was no increase in total Akt or actin levels (Fig. 4). This study demonstrates that increases in cholangiocyte cell volume activate PI 3-kinase activity as measured by Akt phosphorylation. Additionally, the rapid activation of PI 3-kinase following hypotonic exposure is consistent with an early role in the response to cell volume increases.

Intracellular Dialysis with a Specific Antibody to PI 3-Kinase Inhibits Volume-activated Cl⁻ Currents—To determine whether the inhibitory effects of wortmannin and LY294002 on volume-activated Cl⁻ channel activity represents a specific effect on PI 3-kinase, an alternative strategy utilizing intracellular dialysis with specific antibodies to the 110-kDa catalytic subunit of PI 3-kinase was evaluated. These antibodies have been shown to inhibit growth factor-stimulated PI 3-kinase activity in cultured fibroblasts (31). For these studies, the antibodies were delivered to the cell interior by inclusion in the patch pipette. Intracellular dialysis with anti-PI 3-kinase antibody (5 μg/mL) completely inhibited Cl⁻ currents in response to hypotonic exposure with a maximal average current density of -2.9 ± 0.4 pA/pF (n = 5). In contrast, currents measured during intracellular dialysis with either heat-inactivated antibodies (5 μg/mL) or antibodies to unrelated proteins (β-galactosidase, 5 μg/mL) were similar to controls (-36.7 ± 5.9 pA/pF and -39.4 ± 12.2 pA/pF respectively, n = 5 for each, p < 0.005, Fig. 5). These findings support a specific role of PI 3-kinase in volume-sensitive Cl⁻ channel activation.

Intracellular Dialysis with the Lipid Products of PI 3-Kinase Activates Cl⁻ Currents—PI 3-kinase phosphorylates the D3 position of the inositol ring forming three lipid products: PtdIns-3-P, PtdIns-3,4-P₂, and PtdIns-3,4,5-P₃. PtdIns-3-P is constitutively present in unstimulated cells and changes little upon stimulation. In contrast, PtdIns-3,4-P₂ and PtdIns-3,4,5-P₃ are undetectable under resting conditions but increase rapidly upon stimulation, suggesting that they function as secondary messengers, translating extracellular stimuli to cellular responses (35–37). The development of synthetic lipid products of PI 3-kinase has made the direct study of the PI 3-kinase-dependent cellular processes including membrane ruffling and chemotaxis (38) and cell survival and gluconeogenesis through Akt regulation (32, 39).

To determine whether the lipid products of PI 3-kinase lead to Cl⁻ channel opening, the synthetic lipids PtdIns-3-P (10 μM), PtdIns-3,4-P₂ (10 μM) and PtdIns-3,4,5-P₃ (10 μM) were individually delivered to the cell interior during isotonic conditions. Whole cell currents (measured at -80 mV) remained small in control cells (-1.6 ± 0.2 pA/pF, n = 22) and cells dialyzed with either inactive lipid PIP-2 (10 μM, -1.4 ± 0.3 pA/pF, n = 10) or PtdIns-3-P (-2.8 ± 1.5 pA/pF, n = 5). In contrast, spontaneous activation of currents was observed during intracellular dialysis with PtdIns-3,4-P₂ (-11.7 ± 4.8 pA/pF, n = 8, p < 0.05 (compared with PIP-2)) and PtdIns-3,4,5-P₃ (-7.9 ± 2.5, n = 6, p < 0.01; Fig. 6A). Intracellular dialysis with both PtdIns-3,4-P₂ and PtdIns-3,4,5-P₃ together (10 μM each) also resulted in spontaneous current activation, although the magnitude of

**FIG. 3. Inhibition of PI 3-kinase decreases transepithelial Cl⁻ secretion.** Short circuit current (Iₛ) across NRC monolayers was measured under voltage-clamp conditions in an Ussing chamber. A, in this representative recording, simultaneous perfusion of the apical and basolateral chambers with hypotonic buffer (30% decrease in NaCl, as represented by the bar) resulted in an increase in the Iₛ (upper tracing). In the presence of wortmannin (50 nM) the Iₛ response to hypotonic exposure was attenuated (lower tracing). B, transepithelial Cl⁻ secretion in response to hypotonic exposure. The y axis values are reported as ΔIₛ (maximum Iₛ − basal Iₛ). Exposure to wortmannin (50 nM) attenuates the swelling-activated ΔIₛ in response to hypotonic exposure (n = 4, p < 0.05).

**FIG. 4. Increases in cholangiocyte cell volume result in Akt phosphorylation.** Under isotonic conditions, Western blotting of Mz-Cha-1 cells with Thr308 antibody (T308), specific against the phospho-tylated form of Akt, readily detected phosphorylated Akt. Hypotonic exposure (40% decrease in NaCl) resulted in increased Akt phosphorylation after 9 min of exposure. Constant actin and total Akt levels reflect equivalent sample loading. The increase in Akt phosphorylation is indicative of PI 3-kinase activation.
the resulting current was not statistically greater than the individual lipids alone (−16.2 ± 1.7 pA/pF, n = 5, p < 0.001 (compared with PIP-2); Fig. 6, A and C). These currents demonstrated characteristics of volume-stimulated Cl− currents, with reversal near 0 mV (E_{rev}), outward rectification, and time-dependent inactivation at depolarizing potentials above +60 mV (Fig. 6, B and D). Moreover, the current response generated by intracellular dialysis with PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 was inhibited in the presence of extracellular apyrase to remove any ATP released from cells (−2.6 ± 0.1 pA/pF, n = 4, p < 0.05, Fig. 6, A and B). These results are consistent with a model where the lipid products of PI 3-kinase contribute to direct modulation of membrane ATP permeability.

PI 3-Kinase Modulates Swelling-activated ATP Release—In other cell types, increases in cell volume result in ATP release, stimulation of purinergic (P2) receptors, and Cl− channel activation (40). In cholangiocytes, activation of P2 receptors by extracellular ATP represents a potent stimulus for Cl− secretion (19). Thus, PI 3-kinase could potentially modulate current activation through stimulation of ATP release, modulation of P2 receptors, or coupling receptor binding to channel opening. To assess the site of action of PI 3-kinase, two strategies were utilized. First, the effect of wortmannin on the current response to exogenous ATP was assessed. For these studies, Mz-Cha-1 cells in hypotonic buffer were exposed to exogenous ATP in the presence of wortmannin (50 nM). If PI 3-kinase modulates P2 receptors or couples receptor binding to Cl− channel opening, wortmannin would be expected to inhibit ATP-dependent current activation. In the presence of wortmannin, hypotonic exposure failed to activate Cl− currents. However, subsequent addition of ATP (10 μM) to the perfusate resulted in instantaneous activation of Cl− currents (representative trace, Fig. 7A), increasing current density from −6.1 ± 2.5 pA/pF to −25.5 ± 7.3 pA/pF (n = 5, p < 0.05; Fig. 7B). These findings indicate that PI 3-kinase is likely to function more proximally in the signaling pathway by modulating local ATP concentrations outside of the cell.

To assess this possibility more directly, the effects of PI 3-kinase inhibition on ATP efflux were evaluated utilizing a luciferin-luciferase assay. NRC cells in isotonic buffer demonstrated a basal release of ATP (0.53 ± 0.08 ALU). Exposure to wortmannin or LY294002 decreased basal ATP release (wortmannin 0.21 ± 0.02 ALU, LY294002 0.20 ± 0.07 ALU, n = 6 for each, p < 0.001; Fig. 6). In control cells, exposure to hypotonic perfusate stimulated ATP efflux (an increase of 0.73 ± 0.07 ALU, at 20% dilution, and an increase of 0.47 ± 0.06 at 40% dilution), as shown in Fig. 8. The increases in swelling-induced ATP release were inhibited by both wortmannin (0.19 ± 0.01 ALU at 20% and 0.19 ± 0.01 ALU at 40%) and LY294002 (0.12 ± 0.01 ALU at 20% and 0.17 ± 0.02 ALU at 40%, n = 6 for each, p < 0.001; Fig. 8). In all studies, the addition of apyrase (2 units/ml) eliminated bioluminescence consistent with ATP scavenging (data not shown). These findings indicate that PI 3-kinase activity contributes to regulation of both basal and swelling-activated ATP release.

**DISCUSSION**

ATP exerts potent regulatory effects on many epithelial cells by binding to one or more purinergic receptors in the plasma membrane. In most epithelia, however, little is known regarding the mechanisms responsible for modulation of nucleotide release. These studies of a model Cl− secretory cell indicate that volume-sensitive changes in PI 3-kinase activity contribute to changes in cellular ATP efflux rates and suggest that the D3 products of phosphoinositol phosphorylation exert the principal biological effects. Thus, PI 3-kinase may contribute to rapid coordination of cellular ATP release and membrane ion permeability and to modulation of biliary secretion and bile flow in response to changing physiologic demands.

The formation of bile by the liver depends upon complementary interactions between hepatocytes, which transport bile acids and other organic solutes into the canalicular space and cholangiocytes, which line the lumen of intrahepatic bile ducts and are responsible for electrolyte and water transport. Extracellular ATP has been proposed to function as a signal coordinating the separate transport functions of hepatocytes and cholangiocytes because (a) ATP is present in bile where it has direct access to the apical membrane of cholangiocytes (41) and (b) nanomolar concentrations of ATP stimulate cholangiocyte secretion by binding to P2Y2 receptors in the apical membrane (19, 42). Both hepatocytes and cholangiocytes may be capable of electrodiffusional release of ATP (41, 43). However, the polarity of release and the signals responsible for its regulation have not been defined. With these issues in mind, several points merit emphasis.

First, these studies provide additional evidence that cholangiocytes themselves are capable of regulated release of ATP. Moreover, significant amounts of extracellular ATP are detectable under basal conditions (constitutive release), and the
amount increases in parallel with changes in cell volume (volume-sensitive release). ATP was always detectable in the media from cultures of both biliary cell models. In NRC monolayers, which form high resistance junctions between cells, the luciferin reaction mixture was added to the apical chamber only. Because luminometric readings reflect movement of ATP across the apical membrane, these findings indicate that increases in cell volume represent a potent stimulus for apical ATP release into bile.

Second, experimental results from several model systems indicate that apical ATP permeability is regulated in part by changes in endogenous PI 3-kinase activity. Utilizing a specific assay, which measures the phosphorylation of the PI 3-kinase effector Akt, we demonstrated that cell volume increases are associated with an increase in PI 3-kinase activity. Additionally, both constitutive and volume-sensitive ATP release was inhibited by the PI 3-kinase inhibitors wortmannin and LY294002. These findings suggest that PI 3-kinase modulates ATP release, but other sites of action are possible as well. It is notable that these inhibitors did not inhibit Cl channel opening stimulated by exogenous ATP. Although the addition of ATP overcomes the inhibition of the volume-stimulated current activation by wortmannin, the magnitude of the current is less than that observed with hypotonic challenge. Several explana-
The D3-phosphorylated products of phosphatidylinositol are thought to contribute importantly to the PI 3-kinase signaling pathway, coupling external stimuli to cellular metabolism. To assess their role in coupling cell volume changes to membrane transport, additional studies were performed using intracellular dialysis with the synthesized lipids PtdIns-3-P, PtdIns-3,4-P_2, and PtdIns-3,4,5-P_3. Intracellular delivery of these lipids through the patch pipette permits careful comparison among cells under conditions where key components of the membrane regulatory apparatus (i.e., receptors, cytoskeleton, and channels) remain largely intact. Intracellular delivery also yields greater homogeneity in the delivered lipid concentration than can be achieved through bath application. In all studies, isotonic conditions were analogous to those activated by cell volume increases. PtdIns-3-P and the control lipid, PIP_2, in the same concentrations had no effect. Moreover, the response to intracellular D3-phospholipids was eliminated by removal of extracellular ATP, again consistent with an effect on cellular ATP release.

The regulatory role of PI 3-kinase in ATP transport and Cl\(^-\) channel activity is of particular interest to cholangiocyte secretion because PI 3-kinase has been shown to be an important modulator of bile flow. In the isolated perfused rat liver model, for example, inhibition of PI 3-kinase by wortmannin decreases both bile salt and phospholipid transport and decreases basal bile flow by 25% (15). The present studies suggest that PI 3-kinase might also play an important physiologic role in the regulation of cholangiocyte Cl\(^-\) secretion, which is thought to account for ~40% of human bile formation (45). In NRC in monolayer culture, increases in cell volume (a) stimulate apical ATP release and (b) increase \(I_{\text{c}1}\), the electrophysiologic signature of Cl\(^-\) secretion in these cells. These effects appear to be related because the secretory response is decreased or eliminated by wortmannin to inhibit ATP release, apyrase to remove ATP from the apical solution, and suramin or reactive blue-2 to inhibit P2 receptor activation (10). Thus, it is attractive to speculate that PI 3-kinase represents a critical intermediary signal coupling changes in cholangiocyte cell volume to secretion of Cl\(^-\) through effects on local ATP concentrations.

Assuming that PI 3-kinase is one of the primary signals regulating volume-sensitive ATP release, several additional points merit further investigation. First, the elements responsible for translating increases in cholangiocyte volume to generation of PI 3-kinase lipid products are yet to be determined. Second, because the molecular identities of the ATP transporting protein and the volume-sensitive Cl\(^-\) channel(s) are not established, the cellular site(s) of action of the D3 phosphorylated lipid messengers is not clear. Lastly, functional interactions between PI 3-kinase and other kinases are likely to be operative, and the sequence of action and relative importance of these kinases has not been established.

Taken together, these findings indicate that there are dynamic functional interactions between cell volume, PI 3-kinase activity, ATP release, and Cl\(^-\) secretion that contribute to ductular bile formation. Similar results in a hepatocyte cell line (25) suggest that PI 3-kinase may represent a general signal involving both cell types in the bile secretory unit. Consequently, cell volume may represent an important signal involved in “hepato-biliary coupling” through release of ATP into the lumen of intrahepatic ducts. Further characterization of
the mechanisms involved may provide novel strategies for stimulation of ductular secretion and bile flow in cholestatic liver diseases.

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