Initiation of Purinergic Signaling by Exocytosis of ATP-containing Vesicles in Liver Epithelium*

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Extracellular ATP represents an important autocrine/paracrine signaling molecule within the liver. The mechanisms responsible for ATP release are unknown, and alternative pathways have been proposed, including either conductive ATP movement through channels or exocytosis of ATP-enriched vesicles, although direct evidence from liver cells has been lacking. Utilizing dynamic imaging modalities (confocal and total internal reflection fluorescence microscopy and luminescence detection utilizing a high sensitivity CCD camera) at different scales, including confluent cell populations, single cells, and the intracellular submembrane space, we have demonstrated in a model liver cell line that (i) ATP release is not uniform but reflects point source release by a defined subset of cells; (ii) ATP within cells is localized to discrete zones of high intensity that are ~1 μm in diameter, suggesting a vesicular localization; (iii) these vesicles originate from a bafilomycin A1-sensitive pool, are depleted by hypotonic exposure, and are not rapidly replenished from recycling of endocytic vesicles; and (iv) exocytosis of vesicles in response to cell volume changes depends upon a complex series of signaling events that requires intact microtubules as well as phosphoinositide 3-kinase and protein kinase C. Collectively, these findings are most consistent with an essential role for exocytosis in regulated release of ATP and initiation of purinergic signaling in liver cells.

Increased concentrations of ATP in bile sufficient to activate P2 receptors in the apical membrane of targeted cholangiocytes, resulting in a robust secretory response through activation of Cl− channels in the apical membrane. Moreover, multiple signals including intracellular calcium, cAMP and bile acids appear to coordinate ATP release, which has been recognized recently as a final common pathway responsible for biliary secretion (3–5). Accordingly, definition of the mechanisms involved in ATP release represents a key focus for efforts to modulate liver function and the volume and composition of bile.

Previous studies indicate that increases in cell volume serve as a potent stimulus for physiologic ATP release in many epithelia and in liver cells increase extracellular nucleotide concentrations 5–10-fold (6). Two broad models for ATP release by nonexcitatory cells have been proposed, including (i) opening of ATP-permeable channels and/or (ii) exocytosis of ATP-containing vesicles (7). There is evidence, for example, for conductive movement of ATP across the plasma membrane, consistent with a channel-mediated mechanism, and connexin 36 hemichannels (8), ATP-biding cassette proteins, and P2X7 receptor proteins (9) each have been proposed to function as ATP-permeable transmembrane pores where opening permits movement of ATP from the cytoplasm to the extracellular space (10). Alternatively, ATP can be co-packaged into vesicles with other signaling molecules in endothelial and chromaffin cells, and exocytosis results in rapid point source increases in extracellular ATP concentrations (11, 12). Quinacrine taken up by the cell is concentrated in ATP-containing vesicles, and fluorescence imaging of intracellular ATP stores in pancreatic acinar cells shows a punctate distribution consistent with a vesicular localization (13). Given the diverse functions of ATP as an agonist, it is likely that more than one pathway is operative with substantial differences among cell types in the mechanisms involved.

In the liver, expression of the ATP-binding cassette protein MDRI increases ATP release, but the effects of P-glycoproteins on ATP release can be dissociated from P-glycoprotein substrate transport, suggesting that MDRI per se is not likely to function as an ATP channel (14). Similarly, in biliary cells, the related cystic fibrosis transmembrane conductance regulator (CFTR) is expressed in the apical membrane and plays an important regulatory role in ATP release through a mechanism not yet defined (3, 15). Recent indirect observations suggest an important role for vesicular pathways in hepatic ATP release. In a cholangiocyte cell line, increases in cell volume stimulate an
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and hypotonic conditions, as described above. TIRF imaging was performed on an Olympus IX71 microscope equipped with an Olympus TIRF module. Quinacrine TIRF was excited with the 488-nm line of a 10-milliwatt argon laser (Melles Griot) through a ×60 PlanApo-N 1.45 numerical aperture oil immersion lens or a PLAPO ×100 1.45 numerical aperture TIRF microscope oil immersion lens as described (29, 30). Laser intensity was modulated by neutral density filters and Uniblitz electronic shutters (Vincent Associates, Rochester, NY). Time-lapse images were acquired every 300 ms with exposure times of 100–200 ms using SlideBook (Intelligent Imaging Innovations, Inc., Denver, CO) to control a Hamamatsu Orca II ERG camera (Hamamatsu Photonics) and the electronic shutters.

Lactate Dehydrogenase (LDH) Release—LDH measurements were performed pre- and posthypotonic exposure using an enzymatic colorimetric cytotoxicity assay (CytoTox 96, Promega, Madison, WI). Calibration of the assay was performed with cell-free controls and reagents only (no LDH release). LDH standard at a dilution of 1:5000 (amount of LDH in ~13,000 lysed cells), and after exposure of cells to lysis solution (maximum LDH release).

Reagents—Wortmannin was obtained from Calbiochem/EMD Biosciences (La Jolla, CA), and calphostin C was obtained from MP Biomedicals (Solon, OH). All other reagents were obtained from Sigma. None of the reagents affected L-L luminescence as measured during cell-free conditions.

Statistics—Results are presented as the means ± S.E., with n representing the number of culture plates or repetitions for each assay as indicated. Statistical analysis included Fisher's paired and unpaired t test and analysis of variance for multiple comparisons to assess statistical significance as indicated. p values of <0.05 were considered to be statistically significant.

RESULTS

Identification of Point Source Bursts of ATP Release from Liver Cells—Increases in liver cell volume result in an increase in bulk ATP concentration in the overlying media from ~10 to ~300 nM as measured by luciferin-luciferase bioluminescence (31). This response represents an integrated signal from HTC cells as focal increases in bioluminescence generated by ATP-dependent luciferin breakdown by luciferase (see supplemental Fig. S1). Time-lapse images of a representative study. Hypotonic solution was perfused onto confluent cells (shown in the first frame, bright field image; scale bar, 50 μm) from a pipette immediately above the cells and to the right of view (indicated by small gray triangle in the last frame). The first image was obtained 2 s after hypotonic addition, and sequential images (from left to right and top to bottom) represent 500-ms intervals. Scale bar, 100 μm. B, relative change in bioluminescence of the example shown in A. Total bioluminescence is shown as a solid line, and one individual burst event is shown as a dotted line. The maximal burst diameter was measured for 31 bursts, and the mean ± S.E. is shown in the inset.

FIGURE 1. Point source ATP release. ATP release was detected from confluent HTC cells as focal increases in bioluminescence generated by ATP-dependent luciferin breakdown by luciferase (see supplemental Fig. S1). A, time-lapse images of a representative study. Hypotonic solution was perfused onto confluent cells (shown in the first frame, bright field image; scale bar, 50 μm) from a pipette immediately above the cells and to the right of view (indicated by small gray triangle in the last frame). The first image was obtained 2 s after hypotonic addition, and sequential images (from left to right and top to bottom) represent 500-ms intervals. Scale bar, 100 μm. B, relative change in bioluminescence of the example shown in A. Total bioluminescence is shown as a solid line, and one individual burst event is shown as a dotted line. The maximal burst diameter was measured for 31 bursts, and the mean ± S.E. is shown in the inset.
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Evidence for ATP-enriched Vesicles—This temporal profile of extracellular ATP kinetics is most compatible with point source release and rapid ATP diffusion/hydrolysis. In an effort to visualize candidate vesicles containing ATP, cells were preincubated with quinacrine, which has a high affinity for ATP and, when exposed to blue light, produces a concentration-dependent fluorescence (13). Fig. 2, using TIRF microscopy to visualize the submembrane space of ~100 nm adjacent to the coverslip, demonstrates that quinacrine visualization of cellular ATP stores is not uniform. Rather, fluorescence is detected as discrete foci 0.5–1.0 μm in diameter, which vary in intensity over a 4-fold range. When visualized in real time, these focal regions of high intensity are not static but vacillate in a Brownian manner. Further, occasional vesicles move toward the membrane and demonstrate a transient increase in fluorescence intensity followed by rapid disappearance (see supplemental Fig. S2). This transient increase in intensity is analogous to exocytosis of pancreatic secretory vesicles loaded with acridine orange (32), suggesting concentration of quinacrine (and presumably ATP) within a population of vesicles and subsequent exocytic release of quinacrine/ATP into the extracellular space (Fig. 2B). Although Figs. 1 and 2 are visually similar, Fig. 1 corresponds to macroscopic visualization of extracellular ATP diffusing over ~200 μm, and Fig. 2 detects intracellular stores packaged into discrete domains of ~1 μm. Under basal conditions (23 °C), these release events occurred spontaneously at a rate of 0.13 ± 0.06 events/cell/min (Fig. 2C). Increases in cell volume (33% decrease in osmolality) increased the rate to 0.88 ± 0.27 events/cell/min (n = 35, p < 0.01). Moreover, increasing temperature to 35 °C increased both basal (0.73 ± 0.09 events/cell/minute) and volume-sensitive (1.48 ± 0.19 events/cell/min) responses (n = 41 for each, p < 0.01).

Total Cellular ATP Vesicles—Similar results were obtained when quinacrine-enriched vesicles were assessed using digital reconstruction of spinning disk confocal z stacks to estimate the size of the vesicular pool involved (Fig. 3A). In the example shown, the plasma membrane is shown in red (FM4-64), and vesicles are shown in green (quinacrine). Exposure to hypotonic...
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FIGURE 3. Confocal imaging and three-dimensional reconstruction of HTC cells dually labeled with quinacrine and FM4-64. A, representative single cell. Prehypotonic (top) and 1 min posthypotonic (33%) (bottom) exposure are shown. Large grid squares represent 1 μm. B, representative confluent monolayer. Prehypotonic (top) and 1 min posthypotonic (33%) (bottom) exposure are shown. A spot detection algorithm was applied to count small (0.4–0.99 μm, gray spheres) and large (≥1 μm, blue spheres) vesicles during basal conditions and 1 min after equal volumes of either isotonic or hypotonic buffers were applied. Representative responding cells (defined as a loss of ≥5% of total vesicles) and nonresponding cell are outlined in yellow and white boxes, respectively. Scale bar, 10 μm. C, cumulative data of responding cells reported as percentage of remaining vesicles per cell. Note that only responding cells are included in the analysis. The numbers of total (dark gray bar), large (black bar), and small (light gray bar) vesicles all decreased in response to hypotonic exposure. *, p < 0.01 versus isotonic for each (n = 14).

medium decreased the total number of quinacrine-stained vesicles in responding cells by 76 ± 8% (n = 14 cells); which represented a decrease of 76 ± 6% of small (0.4–0.99-μm) and a decrease of 58 ± 8% of large (≥1-μm) vesicle populations (Fig. 3C). Similar to the point source ATP bursts described above, not all cells within a given area reacted similarly, with a loss of quinacrine vesicles, in response to the hypotonic stimulus. In confluent cell preparations, 69 ± 14% of cells responded to a hypotonic stimulus with a loss of quinacrine-labeled vesicles, as shown in this representative example (Fig. 3B). Together, these findings are compatible with previous studies demonstrating close coupling between volume-stimulated exocytosis and ATP release. Moreover, the findings suggest that the ATP-containing vesicular pool might be limited in size and subject to depletion over the time course of these studies. Loss of quinacrine-stained vesicles was not due to photobleaching because cells exposed to isotonic solution and then imaged under identical conditions did not lose fluorescence (Fig. 3C).

ATP Vesicle Formation Is Dependent on V-type ATPase—To determine the origin of the ATP-enriched vesicles, a dual staining strategy was employed with quinacrine and FM4-64 to label endosomes (Fig. 4). Two staining protocols were utilized: (i) concurrent FM4-64 and quinacrine staining in which cells were incubated with both dyes and imaged at defined time points for up to 45 min and (ii) sequential labeling in which cells were first exposed to FM4-64 for 15–120 min and then dye was removed, followed by exposure to quinacrine and imaging at the defined time points. Utilizing these protocols, two vesicle populations were observed: endocytic vesicles due to incorporation of FM4-64-stained plasma membrane and a second pool of quinacrine-labeled vesicles that was distinct from the FM4-64 endocytic pool. No dual staining was observed within 30 min of labeling (Pearson’s coefficient = 0.42) with either of the protocols. However, co-localization was observed at 45 min with 96% of the quinacrine-labeled vesicles co-localizing with the FM4-64-labeled vesicles (Pearson’s coefficient = 0.85). The same staining protocol was utilized after cells were exposed to hypotonicity (33%) to determine if endosomal or rapid refilling occurs after an ATP-depleting stimulus. Once again, no dual labeling was identified until 30 min postexposure (data presented in supplemental Tables S1 and S2). Together, these studies demonstrate that the quinacrine-labeled vesicles do not co-localize with the FM4-64-labeled vesicles until after 30 min, suggesting that these vesicles may derive from a late endosomal compartment. No co-localization was observed at early time points (before 30 min), excluding rapid refilling of early endosomes as a source of the ATP-enriched compartment.

Bafilomycin A1, a macrolide antibiotic, is a potent inhibitor of V-type ATPase and impairs storage of ATP in chromaffin granules and secretory vesicles in astrocytes (28). Incubation of cells with bafilomycin (4 μM × 30 min) significantly inhibited the appearance of quinacrine-stained ATP vesicles 1.12 ± 0.48 versus control 31.6 ± 3.35 vesicles/unit area (square pixels), n = 8 each, p < 0.01 (Fig. 4B, bottom). In complementary real-time measurements of ATP release, bafilomycin blocked basal (57.9 ± 9.1 ALU), mechanically stimulated (67.6 ± 11.1 ALU), and volume-stimulated (92.6 ± 17.1 ALU) ATP release versus control (219.3 ± 4.3, 251.4 ± 7.8, and 384.4 ± 9.1 ALU, respectively, n = 7 trials each, p < 0.01 (Fig. 4C)). Together, these studies demonstrate that the V-type ATPase, a necessary component of vesicle formation and trafficking, contributes to liver cell ATP release through effects on ATP uptake into vesicles.

Regulation of Exocytosis and ATP Release—Previous studies using measurements of ATP in bulk solution suggest that volume-sensitive exocytosis and ATP release is potentiated by protein kinase C (16) and requires intact phosphoinositide 3-kinase signaling (6). If the vesicles visualized by quinacrine play a role in this process, then parallel regulation of exocytosis and ATP release would be anticipated. The strategy for addressing this possibility is shown in Fig. 5. First, following exposure of cells to brefeldin A (4–6 h), an inhibitor of protein translocation through the Golgi, cells were exposed to quinacrine to label vesicles and imaged by TIRF microscopy. Brefeldin exposure decreased the number of quinacrine-stained vesicles under
basal conditions (11.61 ± 1.28 vesicles/unit area (square pixels)) versus control (31.62 ± 3.35 vesicles/unit area (square pixels)), suggesting depletion of the submembrane pool in question (Fig. 5A). Studies utilizing digital reconstruction of spinning disk confocal z-stacks demonstrated a similar loss of the cellular pool of quinacrine-stained vesicles after brefeldin (decrease of 35.1 ± 7.3% from basal, n = 11, p < 0.01 (Fig. 5, D and E)). Interestingly, the effect was most pronounced on the pool of small (~1-μm) vesicles (decrease of 50.1 ± 5.3% from basal, n = 11, p < 0.01).

Dynamic confocal imaging after hypotonic exposure revealed that brefeldin significantly inhibited the normal loss of quinacrine-stained vesicles observed in control cells (decrease of only 8.7 ± 2.8% versus 67.9 ± 10.1% of total vesicles, n = 11, p < 0.01 (Fig. 5F)).

In parallel, the dynamics of ATP release in bulk solution were measured using luminometry (integrated signal from >10⁶ cells/plate (Fig. 5, G and H)). Under control conditions, the addition of isotonic solution as a control for mechanical stimulation caused a small increase in detectable ATP (from 1.22 ± 0.15 to 22.8 ± 4.9 ALU), whereas exposure to the same volume of hypotonic solution to increase cell volume caused a much greater release (365.7 ± 23.2 ALU at 2 min, n = 9, p < 0.05) as described previously (6). Preincubation with brefeldin A using conditions sufficient to deplete quinacrine staining inhibited the response to isotonic (8.4 ± 3.1 ALU, n = 9, p < 0.05) and hypotonic exposure (110.3 ± 39.4 ALU, n = 9, p < 0.05, Fig. 5, G and H).

Using a similar approach, the effects on quinacrine vesicles of inhibitors of vesicular formation and trafficking were assessed by TIRF and confocal microscopy and compared with parallel studies of ATP release to develop insights into the signaling events important to ATP release. Preincubation with nocodazole (10 μM for 15 min), a microtubule-depolymerizing agent; or wortmannin (50 nM for 15 min),
an inhibitor of phosphoinositide 3-kinase, each inhibited the volume-stimulated exocytic response seen under control conditions (increase of fluorescence events/cell/min (Fig. 5C)). This inhibition could not be explained by a decrease in the basal number of vesicles per unit area (Fig. 5B). In parallel, these same agents decreased volume-sensitive ATP release as well (Fig. 5H). Similarly, the carboxylic ionophore monensin, which is known to dissipate the transmembrane pH gradients in Golgi and lysosomal compartments, inhibited volume-stimulated ATP release from cell monolayers (Fig. 5H). Thus, quinacrine appears to be a good marker for ATP-containing vesicles, and there is close correlation between exocytic events as visualized by TIRF microscopy, total vesicle counts by spinning disk confocal microscopy, and measurement of ATP in solution. Further, intact mechanisms for vesicle formation, acidification, and trafficking are required for volume-sensitive ATP release.

Regulation of Exocytosis by Protein Kinase C (PKC)—Increases in liver cell volume result in activation of phorbol-sensitive PKC isoforms, which in turn serve as a regulatory signal leading to volume-sensitive exocytosis and ATP release (16). In order to characterize the relationship between the quinacrine-enriched vesicles demonstrated herein and the more generalized exocytosis visualized by FM1-43, cells were loaded with quinacrine and again visualized by TIRF microscopy (Fig. 6), and the effects of PKC signaling were assessed. Prior exposure to phorbol 12-myristate 13-acetate (PMA) to activate protein kinase C stimulated volume-sensitive (33% hypotonicity) exocytosis (2.99 ± 0.28 events/cell/min, n = 28) rates ~4-fold above control (0.73 ± 0.09 events/cell/min, n = 35, p < 0.05 (Fig. 6A)). In parallel studies, exposure to PMA alone had no effect on basal ATP release but enhanced significantly the amount of ATP released following hypotonic expo-
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DISCUSSION

Although essentially all epithelia are capable of physiologic ATP release, definition of the mechanisms involved has proven to be challenging (7). The principal findings of these studies in a liver cell line are that (i) ATP release is not uniform but reflects point source release by a defined subset of cells; (ii) ATP within cells is localized to discrete zones of high intensity that are ~1 μm in diameter, suggesting a vesicular localization; (iii) these vesicles originate from a bafilomycin A₁-sensitive pool, are depleted by hypotonic exposure, and are not rapidly replenished from recycling of endocytic vesicles; and (iv) exocytosis of quinacrine-labeled vesicles in response to cell volume depends upon a complex series of signaling events that requires intact microtubules as well as PI-3 kinase and protein kinase C. Collectively, these findings are most consistent with an essential role for exocytosis in regulated release of ATP and initiation of purinergic signaling in liver cells. They do not, however, rule out the possibility that exocytosis is involved in trafficking of ATP-permeable channels as well.

Following the demonstration of ATP-containing vesicles in astrocytes, evidence has emerged to suggest that epithelial ATP release might also be related, at least in part, to exocytosis. Brefeldin A, which blocks trafficking between endoplasmic reticulum and Golgi, impairs ATP release in oocytes (33) and urogenital epithelium (34), and phosphoinositide 3-kinase signaling in urogenital epithelium (34), and phosphoinositide 3-kinase signaling in liver and Rho kinase and tyrosine kinase signaling in urogenital epithelium (34), and phosphoinositide 3-kinase signaling in liver and Rho kinase and tyrosine kinase signaling in urogenital epithelium (34), and phosphoinositide 3-kinase signaling in liver and Rho kinase and tyrosine kinase signaling in urogenital epithelium (34). The pattern of distribution of quinacrine fluorescence revealed multiple (often 40 per cell) regions of high intensity consistent with a pre-

FIGURE 5. Regulation of exocytosis and ATP release. A, representative TIRF image of the submembrane space of a single HTC cell, labeled with quinacrine according to protocol, during control conditions and after incubation with brefeldin A (10 μM for 1 h). B, cumulative data demonstrating basal number of vesicles in submembrane space visualized by TIRF. Incubation with brefeldin A (n = 21) or bafilomycin A₁ (4 μM × 30 min, n = 8) significantly decreased the basal number of vesicles, *p < 0.01 versus basal (n = 21). Nocodazole (n = 25) or wortmannin (n = 30) did not affect basal number of vesicles in submembrane space. C, cumulative data demonstrating number of evocytic events/cell/min during basal conditions and after hypotonic exposure (33% decrease osmolality) during TIRF. Incubation with brefeldin A (10 μM, n = 13), nocodazole (10 μM, n = 25), or wortmannin (50 nM, n = 30) significantly inhibited the number of evocytic events, *p < 0.01 compared with control (n = 21). D, representative confocal images of confluent HTC cells dually labeled with FM4-64 and quinacrine. E, cumulative data demonstrating number of vesicles remaining after hypotonic exposure. **p < 0.01 versus basal. Both brefeldin A (n = 11) and nocodazole (n = 10) significantly inhibited the loss of quinacrine-stained vesicles. #p < 0.05 versus hypotonic control (n = 11). G, bulk ATP release (arbitrary light units) was measured from confluent HTC cells after isotonic and hypotonic (33%) exposures in control (black circles) or after incubation with brefeldin A (open circles). Each point represents mean ± S.E. (n = 6 each). H, cumulative data demonstrating effect of inhibitors of vesicle formation and trafficking on bulk ATP release. Values represent percentage of maximal control ATP release at 2 min. Brefeldin A, nocodazole, and monensin (100 μM) significantly inhibited ATP release in response to hypotonic exposure. *p < 0.05 versus control, n = 5–6 each.
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FIGURE 6. Regulation of exocytosis and ATP release by PKC. A, HTC cells were labeled with quinacrine and imaged via TIRF microscopy according to the protocol in Fig. 2 at 23 °C. Cumulative data represent the number of exocytic events/cell/min. Incubation with PMA (1 μM × 10 min, n = 28) significantly increased the number of hypotonic buffer-stimulated (33% decrease in osmolality) exocytic events versus control (n = 35). *, p < 0.01 versus basal without PMA; **, p < 0.05 versus hypotonic control. B, bulk ATP release (arbitrary light units) was measured from confluent HTC cells after isotonic and hypotonic (33%) exposures in control (open circles) or after incubation with PMA (closed circles). Filled triangles, isotonic exposure followed by PMA exposure (without hypotonic exposure). Each point represents mean ± S.E. (n = 5 trials each). C, cumulative data demonstrating effects of PKC stimulation (PMA 1 μM × 10 min) or inhibition by chelerythrine (20 μM × 15 min), calphostin C (3 μM × 15 min), or PMA (1 μM × 24 h) on hypotonic buffer-stimulated ATP release. Data represent mean ± S.E. of maximal ATP release (n = 5–8 for each). *, hypotonic buffer-stimulated ATP release was significantly increased by PMA, p < 0.05 versus hypotonic control; **, hypotonic buffer-stimulated ATP release was significantly decreased by inhibition of PKC, p < 0.05 versus hypotonic control.

dominant vesicular localization. Under control conditions, these showed spontaneous three-dimensional Brownian type motion and a low rate of spontaneous exocytosis. Hypotonic exposure in amounts sufficient to increase bulk extracellular ATP increased the observed frequency of exocytic events ~5-fold and decreased the number of quinacrine-labeled vesicles to ~20% of initial values.

Several additional observations support the conclusion that vesicular release of ATP is probably related to the observed increase in extracellular ATP concentrations. First, both constitutive and volume-stimulated release were temperature-sensitive, consistent with an exocytic mechanism (43). Second, the number of ATP vesicles and the amount of ATP released were inhibited by incubation with bafilomycin or brefeldin A to inhibit vesicular acidification or trafficking, respectively (33, 34, 40). Third, intact microtubules and phosphoinositide 3-kinase signaling are required for both vesicular exocytosis and ATP release. These conclusions are strengthened by the combined use of several modalities at different distance scales showing parallel regulation of exocytosis and ATP release. It is acknowledged that in bioluminescence studies, the point source pattern of ATP bioluminescence could result from channel-mediated ATP release, exocytosis of ATP containing vesicles, or exocytosis of ATP-permeable channels. Although the current studies do not resolve the molecular basis of ATP transport, they do support the conclusions that ATP is concentrated within vesicles and that intact exocytic mechanisms are required for volume-stimulated ATP release.

These findings are of interest in view of the recent identification of a volume-sensitive pool of vesicles in different liver cell models. Biliary cells, for example, possess a dense population of vesicles ~140 nm in diameter in the subapical space (44), and increases in cell volume increase the rate of exocytosis ~10-fold to values sufficient to replace 15–30% of plasma membrane surface area within minutes, through phosphoinositide 3-kinase-dependent mechanism (16). Both the rate of exocytosis and the amount of ATP released are potentiated by prior activation of PKC, suggesting that PKC is involved in the recruitment of these vesicles to a readily releasable state (16). These present observations support an analogous paradigm. Specifically, acute exposure to PMA to activate PKC had small effects on the rate of exocytosis of quinacrine-stained vesicles and no effect on the appearance of ATP in solution, indicating that activation of ATP alone is not sufficient to activate the full response. However, prior exposure to PMA greatly increased the response to hypotonic exposure as assessed by TIRF microscopy of quinacrine vesicles and measurement of ATP in solution. These observations suggest that PKC serves to prime a pool of ATP-containing vesicles, converting them from a nonreleasable state to a readily releasable state, although there is no direct morphological evidence to support this conclusion (16).

In summary, in this model liver cell, increases in cell volume stimulate exocytosis of a pool of vesicles enriched in ATP. The localized increase in extracellular ATP concentrations appears rapidly and extends over ~10 cell diameters, and within seconds, concentrations fall below detectable levels. The findings support a model wherein vesicular exocytosis is closely regulated to modulate the concentrations of ATP outside the cell in response to changing physiological demands. Moreover, because ATP can be metabolized into other purinergic agonists and liver cells express an array of purinergic receptor subtypes,
regulation of ATP availability is likely to play important roles in a range of liver cell and organ functions.

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