special communication

Cell swelling-induced ATP release and gadolinium-sensitive channels

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Boudreault, Francis, and Ryszard Grygorczyk. Cell swelling-induced ATP release and gadolinium-sensitive channels. Am J Physiol Cell Physiol 282: C219–C226, 2002. First published September 5, 2001; 10.1152/ajpcell.00317.2001.—ATP release induced by hypotonic swelling is an ubiquitous phenomenon in eukaryotic cells, but its underlying mechanisms are poorly defined. A mechanosensitive (MS) ATP channel has been implicated because gadolinium (Gd3+), an inhibitor of stretch-activated channels, suppressed ATP efflux monitored by luciferase bioluminescence. We examined the effect of Gd3+ on luciferase bioluminescence and on ATP efflux from hypotonically swollen cells. We found that luciferase was inhibited by \( < 10 \mu M \) Gd3+, and this may have contributed to the previously reported inhibition of ATP release. In ATP efflux experiments, luciferase inhibition could be prevented by chelating Gd3+ with EGTA before luminometric ATP determinations. Using this approach, we found that 10–100 \( \mu M \) Gd3+, i.e., concentrations typically used to block MS channels, actually stimulated hypotonically induced ATP release from fibroblasts. Inhibition of ATP release required at least 500, 200, or 100 \( \mu M \) Gd3+ for fibroblasts, A549 cells, and 16HBE14o− cells, respectively. Such biphasic and cell-specific effects of Gd3+ are most consistent with its action on membrane lipids and membrane-dependent processes such as exocytosis.

mechanosensitive adenosine 5′-triphosphate release; luciferase bioluminescence

ATP release induced by mechanical stimuli, such as hypotonic swelling, shear stress, or mechanical strain, has been observed in all eukaryotic cells examined (4, 16, 20–22, 27, 29, 31). Extracellular ATP and other nucleotides then interact with purinergic P2Y and P2X receptors to regulate a broad range of physiological responses, including vascular tone, muscle contraction, cell proliferation, mucociliary clearance, synaptic transmission, and platelet aggregation (5, 7, 12). The mechanism of mechanosensitive (MS) ATP release is not known, and several mechanisms have been proposed, including the fusion of ATP-enriched protein transport vesicles, ATP transporters, and MS ATP channels (6, 20, 23, 28, 29). The latter hypothesis was based on the observation that gadolinium (Gd3+), an inhibitor of stretch-activated cation channels, inhibited swelling-induced ATP efflux when it was monitored by luciferase bioluminescence. In particular, for epithelial cells, it was proposed that the cystic fibrosis transmembrane conductance regulator (CFTR) facilitates swelling-induced ATP release through a separate ATP-permeable channel and contributes to cell volume regulation. An MS channel was implicated because ATP release was blocked by Gd3+ (6).

ATP-dependent luciferase bioluminescence allows the detection of femtomoles of ATP; however, light output from the reaction depends on a number of factors, including ionic strength, pH, temperature, and the concentration of divalent cations (25), all of which may vary during the experiment. For example, experimental manipulations aimed at modulating ATP efflux from cells, such as application of hypotonic media or putative inhibitors, may directly interfere with the bioluminescence reaction and hinder accurate ATP detection. The trivalent lanthanide Gd3+ blocks mechanogated channels and has diverse, often nonspecific effects that include blockage of other channel types, induction of liposome fusion, and pore formation in erythrocytes (2, 9, 14, 18, 19, 32). Many of these actions may originate from binding of Gd3+ to phospholipids and alteration of physical properties of the cell membranes (13). In addition to direct and indirect inhibition of ion channels, lanthanides have been reported to inhibit several enzymes, including calcium ATPases, proteinases, and kinases (14, 15, 33). Use of Gd3+ is further complicated by its strong binding to certain anions that are often present in physiological and cell culture solutions such as phosphate, carbonate, sul-
fate, EDTA, albumin, and ATP (8, 14). Thus the concentration of free Gd\(^{3+}\) in the presence of these anions could be significantly overestimated, leading to erroneous conclusions (8).

In this study, we sought to examine the effect of Gd\(^{3+}\) on ATP detection by luciferase-luciferin bioluminescence and on ATP efflux from hypotonically swollen cells. We found that ATP-dependent luciferase bioluminescence was directly inhibited by \(<10 \mu M\) free Gd\(^{3+}\) and may have contributed to the previously reported apparent inhibition of ATP efflux from several cell types. When luciferase inhibition was prevented by chelating any Gd\(^{3+}\) before luminometric ATP determinations, we found that Gd\(^{3+}\) at 10–100 \(\mu M\) stimulated ATP efflux from hypotonically swollen 3T3 fibroblasts. Inhibition of ATP efflux from fibroblasts and lung epithelial A549 cells by Gd\(^{3+}\) was observed at 200–500 \(\mu M\), i.e., concentrations significantly higher than those used typically to directly block MS ion channels. Our results do not support the view that Gd\(^{3+}\)-sensitive channels are involved in ATP release and suggest that indirect and nonspecific effects of Gd\(^{3+}\) on lipid membrane and membrane-dependent processes should be considered.

METHODS

Cell culture. Adult human lung carcinoma A549 cells and NIH/3T3 fibroblasts (American Type Culture Collection) were grown in DMEM supplemented with 10% fetal bovine serum, 20 mM L-glutamine, 60 \(\mu g/ml\) penicillin G, and 100 \(\mu g/ml\) streptomycin. Human bronchial epithelial 16HBE14o cells, a generous gift from Dr. D. Gruenert, were cultured as described (10). All constituents of the culture media were purchased from Sigma. During the assay, 50 \(\mu l\) of the medium covering the cells was first treated with EGTA plus MgSO\(_4\) to chelate any Gd\(^{3+}\) before luminometric measurements, as described in more detail in RESULTS. For experiments requiring prolonged luminescence measurement, the luminometer was connected to a computer, and the luminescence signal was recorded and displayed in real time with software provided by the luminometer manufacturer and Microsoft Excel.

Cell viability test. The uptake of ethidium bromide, a nucleic marker, was monitored in selected experiments to evaluate cell viability and the possible contribution of cell lysis to ATP release. Cell monolayers were incubated in the presence of 0.15–0.3 \(\mu g/ml\) of ethidium bromide, and red-stained dead cells were counted by epifluorescence microscopy.

Solutions and chemicals. Physiological NaCl solution contained (in mM) 140 NaCl, 5 KCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 10 HEPES, and 10 glucose, pH 7.4, adjusted with NaOH. For hypotonic cell swelling, toxicity of the NaCl solution was reduced by adding an appropriate volume of distilled water or, in the majority of experiments, a solution that contained 1 mM MgCl\(_2\) and 1 mM CaCl\(_2\) to preserve the concentration of divalent cations during hypotonic shock. GdCl\(_3\) hexahydrate (Sigma) was prepared as a 100 mM stock solution in physiological extracellular saline with the luciferase reagent, and the average light signal was measured for 10 s with a TD20/20 luminometer (Turner Design, Sunnyvale, CA). ATP content was determined using [ATP] vs. luminescence standard curves, which were made for each experimental solution and each luciferase reagent preparation. In experiments in which Gd\(^{3+}\) was used to block ATP efflux from the cells, samples of extracellular media were first treated with EGTA plus MgSO\(_4\) to chelate any Gd\(^{3+}\) before luminometric measurements, as described in more detail in RESULTS. For experiments requiring prolonged luminescence measurement, the luminometer was connected to a computer, and the luminescence signal was recorded and displayed in real time with software provided by the luminometer manufacturer and Microsoft Excel.

RESULTS

Gd\(^{3+}\) directly inhibits luciferase bioluminescence. Hypotonicity-induced ATP release experiments are often performed by adding luciferase reagent directly to the cell’s extracellular media and measuring ATP-dependent bioluminescence in real time. However, change in salt concentration during hypotonic shock as well as the addition of putative ATP-release blockers could have a direct effect on the luciferase bioluminescence reaction. This is illustrated in Fig. 1A, where we simulated a typical hypotonicity-induced ATP efflux experiment in the absence of cells. The bioluminescence reaction was initiated in a test tube containing physiological extracellular saline with the luciferase reagent, by adding 10 or 100 nM ATP. Approximately 10 min later, when ATP-dependent luciferase bioluminescence reached a relatively stable level, the NaCl was diluted by 30% with distilled water containing the luciferase reagent to mimic the hypotonic shock applied to cells without diluting the luciferase reagent. This resulted in a steplike enhancement of light output despite concurrent dilution of ATP. Subsequent application of 50 \(\mu M\) GdCl\(_3\) caused \(-50\%\) inhibition of bioluminescence. These results demonstrate that Gd\(^{3+}\) and changes in NaCl concentration have a direct effect on luciferase bioluminescence and may contribute to an apparent modulation of ATP efflux observed with cells. It is noteworthy that the enhancement and inhibition of bioluminescence were smaller when 10 nM
ATP was used instead of 100 nM (Fig. 1A, traces labeled “low” ATP and “high” ATP, respectively). These effects, therefore, may go unnoticed in experiments when ATP concentration in the media is low. The direct inhibitory effect of Gd$^{3+}$ on luciferase bioluminescence was dose dependent and, under our experimental conditions, required relatively high concentrations similar to those reported previously to inhibit ATP efflux from cells, typically $\geq 100 \mu$M (Fig. 1B).

The extent of bioluminescence inhibition by Gd$^{3+}$ may vary with time and, to some extent, it also depends on the experimental protocol used. For example, somewhat different inhibition was observed when Gd$^{3+}$ was first incubated with luciferase and bioluminescence was recorded upon the subsequent addition of ATP, compared with the protocol in which Gd$^{3+}$ was preincubated with ATP before addition of the luciferase reagent. This latter case is illustrated in Fig. 1C, where the dose-inhibition curves of luciferase luminescence were determined at different time points after GdCl$_3$ was mixed with ATP. It shows that the GdCl$_3$ concentration required to produce 50% inhibition of bioluminescence was $\approx 300$ $\mu$M at 0.5 min but decreased significantly to 50 $\mu$M at 150 min. Longer incubation of ATP with Gd$^{3+}$ for up to 24 h did not significantly change this inhibition (not shown). Time-dependent inhibition may be relevant in ATP efflux assays when aliquots of extracellular media are taken at different time points, but their ATP content is measured later, after all the samples are collected.

Several commercially available luciferase reagents contain EDTA. In the experiments shown in Fig. 1, we used glycine-buffered firefly extract with added luciferin (Sigma, catalog no. F3641), which contains $\approx 0.5$ mM EDTA when dissolved at a concentration of 1 mg/ml. Because of strong binding of Gd$^{3+}$ to EDTA (14), the actual concentration of free Gd$^{3+}$ responsible for the observed inhibition of the luciferase reaction could be significantly lower. Indeed, this was confirmed in the experiment illustrated in Fig. 2. When nomi-
nally EDTA-free luciferase-luciferin mixture was used in an experiment similar to that shown in Fig. 1B, 50% inhibition of bioluminescence was observed with 10 μM Gd\(^{3+}\). This inhibition could be prevented by adding 250 μM EDTA. Data are representative of 3 experiments. B: comparison of bioluminescence dose-inhibition curves obtained in the absence and presence of 250 μM EDTA. The observed shift of the dose-inhibition curve is consistent with ~1:1 binding of Gd\(^{3+}\) to added EDTA. To avoid the time dependence shown in Fig. 1C, the protocol used here was slightly modified. In the experiment in Fig. 1C, Gd\(^{3+}\) and ATP were first mixed together and incubated for different time periods before being added to the test tube containing the luciferase reagent. In this experiment, Gd\(^{3+}\) was preincubated with the luciferase reagent for 5 min, and then peak luminescence was recorded immediately upon the addition of 10 nM ATP. Luminescence was normalized to that observed in the absence of Gd\(^{3+}\).

**Chelation of Gd\(^{3+}\) by EDTA prevents inhibition of luciferase in ATP efflux experiments.** Strong binding of Gd\(^{3+}\) by EDTA or EGTA may be used to remove Gd\(^{3+}\) from aliquots of extracellular media before determining ATP content by luminometry. This could allow studies of Gd\(^{3+}\) effects on ATP efflux from cells while avoiding its inhibitory action on luciferase bioluminescence. Figure 3 shows that incubation of Gd\(^{3+}\)-containing media with 25 mM EGTA plus 5 mM MgSO\(_4\) for 30 min chelated any Gd\(^{3+}\) and almost completely removed inhibition of bioluminescence. We used this method in subsequent studies of swelling-induced ATP efflux from cells.

**Reexamination of the effect of Gd\(^{3+}\) on ATP release from hypotonically swollen cells.** Figure 4 shows the accumulation of ATP in extracellular media bathing NIH/3T3 fibroblasts, human lung carcinoma A549 cells, and human bronchial epithelial 16HBE14o− cells, determined 25 min after hypotonic shock. Hypotonic shock was applied in the absence or presence of increasing concentrations of Gd\(^{3+}\), from 0 to 500 μM. After Gd\(^{3+}\) was chelated with EGTA plus MgSO\(_4\), ATP content in aliquots of extracellular media was determined by luminometry. Figure 4A demonstrates that in response to 50% hypotonic shock, the fibroblasts released approximately fivefold more ATP in the presence of 10–200 μM Gd\(^{3+}\) compared with the hypotonic shock applied in the absence of Gd\(^{3+}\). At 500 μM Gd\(^{3+}\), the amount of released ATP declined and was similar to that observed in the absence of Gd\(^{3+}\). Similar behavior was observed with A549 cells (Fig. 4B). The amount of ATP released in the presence of 30 μM Gd\(^{3+}\) was reproducibly, although statistically not significantly, increased and it was inhibited ~80% by 200 μM Gd\(^{3+}\). For 16HBE14o− cells, slight inhibition of hypotonically induced ATP release could be noticed already with 30

![Image](http://ajpcell.physiology.org/)
μM Gd$^{3+}$, but statistically significant inhibition required ≥100 μM (Fig. 4C). Hypotonic shock and Gd$^{3+}$ did not cause any detectable cell lysis during these experiments, as evaluated by ethidium bromide uptake and fluorescence microscopy (see METHODS). Under control conditions, typically 3–10 cells per 1,000 were found to be stained. The number of dead cells, however, did not increase during a 25-min exposure to 50% hypotonic shock.

**DISCUSSION**

The mechanisms underlying cell swelling-induced ATP release remain poorly defined. In this study, we have tested the hypothesis that Gd$^{3+}$-inhibitable channels are involved by evaluating the direct effect of Gd$^{3+}$ on luciferase bioluminescence and on ATP efflux from cells. We also have taken into account the tight binding of Gd$^{3+}$ to ATP and to other anions, such as EDTA, that are often present in luciferase reagent.

In cell-free experiments, we found that the luciferase bioluminescent reaction is directly blocked by <10 μM Gd$^{3+}$ and enhanced in hypotonic solution. Reduction of the luciferase assay sensitivity by Gd$^{3+}$ and its increase in hypotonic solution also were noticed in a recent study on MS ATP release from *Xenopus* oocytes (23). Block of luciferase bioluminescence by Gd$^{3+}$ may be responsible, at least in part, for the reported inhibition of ATP efflux from cells. Lanthanides have been previously reported to inhibit several enzymes, including proteinase, phosphoglycerate kinase, hexokinase, pyruvate kinase, calcium ATPase, and malic enzyme (14, 15, 33). It has been suggested that the mechanism of inhibition of several kinases involves competitive replacement of Mg$^{2+}$ by lanthanides in the biologically active Mg-ATP complex (14, 15). A similar mechanism may be responsible for the inhibition of luciferase bioluminescence observed in our study. Negatively charged phosphates on ATP bind more tightly to Gd$^{3+}$ than to Mg$^{2+}$ or Ca$^{2+}$. The logarithm of the association constant ($K_a$) for the ATP-metal ion complex is 7.1 for Gd$^{3+}$ at pH 8 (14), compared with 5.8 for Mg$^{2+}$ and 3.8 for Ca$^{2+}$ (24). This favors competitive displacement of Mg$^{2+}$ by Gd$^{3+}$ in the Mg-ATP complex. Inhibition of luciferase activity by the formation of biologically in-

![Fig. 4. Effect of Gd$^{3+}$ on ATP accumulation in the extracellular media of hypotonically swollen cells. ATP efflux was stimulated by applying 50% hypotonic shock to NIH/3T3 fibroblasts (A), lung carcinoma A549 cells (B), and bronchial epithelial 16HBE14o- cells (C) in the absence or presence of different GdCl$_3$ concentrations. This was achieved by diluting extracellular salts with hypotonic solution (see METHODS), which contained GdCl$_3$ at final concentrations as indicated. Twenty-five minutes after hypotonic shock, 25-μl aliquots of extracellular media were taken from control (no Gd$^{3+}$) and Gd$^{3+}$-treated cells and then mixed with 25 μl of solution containing EGTA + MgSO$_4$ to chelate Gd$^{3+}$. After a 15-min incubation, 50 μl of the luciferase reagent was added to each sample, and peak luminescence was recorded. ATP that accumulated in the extracellular media was expressed in % of control after subtraction of basal ATP content observed before hypotonic shock. The control values of ATP released by hypotonic shock, i.e., in the absence of Gd$^{3+}$, were 1.5 ± 0.6, 17 ± 4, and 11 ± 5 pmol/10$^5$ cells for 3T3 fibroblasts, A549 cells, and 16HBE14o- cells, respectively. Data are the average ± SE of 6 cell monolayers from 2–3 independent experiments. Data points show statistically significant differences (*) compared with the control (ANOVA and Fisher's protected least significant differences test, $P < 0.05$). For 3T3 fibroblasts, the accumulated ATP was significantly enhanced by 10–200 μM Gd$^{3+}$ ($P = 0.01$), but it was not different from control for 500 μM Gd$^{3+}$. For A549 cells, the accumulated ATP was statistically not different from the control for Gd$^{3+}$ concentrations of up to 100 μM but showed significant inhibition ($P < 0.0001$) at 200 and 500 μM. For 16HBE14o- cells, all tested concentrations of Gd$^{3+}$ appear to inhibit ATP accumulation, although the effect was only statistically significant ($P < 0.001$) at concentrations of 100 μM and higher.

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active Gd-ATP complex rather than direct binding of Gd$^{3+}$ to the luciferase enzyme agrees with our observation that after inhibition of bioluminescence by Gd$^{3+}$, the addition of fresh Mg-ATP to the reaction mixture produced a strong luminescence signal (data not shown).

Inhibition of bioluminescence required high concentrations of Gd$^{3+}$ (100–200 μM) when the luciferase reagent contained EDTA. However, when a nominally EDTA-free luciferase reagent was used, we observed a 50% block of bioluminescence by only 10–17 μM Gd$^{3+}$ (Fig. 2, A and B, respectively). This is because Gd$^{3+}$ strongly binds to EDTA and EGTA, with affinities even higher than for ATP (pK$_a$ of 17.4 and 17.5, respectively) (24). The presence of these molecules or other anions of high affinity for Gd$^{3+}$ in the luciferase reaction solution will affect the concentration of free Gd$^{3+}$ available to form the Gd-ATP complex and shift the threshold of inhibition to apparently higher GdCl$_3$ concentrations. This is demonstrated in Fig. 2B where the addition of 250 μM EDTA shifted the dose-inhibition curve to the right, a result consistent with 1:1 binding of EDTA and Gd$^{3+}$. Chelation of Gd$^{3+}$ may explain why inhibition of luciferase bioluminescence was not detected in cell-free tests in some studies. Furthermore, because GdCl$_3$ is typically >1,000-fold in excess of ATP (10 μM vs. 10 nM), it could be estimated that even when 95% of Gd$^{3+}$ is chelated by anions, essentially 100% of ATP will be bound in a Gd-ATP complex, leading to complete inhibition of the luciferase reaction. This may account for the very steep dose dependence of inhibition seen in Fig. 2B. As a consequence, minor differences in Gd$^{3+}$ binding capacities of solutions used in experiments with and without cells may lead to entirely different results, depending on whether any Gd$^{3+}$ was available to form the Gd-ATP complex. In some studies, 200–500 μM Gd$^{3+}$ was required to produce noticeable inhibition of bioluminescence, while in others it was blocked by 10–30 μM Gd$^{3+}$. The concentration typically used to block mechanogated channels and luciferase bioluminescence in our experiments (20, 28–30). These differences may reflect the varying composition of experimental solutions and their different capacity to bind Gd$^{3+}$. Although we have observed luciferase inhibition by as low as 10 μM GdCl$_3$, the concentration of free Gd$^{3+}$ in these experiments was certainly lower than that of total added GdCl$_3$ due to binding by other anions present in this luciferase reagent, such as sulfate, Tris, albumin, glycine (see METHODS), and traces of EDTA, which were left from the luciferase purification step (~100 nM according to the manufacturer).

To examine the effect of Gd$^{3+}$ on ATP efflux from cells while avoiding its inhibitory influence on the luciferase reaction, the samples of extracellular media containing Gd$^{3+}$ in this study were treated with EGTA plus MgSO$_4$ to chelate any Gd$^{3+}$ before the luminometry determinations of ATP content. Usually, to properly determine ATP content in extracellular solutions of different compositions, it is sufficient to make luminescence-ATP calibration curves for each experimental solution tested. This approach, however, will not work reliably for solutions containing Gd$^{3+}$ because it binds directly to ATP and often leads to complete loss of the bioluminescence signal. This is further complicated by the fact that inhibition is time dependent (Fig. 1C). This time dependence may be due to slow equilibration/redistribution of Gd$^{3+}$ between ATP and other Gd-binding anions such as phosphate, sulfate, carbonate, or EDTA, often present in the reaction solution.

The present study revealed that the effect of Gd$^{3+}$ on cell swelling-induced ATP release was cell-type specific and showed biphasic dependence for some cells. ATP release by fibroblasts was enhanced by low concentrations of Gd$^{3+}$ but was inhibited by higher concentrations, although differently for different cell types. Our results vary from those of earlier studies, which did not detect stimulation of ATP release, but reported 50–70% inhibition by ~200 μM Gd$^{3+}$, regardless of the cell type tested, including fibroblasts, human bronchial epithelial 16HBE14o- cells, rat hepatocytes, and cholangiocytes (28, 30). We believe that these differences could be attributed to direct inhibition of luciferase bioluminescence combined with partial chelation of the Gd$^{3+}$ added in those studies.

One possible mechanism of the enhanced ATP accumulation that we observed in the presence of Gd$^{3+}$ could be the inhibition of ATP hydrolysis by ectonucleotidases. This mechanism, however, is unlikely to contribute significantly, since ATP hydrolysis by cell ectonucleotidases is relatively slow at the ATP concentrations observed in our experiments (16). Thus an increased rate of ATP release in the presence of Gd$^{3+}$ is likely responsible for the enhanced ATP accumulation. This phenomenon may be related to the reported fusogenic effects of low concentrations of lanthanides (2), which could facilitate fusion of ATP-containing vesicles with the plasma membrane. Such a mechanism would, however, require extracellularly applied Gd$^{3+}$ to have access to the cytosolic side of the membrane. Although cell membranes are considered to be impermeable to lanthanides (14), a recent study suggests that La$^{3+}$, and to some extent Gd$^{3+}$, could be rapidly taken up into the cytosol of Chinese hamster ovary cells (17).

ATP release is clearly inhibited at higher Gd$^{3+}$ concentrations of 200–500 μM. Although 10 μM Gd$^{3+}$ is often sufficient to block mechanogated channels (19), the effects of higher Gd$^{3+}$ concentrations may be explained in terms of Gd$^{3+}$ interaction with the outer surface of cell membranes. Lanthanides have a high affinity for cellular membrane phospholipids, with K$_d$ values in the micromolar range (14). Binding of lanthanides to the outer surface of cell membranes not only decreases its negative surface charge but also produces structural changes in the membrane/water interface, lipid packing, and phase equilibrium (13). Membrane stiffening caused by Gd$^{3+}$ could decrease a cell’s osmotic sensitivity. For example, lanthanides have been reported to decrease osmotic fragility of erythrocytes, thus protecting them from hypotonic hemolysis (14).
The present data do not entirely exclude the possibility that stretch-activated channels may contribute to MS ATP release from some cell types. Although Gd\(^{3+}\) and other lanthanides were often found to have biphasic stimulator/inhibitory effects on exocytotic release of various neurotransmitters (1, 14), it should be noted that small stimulation of MS channel activity by low concentrations and inhibition by higher concentrations of Gd\(^{3+}\) were reported in Escherichia coli protoplasts (11). However, in Xenopus oocytes, which also possess MS and Gd\(^{3+}\)-sensitive channels, electrophysiological studies have demonstrated that mechanical stimuli much stronger than that required to cause ATP release failed to activate an increase in membrane conductance, ruling out the involvement of such channels in these cells (23, 27, 34). Further investigations are required to verify whether similar responses could be seen in cell lines tested in this study. Besides Gd\(^{3+}\), certain anion channel blockers, such as 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) and DIDS, were also reported to inhibit ATP release (6, 20, 26, 29). However, our preliminary experiments showed that 100 μM NPPB caused ~20% inhibition of the luciferase bioluminescence (data not shown). Furthermore, DIDS was also recently reported to directly interfere with luciferase activity (3). It may be desirable, therefore, to systematically reexamine the effect of various Cl\(^-\) channel blockers on luciferase bioluminescence before any firm conclusions can be drawn regarding their block of ATP release.

In summary, our data showing cell-specific and, for some cells, biphasic effects of Gd\(^{3+}\) on swelling-induced ATP release do not support the view that channels directly inhibitable by Gd\(^{3+}\) are involved. Rather, they are consistent with indirect effects of Gd\(^{3+}\) on lipid membranes. Therefore, other mechanisms should also be considered, such as the recently proposed exocytotic release due to fusion of ATP-enriched vesicles involved in transporting proteins from the Golgi complex to the cell surface (23).

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