Regulated changes in cell volume represent a signal that modulates a broad range of cell and organ functions. In HTC hepatoma cells, increases in volume are coupled to membrane ion permeability through a pathway involving (i) ATP efflux, (ii) autocrine stimulation of P2 receptors, and (iii) increases in anion permeability and Cl⁻ efflux, contributing to recovery of volume toward basal values. Based on recent evidence that cell volume increases also stimulate phosphoinositide kinases, the purpose of these studies was to determine if phosphatidylinositol 3-kinase (PI 3-kinase) modulates these pathways. Exposure of cells to hypotonic buffer (20 or 40% less NaCl) caused an initial increase in cell volume and stimulated a rapid increase in ATP release. Subsequent opening of Cl⁻ channels was followed by recovery of cell volume toward basal values, despite the continuous presence of hypotonic buffer. Inhibition of PI 3-kinase with wortmannin (Kᵢ = 3 nm) significantly inhibited both the rate of volume recovery and activation of Cl⁻ currents; similar results were obtained with LY294002 (10 μM). Additionally, current activation was inhibited by intracellular dialysis with antibodies specific for the 110-kDa catalytic subunit of PI 3-kinase. Since release of ATP is a critical element in the volume-regulatory pathway, the role of PI 3-kinase on volume-stimulated ATP release was assessed. Both wortmannin and LY294002 decreased basal and volume-stimulated ATP permeability but had no effect on the current response to exogenous ATP (10 μM). These findings indicate that PI 3-kinase plays a significant role in regulation of cell volume and suggest that the effects are mediated in part through modulation of cellular ATP release.

However, the function and the targets of these lipid products are not fully known. PI 3-kinase is a heterodimer composed of a 110-kilodalton catalytic peptide and an 85-kilodalton regulatory peptide, which are tightly associated (1, 8, 9). This protein has been purified from rat liver (10), and PI 3-kinase activity has been shown to increase in response to a number of hormonal and growth factor stimuli, including insulin, platelet-derived growth factor, insulin-like growth factor, epidermal growth factor, colony-stimulating factor, and hepatocyte growth factor (2, 11, 12). Although the physiologic role of PI 3-kinase and its lipid products has not been completely defined, it has been implicated in such diverse processes as cellular growth and transformation (12, 13), glucose uptake and transport (14–16), membrane ruffling (17, 18), actin rearrangement (14, 19), and vesicular trafficking (20–22).

Recently, physiologic increases in cell volume have also been shown to be a potent stimulus for PI 3-kinase activation (23). Regulation of cell volume is mandatory for maintenance of cellular integrity; in addition, the hydration state may represent a means of coupling changes in membrane transport to other organ level functions. In hepatocytes, for example, cell volume increases reproduce many of the metabolic effects of insulin, including stimulation of bile acid secretion, glycogen, and protein synthesis, and gene expression (24, 25). These and other observations have led to the concept that changes in cell volume per se may represent a signal regulating liver function (26, 27).

In model liver cells, increases in cell volume stimulate an adaptive response that involves opening of membrane Cl⁻ channels through an ATP-dependent mechanism (Fig. 1). The resulting efflux of Cl⁻ favors water loss and recovery of cell volume toward basal values. Interestingly, liver cell volume increases stimulate parallel activation of multiple kinases, including PI 3-kinase, tyrosine kinase, and mitogen-activated protein kinases (1, 23, 28). However, little is known regarding the cellular site(s) of action of these kinases, and the cellular signals involved in volume-dependent Cl⁻ channel regulation in liver have not been defined. Consequently, the purpose of these studies was to assess the potential role of PI 3-kinase in recovery from cell swelling and in cell volume-dependent changes in membrane ion permeability.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—All studies were performed in HTC cells, a model rat hepatoma cell line that expresses ion channels and purinergic receptors similar to those found in primary rat hepatocytes. Increases in HTC cell volume stimulated by uptake of alanine (29) or exposure to hypotonic buffer (30, 31) are followed by ATP efflux, receptor activation, and opening of membrane Cl⁻ channels (30, 33). Cells were passaged at weekly intervals and maintained in HCO₃⁻-containing minimal essential medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin...
30–45 min. Aliquots (pended in 3 ml of isotonic buffer, and incubated with gentle agitation for in cell culture medium, centrifuged for 1 min at in subconfluent culture were harvested with 0.05% trypsin, suspended in cellular (pipette) solution for whole-cell recordings contained 130 mM KCl, glucose, and 10 mM HEPES/NaOH (pH 7.40). The standard intracellular (pipette) solution for whole-cell recordings contained 130 mM KCl, 10 mM NaCl, 2 mM MgCl2, 10 mM HEPES/ROH, 0.5 mM CaCl2, and 1 mM EGTA (pH 7.3), corresponding to a free [Ca2+] of ~100 nM (37). Patch pipettes were pulled from Corning 7052 glass and had a resistance of 3–10 megohms. Recordings were made with an Axopatch ID amplifier (Axon Instruments, Foster City, CA) and were digitized (1 kHz) for storage on a computer and analyzed using pCLAMP version 6.0 programs (Axon Instruments, Burlingame, CA) as described previously (36, 38). Pipette voltages refer to the bath. Current-voltage relations were measured between |120 and +100 mV in 20-mV increments (400-ms duration, 2 s between test potentials). In the whole-cell configuration, pipette voltage corresponds to the membrane potential, and upward deflections of the current trace indicate outward membrane current. Changes in membrane Cl permeability were assessed at a test potential of |80 mV (ECl) to minimize any contribution of K currents (30).

Cell Size Measurements—Mean cell volume was measured in cell suspensions by electronic cell sizing (Coulter Multizizer, 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 medium, centrifuged for 1 min at ~1000 g, resuspended in 3 ml of isotonic buffer, and incubated with gentle agitation for 30–45 min. Aliquots (~500 μl) of cell suspension were added to 20 ml of isotonic or hypotonic (40% less NaCl) buffer. Measurements of ~20,000 cells at specified time points after exposure to isotonic or hypotonic buffer were compared with basal values (time 0). Changes in values are expressed as relative volume normalized to the basal period. As a measure of volume recovery, the percentage of regulatory volume decrease was calculated as (peak relative volume at 3 min – relative volume at measured time point)/peak relative volume – 1) × 100. Experimental reagents were added as indicated.

ATP Bioluminescence Assay—Cells were grown to confluence in a 35-mm dish, washed twice with phosphate-buffered saline, and incubated with Opti-MEM I reduced serum medium plus luciferase-luciferin reagent (2 mg/ml, lyophilized reagent; Sigma). The dish was placed on a platform, lowered into the recording chamber of a Turner model TD20/20 luminometer, and studied immediately in real time. Since background luminescence (cells and medium without luciferase-luciferin reagent) is less than 0.1 arbitrary light unit (ALU), ATP released from cells into the media catalyzes the luciferase-luciferin reaction. Bioluminescence was measured in continuous 15-s photon collection intervals. To induce cell volume increases, the extracellular buffer was diluted 20 or 40% as indicated by the addition of water. In control studies, an equal volume of isotonic buffer was added to assess possible ATP release due to mechanical stimulation (39). The small changes in bioluminescence associated with isotonic exposures were <10% of values associated with hypotonic exposure (data not shown).

Reagents—Wortmannin (Sigma) and LY294002 (Calbiochem) were used as PI 3-kinase inhibitors (40–42). For all studies with wortmannin and LY294002, cells were preincubated with the respective inhibitor for 10 min prior to hypotonic exposure. In separate patch clamp studies, PI 3-kinase was inhibited by intracellular dialysis with a purified rabbit polyclonal antibody recognizing a sequence corresponding to residues 1054–1068 of the 110-kilodalton α-catalytic subunit of PI 3-kinase (Upstate Biotechnology, Inc.) (43). Polyclonal rabbit antibody to β-galactosidase was utilized as a control antibody (5 Prime→3 Prime, Inc., Boulder, CO). ATP and other reagents were obtained from Sigma.

Statistics—Results are presented as the mean ± S.E., with n representing the number of cells for patch clamp studies and the number of cultures 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 Cell Volume Recovery from Swelling—Exposure of cells to hypotonic buffer (40% decrease in NaCl, ~205 mosm) caused a rapid initial increase in relative volume to 1.18 ± 0.01 (n = 5, p < 0.001) within 3 min. The increase was followed by gradual recovery toward basal values despite the continued exposure to hypotonic buffer (Fig. 2). To evaluate whether PI 3-kinase contributes to cell volume recovery, analogous studies were performed in the presence of wortmannin. Preincubation with wortmannin (50 nM) resulted in a greater initial volume increase to 1.24 ± 0.01 immediately after hypotonic exposure and caused significant inhibition of volume recovery at all subsequent time points. The relative volume of 1.14 ± 0.01 at 30 min in the presence of wortmannin significantly exceeded control values of 1.07 ± 0.01 (n = 5, p < 0.001, Fig. 2). These findings indicate that inhibition of PI 3-kinase impairs recovery from cell swelling.

Effect of Kinase Inhibition on Cl Efflux—In HTC cells, cell volume recovery from swelling depends in part upon opening of Cl channels in the plasma membrane. To assess whether PI 3-kinase contributes to channel opening, whole-cell currents were measured under basal conditions and following cell volume increases induced by hypotonic exposure. IC was measured at a test potential of ~80 mV and values were reported as current density (pA/pF) to normalize for differences in cell size as recently described (30). Results are compared with control studies (basal and swelling-induced IC) measured on the same day to minimize any effects of day-to-day variability in current amplitude.

Under basal conditions with standard intra- and extracellular buffers, IC was small (<100 pA, 5 pA/pF). Exposure to hypotonic buffer (20% decrease in bath NaCl, ~230 mosm), resulted in activation of currents in >90% of cells within 2–4 min (representative trace shown in Fig. 3), increasing current density from ~4.6 ± 0.4 pA/pF to ~28.1 ± 8.8 pA/pF at ~80 mV (p < 0.001, n = 10). Swelling-activated currents exhibited characteristic biophysical features, with reversal near 0 mV (ECl), outward rectification, and time-dependent inactivation.

FIG. 1. Autocrine signaling by ATP release contributes to cell volume regulation. A proposed model of Cl channel activation in HTC cells is shown, involving (i) ATP release stimulated by increases in cell volume, (ii) P2 receptor binding, and (iii) Cl channel opening. The resulting Cl efflux favors water loss and cell volume recovery.
Regulation of Cell Volume by PI 3-Kinase

Fig. 3. Wortmannin inhibits volume-activated Cl\(^-\) currents. Whole-cell currents were measured under basal conditions and during increases in cell volume stimulated by hypotonic exposure (20% less NaCl, as indicated by the bar). Currents at −80 mV (downward deflection of the tracing) correspond to \(I_{\text{Cl}}\). A, in control cells (top), hypotonic exposure stimulated a reversible increase in currents. Incubation with wortmannin (50 nM, lower tracing) inhibited current activation by hypotonic exposure. B, the presence of wortmannin (50 nM) failed to inhibit Cl\(^-\) current activation by exogenous ATP (10 μM).

at depolarizing potentials above +60 mV, as described previously (39). Cl\(^-\) currents were sustained for the duration of hypotonic exposure and were fully reversible within 5 min of return to isotonic perfusate (Fig. 3A). In the presence of wortmannin (50 nM), the response to hypotonic exposure was completely inhibited: the maximum Cl\(^-\) current density was \(−1.2 ± 0.2 \text{ pA/μF} \quad (n = 9, p < 0.001, \text{Figs. 3A and 4A})\). Similar results were obtained with LY294002 (10 μM), a structurally unrelated PI 3-kinase inhibitor (−1.7 ± 0.1 pA/μF, \(n = 4, p < 0.001, \text{Fig. 4A}\)).

Additional studies were performed to evaluate the concentration dependence of wortmannin. Currents were measured in the presence of wortmannin, and values were normalized to peak currents measured in the absence of wortmannin and expressed as \(I_{\text{max}}\). Inhibition was concentration dependent, with no effect at values < 0.5 nM and nearly complete inhibition at concentrations ≥ 5 nM. The apparent \(K_i\) for wortmannin inhibition of swelling-activated Cl\(^-\) currents was −3 nM (\(n = 6\) for each point, Fig. 4B).

Intracellular Dialysis with a Specific Antibody to PI 3-Kinase Inhibits Volume-activated Cl\(^-\) Currents—Both wortmannin and LY294002 are thought to be selective inhibitors of PI 3-kinase, but the potential for inhibition of other kinases cannot be fully excluded. Consequently, an alternative strategy was used to assess the specificity of PI 3-kinase using intracellular dialysis with antibodies to the 110-kDa catalytic subunit of PI 3-kinase. This antibody has been shown to inhibit growth factor-stimulated PI 3-kinase activity in cultured fibroblasts (43). 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 ± 1.0 pA/μF. In contrast, currents during intracellular dialysis with antibodies to β-galactosidase (5 μg/ml) were similar to controls (−42.1 ± 5.6 pA/μF, \(n = 6, p < 0.001, \text{Fig. 5}\)). These findings support a specific role of PI 3-kinase in volume-stimulated Cl\(^-\) channel activation.

PI 3-Kinase Modulates Swelling-activated ATP Release—As depicted in Fig. 1, PI 3-kinase could potentially modulate current activation and cell volume recovery 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 ATP was assessed. For these studies, 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, the presence of wortmannin would be expected to inhibit ATP-dependent current activation. In the presence of wortmannin, hypotonic exposure failed to activate Cl\(^-\) currents. However, the subsequent addition of ATP (10 μM) to the perfusate resulted in instantaneous activation of Cl\(^-\) currents (representative trace, Fig. 3B), increasing current density from −1.2 ± 0.4 pA/μF to −37.4 ± 13.1 pA/μF (\(n = 5, p < 0.05, \text{Fig. 4A}\)). Second, both removal of extracellular ATP and inhibition of PI 3-kinase with wortmannin delay cell volume recovery from swelling in Coulter counter studies. To assess whether these effects are related, additional studies were performed to assess the rate of recovery from swelling (percentage of regulatory volume decrease) under control conditions in the presence of wortmannin to inhibit PI 3-kinase and in the presence of wortmannin plus exogenous ATP added to the extracellular bath. The presence of exogenous ATP (10 μM) bypassed the inhibitory effects of wortmannin and restored volume recovery with values no different from controls at all subsequent time points (\(n = 5\) for each time point, \(p < 0.01, \text{Fig. 6}\)). 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 as shown in Figs. 7 and 8. In Fig. 7, results are expressed as a change from baseline by subtraction of values observed in parallel control studies where cells were exposed to isotonic buffer rather than hypotonic buffer. In Fig. 8, values are presented in absolute numbers without correction for basal release. Cells in isotonic

Fig. 4. PI 3-kinase inhibitors prevent activation of Cl\(^-\) currents by cell volume increases. A, exposure of HTC cells to hypotonic buffer (20% less NaCl) increased Cl\(^-\) current density from −4.6 ± 0.4 pA/μF to −28 ± 3.8 pA/μF (\(n = 10, p < 0.001\)). Both wortmannin (50 nM, \(n = 9, p < 0.001\)) and LY294002 (10 μM, \(n = 4, p < 0.001\)) inhibited this response. Wortmannin had no effect on the response to exogenous ATP (1 μM, \(n = 5, p < 0.05\)). Data represent the mean ± S.E. current density at −80 mV. B, inhibition of volume-activated Cl\(^-\) currents by wortmannin is concentration-dependent with an apparent \(K_i\) of ~3 nM. Each point represents the mean ± S.E. of ≥6 cells. Currents were normalized to average peak currents at −80 mV measured in control cells during hypotonic exposure (\(I_{\text{max}}\).)
buffer demonstrated spontaneous release of ATP under basal conditions (18.59 ± 2.91 ALU). Exposure of cells to hypotonic perfusate to stimulate cell swelling induced ATP efflux (29.11 ± 5.58 ALU at 20% dilution and 35.7 ± 8.06 ALU at 40% dilution), as shown in Fig. 8. Values remained elevated above basal levels for the duration of hypotonic exposure (4 min). Basal values were decreased significantly by both wortmannin (9.67 ± 1.16 ALU) and LY294002 (9.96 ± 1.44 ALU, n = 5 for each; p < 0.05; Fig. 8). Similarly, the response to hypotonic exposure was also inhibited by both wortmannin (12.84 ± 1.42 ALU at 20% dilution, and 15.8 ± 2.04 ALU at 40% dilution) and LY294002 (13.82 ± 2.21 ALU at 20% dilution and 13.43 ± 2.26 ALU at 40% dilution, n = 5 for each; p < 0.05; Fig. 8). These findings indicate that PI 3-kinase activity contributes to regulation of both basal and swelling-activated ATP release.

**DISCUSSION**

In hepatocytes and other epithelial cells, physiologic changes in cell volume are closely coupled to membrane ion permeability and directly modulate a broad range of cell and organ functions (44). For example, increases in cell volume produced by hypotonic exposure mimic many of the effects of insulin, stimulating protein and glycogen synthesis, bile flow, and exocytosis through selective effects on gene and protein expression (1, 23–25). The present studies indicate that PI 3-kinase may play a critical intermediary role in this process and suggest that the effects of PI 3-kinase are mediated in part through regulation of electrodiffusional movement of ATP across the plasma membrane.

PI 3-kinases catalyze phosphoinositidol at the D-3 position of the inositol ring, leading to formation of at least three phosphoinositides that are presumed to function as intracellular second messengers (3, 5, 7). Tyrosine kinase-regulated PI 3-kinase is composed of a 110-kilodalton catalytic subunit that binds ATP, and its function is modified by interactions with a separate 85-kilodalton regulatory subunit. Both wortmannin and LY294002 effectively inhibit kinase activity (40–42).

In liver cells, the lipid products of PI 3-kinase are not present under basal conditions. However, exposure to insulin or increases in cell volume lead to rapid kinase activation (23).
Regulation of Cell Volume by PI 3-Kinase

Volume-stimulated ATP release. Peak values (expressed as ALU) of ATP released from cell monolayers into media under basal and hypotonic conditions are shown. Preincubation (5 min) of cells with either wortmannin (50 nM) or LY294002 (10 μM) significantly decreased basal ATP permeability (n = 5 for each, p < .05). Exposure to hypotonic buffer (20 or 40% dilutions) stimulated an increase in ATP release from control cells, which was significantly inhibited by both wortmannin and LY294002 (n = 5 for each; p < 0.05).

Inhibition of PI 3-kinase by wortmannin or LY294002 prevents the increases in glycosynase activity, acetyl-CoA carboxylase, and bile acid excretion normally caused by cell volume increases, suggesting that PI 3-kinase activation represents one of the signals coupling changes in cell volume to cell metabolism and transport (23, 45). Moreover, results in different models have implicated PI 3-kinase as a modulator of vesicular trafficking, cytoskeletal organization, and bile formation, processes also directly influenced by physiologic changes in cell volume (46).

In these studies of HTC cells, observations using a variety of techniques support a broader role for activation of PI 3-kinase as an early and important step coordinating changes in cell volume and membrane Cl− permeability. Inhibition of PI 3-kinase significantly impairs cell volume recovery after hypotonic exposure and uncouples cell volume from changes in membrane Cl− permeability.

These findings appear to be specific for PI 3-kinase. The inhibitory effects of wortmannin are detectable in low nanomolar concentrations (K_i ~ 3 nM) and in individual cells are partially reversible. Moreover, similar inhibitory effects are caused by LY294002, a structurally unrelated compound that also inhibits the ATP binding activity of p110 (41, 47). Despite the potency of these compounds, it is acknowledged that inhibitors can have unanticipated effects on other signaling pathways as well; wortmannin, for example, has recently been shown to inhibit a separate PI 4-kinase in higher concentrations (48, 49). Consequently, an alternative strategy was utilized to inhibit PI 3-kinase by intracellular dialysis with antibodies that bind selectively to the p110 catalytic subunit. These antibodies have previously been shown to block growth factor-stimulated PI 3-kinase effects in cultured fibroblasts (43). When antibodies were allowed to equilibrate with the cell interior by inclusion in the patch pipette, current activation following hypotonic exposure was inhibited. Antibodies unrelated to PI 3-kinase had no effect. These findings are likely to reflect specific inhibition of PI 3-kinase activity through antibody binding to a critical functional site on the cell interior.

Several observations indicate that the effects of PI 3-kinase are mediated in part through modulation of cellular ATP release. Previous studies of HTC cells indicate that increases in cell volume lead to electrophysiological phenomena of ATP. The localized increase in extracellular ATP is thought to activate P2 receptors in the plasma membrane coupled to Cl− channels, and the resulting Cl− efflux contributes to restoration of cell volume toward basal values (31, 33). In individual cells, exposure to exogenous ATP (added to the bathing solution) bypasses the inhibitory effect of wortmannin on swelling-induced current activation; and in cell suspensions, supplemental ATP partially reverses the inhibitory effect of wortmannin on cell volume recovery from swelling. Thus, wortmannin is not likely to modulate volume regulatory responses through inhibition of P2 receptors or blockade of membrane Cl− channels.

To assess whether PI 3-kinase is functioning at a more proximal site in the signaling cascade, the effect of cell volume on release of ATP from cells into the supernatant was assessed by a sensitive and specific luminescent assay. This approach has a number of advantages over electrophysiologic methods that are based on detection of currents carried by high (100 mM) concentrations of ATP (33). Specifically, luminescence measuring ATP are performed using intact cells maintained in conventional media under conditions where signaling mechanisms are intact, minimalizing the potential adverse effects of intracellular dialysis with unphysiologic solutions. In addition, the marked increase in sensitivity of this assay permits detection of ATP in the absence of nucleotidase inhibitors or other agents that might modify ATP availability.

Under basal conditions in isotonic buffer, low levels of ATP were always detectable in supernatant media. Increases in cell volume caused a rapid increase in ATP release, and the magnitude of the response was proportional to the transmembrane osmotic gradient. ATP release was not related to apparent cytotoxicity, since the same maneuvers had no effect on trypan blue exclusion, propidium iodide staining, or lactate dehydrogenase release (data not shown). Moreover, ATP release was inhibited by PI 3-kinase inhibitors, supporting a specific process mediated by signaling events. In the presence of wortmannin or LY294002, both basal release and the response to hypotonic exposure were significantly diminished. The most direct
interpretation is that cell volume-dependent activation of PI 3-kinase is necessary for increases in membrane ATP permeability. These findings are of interest in light of recent evidence that diverse cellular processes are directly regulated by ATP release, metabolism, and binding. Indeed, more than 10 purinergic receptors responding to different nucleotides have been defined by pharmacologic and molecular techniques (32). Regulation of nucleotide release by changes in cell hydration may provide one mechanism for autocrine/paracrine signaling coupling changes in cell volume and other cell and organ functions. If so, several points merit further investigation. First, it is notable that PI 3-kinase inhibitors failed to completely suppress basal or swelling-induced ATP release and did not completely prevent recovery from cell swelling (Fig. 2). These observations imply that additional PI 3-kinase-independent mechanisms are operative as well. Given the important role for PI 3-kinase in regulation of endocytic and transcytotic pathways (46), it is attractive to speculate that ATP release may involve two separate pools of transporters, including transporters in the plasma membrane and those in submembrane vesicles. By interference with vesicle trafficking and cytoskeletal organization, wortmannin could decrease cellular ATP release by preventing insertion of new transporters from submembrane vesicles. Thus, wortmannin would be expected to delay but not eliminate the adaptive responses to cell volume increases. While the present studies do not address these possibilities directly, the findings are consistent with emerging observations in other models.

Second, the initial events that couple cell volume increases to activation of PI 3-kinase remain to be identified. Indeed, identifying the proximal signal(s) mediating volume-dependent cellular processes represents a critical focus for many laboratories, and cell volume is known to cause rapid activation of multiple kinases as well as sustained biochemical and genetic effects (1, 23, 27). Since PI 3-kinase utilizes membrane constituents as a substrate, it is possible that changes in the substrate availability or presentation associated with volume may contribute to kinase activity. However, alternative signals including other kinases or stretch-activated ion channels must be considered as well. It is notable, for example, that tyrosine kinases and other G-protein-coupled receptors have also been shown to regulate PI 3-kinase (2).

Third, there are quantitative differences in the time course and/or magnitude of the wortmannin-sensitive parameters involved in cell volume recovery. Wortmannin completely inhibits Cl- current activation in isolated cells, but only partially inhibits ATP release and cell volume recovery. These differences may be related in part to the different experimental techniques used. For example, dialysis of the intracellular space during whole-cell recordings is likely to alter signal transduction and may prevent actual cell volume recovery since the volume of the pipette solution is orders of magnitude greater than the volume of individual cells. While it will be important to address the relationship between ATP release and volume recovery in a more quantitative manner, the inhibitory effect of wortmannin on ATP release, current activation, and volume recovery, measured using different experimental approaches, supports an important role for PI 3-kinase in cell volume regulation. Therefore, PI 3-kinase may be an early and essential signal coupling changes in cell volume to membrane Cl- permeability through effects on cellular ATP release. Given the tissue-specific expression of multiple P2 receptor subtypes, further definition of the mechanisms linking cell volume and ATP release represents an attractive and previously unrecognized target for modulation of the diverse cellular processes regulated by PI 3-kinase.