Swelling-induced, CFTR-independent ATP Release from a Human Epithelial Cell Line

Lack of Correlation with Volume-sensitive Cl\(^{-}\) Channels

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abstract To examine a possible relation between the swelling-induced ATP release pathway and the volume-sensitive Cl\(^{-}\) channel, we measured the extracellular concentration of ATP released upon osmotic swelling and whole-cell volume-sensitive Cl\(^{-}\) currents in a human epithelial cell line, Intestine 407, which lacks expression of cystic fibrosis transmembrane conductance regulator (CFTR). Significant release of ATP was observed within several minutes after a hypotonic challenge (56–80% osmolality) by the luciferin/luciferase assay. A carboxylate analogue Cl\(^{-}\) channel blocker, 5-nitro-2-(3-phenylpropylamino)-benzoate, suppressed ATP release in a concentration-dependent manner with a half-maximal inhibition concentration of 6.3 \(\mu\)M. However, swelling-induced ATP release was not affected by a stilbene-derivative Cl\(^{-}\) channel blocker, 4-acetamido-4\(^{-}\)-isothiocyanostilbene at 100 \(\mu\)M. Glibenclamide (500 \(\mu\)M) and arachidonic acid (100 \(\mu\)M), which are known to block volume-sensitive outwardly rectifying (VSOR) Cl\(^{-}\) channels, were also ineffective in inhibiting the swelling-induced ATP release. Gd\(^{3+}\), a putative blocker of stretch-activated channels, inhibited swelling-induced ATP release in a concentration-dependent manner, whereas the trivalent lanthanide failed to inhibit VSOR Cl\(^{-}\) currents. Upon osmotic swelling, the local ATP concentration in the immediate vicinity of the cell surface was found to reach ~13 \(\mu\)M by a biosensor technique using P2X\(_2\) receptors expressed in PC12 cells. We have raised antibodies that inhibit swelling-induced ATP release from Intestine 407 cells. Earlier treatment with the antibodies almost completely suppressed swelling-induced ATP release, whereas the activity of VSOR Cl\(^{-}\) channel was not affected by pretreatment with the antibodies. Taking the above results together, the following conclusions were reached: first, in a CFTR-lacking human epithelial cell line, osmotic swelling induces ATP release and increases the cell surface ATP concentration over 10 \(\mu\)M, which is high enough to stimulate purinergic receptors; second, the pathway of ATP release is distinct from the pore of the volume-sensitive outwardly rectifying Cl\(^{-}\) channel; and third, the ATP release is not a prerequisite to activation of the Cl\(^{-}\) channel.

key words: adenosine triphosphate • anion channel • osmotic swelling • cell volume regulation

INTRODUCTION

Osmotic cell swelling leads to extrusion of a number of intracellular inorganic and organic osmolytes, thereby accomplishing the regulatory volume decrease (RVD)\(^{1}\) (Lang, 1998; Okada, 1998). Recently, release of intracellular ATP was found to be induced by osmotic swelling in human (Hazama et al., 1998b; Taylor et al., 1998), bovine (Mitchell et al., 1998), and mouse (Hazama et al., 1998b) epithelial cells, as well as in rat hepatoma cells (Wang et al., 1996; Roman et al., 1997). Since ATP exists in the cytoplasm at millimolar concentrations, opening of the large pore may induce a passive efflux of ATP. Actually, it is known that a mitochondrial outer membrane large-conductance voltage-dependent anion channel is permeable to anionic ATP (Rostovtseva and Colombini, 1997). However, the plasma membrane ATP channel has not been identified.

Several studies have suggested that the cystic fibrosis transmembrane conductance regulator (CFTR) Cl\(^{-}\) channel can act as an ATP-permeable channel (Reisin et al., 1994; Schiewbert et al., 1995; Cantiello et al., 1998). The CFTR-mediated ATP release hypothesis has now been challenged by many contradictory reports (Grygorczyk et al., 1996; Li et al., 1996; Reddy et al., 1996; Grygorczyk and Hanrahan, 1997; Sugita et al., 1998). On the other hand, swelling-activated Cl\(^{-}\) chan-
nels are known to show considerable permeability to large organic anions (Strange et al., 1996; Nilius et al., 1997; Okada, 1997). Thus, it is possible that the volume-sensitive Cl\(^{-}\) channel may serve as a pathway for ATP release. As proposed by Wang et al. (1996) and Roman et al. (1997), there is the possibility that activation of the volume-sensitive Cl\(^{-}\) channel is induced by released ATP through stimulation of P2 receptors. In the present study, therefore, we addressed the question of whether the volume-sensitive Cl\(^{-}\) channel serves the pathway of swelling-induced ATP release or its activity is a prerequisite to swelling-induced ATP release, and whether activation of the volume-sensitive Cl\(^{-}\) channel is dependent on swelling-induced ATP release in a human epithelial cell line that lacks CFTR expression.

**MATERIALS AND METHODS**

**Cells**

Human epithelial Intestine 407 cells were cultured in Fischers’ medium supplemented with 10% newborn calf serum, as described previously (Hazama and Okada, 1988). For the luminometric ATP measurements, the cells were plated on 48-well plastic plates. The ATP assay was made at the density of 4 \times 10^5 cells per well. For the biosensor ATP measurements, Intestine 407 cells were cultured on glass coverslips, and then transferred to microscopic chambers. For the patch-clamp whole-cell recordings, Intestine 407 cells were cultured in suspension with agitation for 10–120 min, and then plated on the experimental chamber. PC12 cells that express the P2X\(_1\) receptor channel were cultured, as reported previously (Hazama et al., 1998a).

**Luciferin/Luciferase ATP Assay**

The extracellular bulk ATP concentration was measured by luciferin-luciferase luminesmetry using an AF-100 ATP analyzer and AF-211 reagents (TOA Electronics). The culture medium was totally replaced by 300 \mu l of isotonic PBS containing (mM): 137 NaCl, 2.7 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 8.1 Na\(_2\)HPO\(_4\), and 1.5 KH\(_2\)PO\(_4\) (pH 7.4, 300 mosmol/l kg H\(_2\)O). It is known that ATP release is dramatically stimulated by mechanical stimulation, such as a medium displacement and tilting the monolayer cells or touching to the cells (Grygorczyk and Hanrahan, 1997; Watt et al., 1998). Preliminary experiments showed that this is the case for Intestine 407 cells. However, ATP release induced by replacement of the entire bathing solution subsided to the background level after 30–60 min. Also, mechanical ATP release was found to be avoided by gentle replacement of one third to one half of the solution by a new solution without touching and tilting the monolayer cells. Thus, after a 60-min equilibration, 100 \mu l of the extracellular isotonic solution (300 \mu l) was collected as a control sample and the ATP concentration was measured. Then, 200 \mu l of hypotonic solution was gently added to the remaining 200 \mu l of isotonic solution to reduce the extracellular osmolality down to the desired level (56% osmolality, unless indicated). The hypotonic solutions were prepared by mixing the isotonic PBS with a solution containing 5 mM KCl, 1 mM MgCl\(_2\), 1 mM CaCl\(_2\), and 50 mM HEPES/NaOH (pH 7.4, 35 mosmol/l kg H\(_2\)O). The test samples (100 \mu l) were collected after incubation for the indicated times (usually 15 min) at 37°C and the ATP concentration was measured at room temperature (24–26°C). When necessary, extracellular Ca was removed and 0.1 mM EGTA was added during a hypotonic challenge. The effects of Cl\(^{-}\) channel blockers were observed by adding the drug to the hypotonic solution. Only glibenclamide (500 \mu M) among the employed drugs was found to suppress (by 20–25%) the luciferase detection of ATP, as reported previously (Taylor et al., 1998). Thus, the data of glibenclamide effect were corrected by the calibration curve obtained by reactions with known amounts of ATP added to cell-free isotonic or hypotonic solution with or without 500 \mu M glibenclamide. The effect of pretreatment with antibodies was observed after incubating in the isotonic solution supplemented with 12.5 \mu g/ml antibodies for 60 min, and then applying the hypotonic solution in the absence of antibodies.

**Cell Viability Assay**

After incubating Intestine 407 cells in the above isotonic or hypotonic solution for 30 min at 37°C, cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Mossman, 1983) using the Cell Counting Kit (Dojindo) according to the manufacturer’s instructions. This method is based on the ability of viable (but not dead) cells to cleave the tetrazolium ring of the MTT into a dark blue formazan reaction product.

**Biosensor ATP Assay**

The local concentration of released ATP at the single cell surface was measured by a biosensor technique on single Intestine 407 cells, using PC12 cells expressing ligand-gated cation channels, P2X\(_1\) receptors, as described previously (Hazama et al., 1998a). In brief, after attaining the whole-cell configuration with a PC12 cell, the cell was lightly attached to the surface of an Intestine 407 cell under a microscope. The whole-cell currents were recorded at a holding potential of −50 mV using an Axopatch 200A amplifier (Axon Instruments) at room temperature. From the whole-cell current density thus recorded, the local ATP concentration was estimated by the calibration curve obtained by the response of PC12 cells alone to known amounts of ATP, as described previously (Hazama et al., 1998a). To apply hypotonic stimulation, the bathing solution was changed from isotonic to hypotonic solution by perfusion. The isotonic solution contained (mM): 100 NaCl, 4.2 KCl, 100 mannitol, 8 HEPES, 6 HEPES-Na, 1 CaCl\(_2\), and 1 MgCl\(_2\) (320 mosmol/kg H\(_2\)O, pH 7.4). The hypotonic solution (220 mosmol/kg H\(_2\)O, pH 7.4) was prepared by removing mannitol from the isotonic solution. The pipette (intracellular) solution was composed of (mM): 150 CsCl, 1 MgCl\(_2\), 10 EGTA, and 10 HEPES (280 mosmol/kg H\(_2\)O, pH 7.4 with CsOH).

**WholeCell Cl\(^{-}\) Current Measurements**

Volume-sensitive Cl\(^{-}\) channel currents were recorded by the whole-cell patch clamp technique, as reported previously (Liu et al., 1998). The time course of current activation and recovery was monitored by repetitively applying alternating step pulses of ±40 mV (2-s duration) from a prepotential of 0 mV. To monitor the voltage dependence of the current, stepping pulses (2-s duration) were sometimes applied from a prepotential at −100 mV (0.6-s duration) to test potentials of −80 to −100 mV in 20-mV increments. Currents were recorded using an Axopatch 200A amplifier, filtered at 1 kHz and digitized at 4 kHz. Cs-rich solutions were employed. Isotonic CsCl bathing solution contained (mM): 110 CsCl, 10 HEPES, 8 Tris, 5 MgSO\(_4\), and 100 mannitol (320 mosmol/kg H\(_2\)O, pH 7.4). Cell swelling was induced by hypotonic (260 mosmol/kg H\(_2\)O) CsCl solution in which mannitol was reduced to 40 mM. The pipette (intracellular) CsCl solution contained (mM): 110 CsCl, 2 MgSO\(_4\), 1 Na\(_2\)ATP, 15 NaHPO\(_4\), 10 HEPES, and 100 Tris (280 mosmol/kg H\(_2\)O, pH 7.4 with CsOH).
HEPES, 10 HEPES, 1 EGTA, and 50 mannitol (290 mosm/kg H2O, pH 7.3).

Antibody Production

Since the molecular pathway for swelling-induced ATP release has not as yet been identified, we attempted to produce antibodies against unidentified extracellular epitopes of plasma membrane-associated protein involved in swelling-induced ATP release. Monoclonal antibodies were obtained according to the method described elsewhere (Tsukita et al., 1989). In brief, BALB/c mice were immunized with 10⁶ Intestine 407 cells. Peripheral lymphocytes were fused with myeloma NS-1 cells using 50% polyethylene glycol (PEG 4000). The supernatant from the resulting hybridoma cells was screened to find colonies that had inhibitory effects on ATP release from swollen Intestine 407 cells. The wells that showed positive activity (15 of 288 wells) were immediately expanded and plated in 96-well plates. Positive colonies were found in 12 of 1,470 wells. The positive hybridoma cells were injected to the mice to produce ascites culture. One of these, termed H2D2-5, was employed for the present experiment.

Chemicals

The following agents were added to bathing solutions: 0.1 mg/ml apyrase, 10–30 μM 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), 100 μM 4-acetamido-4'-isothiocyanostilbene (SITS), 500 μM glibenclamide, 100 μM arachidonic acid, and 5–30 μM GdCl₃.

Results

Swelling-induced ATP Release

Luminometric ATP assay showed that Intestine 407 cells respond to a hypotonic challenge not only with cell swelling but also with significant release of ATP. The swelling-induced ATP release was found to start within a minute and continued for over 10 min (Fig. 1 A). From the results collected at 1 min after a hypotonic challenge, the initial rate of swelling-induced ATP release from a single Intestine 407 is estimated to be ~2,500 ATP molecules/s under the assumption that degradation or reuptake of released ATP was negligible.

ATP release increased with decreasing extracellular osmolality (Fig. 1 B). The cell viability was not essentially affected by the hypotonic challenge (Fig. 1 C).

The mean values of bulk ATP concentration in 400 μl ambient solutions of 4×10⁵ cells were 0.47 ± 0.08 nM (n = 39) after a 60-min exposure to isotonic solution and 2.63 ± 0.44 nM (n = 53) after a 15-min exposure to hypotonic (56% osmolality) solution. The mean rate of ATP release from a single Intestine 407 cell during a 15-min exposure to a hypotonic solution is estimated to be ~1,700 ATP molecules/s, and that during a 60-min exposure to isotonic solution is ~60/s.

A biosensor ATP assay method (Hazama et al., 1998a) was employed to assess the ATP concentration at the outer surface of swollen Intestine 407 cells. Hypotonic stimulation failed to evoke currents in PC12 cell alone (data not shown, n = 11). However, when a voltage-clamped PC12 cell was placed close to an Intestine 407 cell, a series of spiky and sustained inward currents were induced within several minutes after exposure to hypotonic solution (69%) at room temperature (Fig. 2 A). ATP release was found to be always coupled to cell swelling under a microscope. In the presence of an ATP-hydrolyzing enzyme, apyrase, in contrast, the inward current response to hypotonic challenge was virtually abolished, as shown in Fig. 2 B. In the absence of apyrase, the average peak response was 7.9 ± 1.2 pA/pF (n = 20), which corresponds to 13.1 μM ATP on a calibration curve of ATP-induced PC12 responses (Fig. 2 C).

Effects of Cl⁻ Channel Blockers on Swelling-induced ATP Release and Cl⁻ Currents

A possible relation of swelling-induced ATP release with the activity of volume-sensitive Cl⁻ channel was investigated.
Swelling-activated ATP Release and Cl\textsuperscript{−} Channel Investigated by Applying a High Concentration of Cl\textsuperscript{−} Channel Blockers. Luminometric ATP assay showed that swelling-induced ATP release was not blocked by 100 \textmu M SITS (Fig. 3), as found previously (Hazama et al., 1998b). Glibenclamide and arachidonic acid were also ineffective at the concentrations by which volume-sensitive Cl\textsuperscript{−} channel currents are known to be almost completely abolished in Intestine 407 cells (Kubo and Okada, 1992; Liu et al., 1998). Glibenclamide insensitivity is in good agreement with a previous observation of swelling-induced ATP release from rabbit ciliary epithelial cells (Mitchell et al., 1998), but is in contrast to those of mechanical stress–induced ATP release from rabbit red blood cells (Sprague et al., 1998) or Ehrlich ascites tumor cells (Pedersen et al., 1999) and cAMP-activated ATP release from CFTR-expressing human epithelial cells (Schwiebert et al., 1995).

Since swelling-induced ATP release was reported to be largely inhibited by a Cl\textsuperscript{−} channel blocker, NPPB, in rabbit ocular ciliary epithelial cells at 100 \textmu M (Mitchell et al., 1998), we then examined the NPPB effect. As shown in Fig. 4 A, NPPB was found to inhibit ATP release from swollen Intestine 407 cells at lower concentrations. The concentration required for 50\% inhibition (IC\textsubscript{50}) was 6.3 ± 0.3 \textmu M (Fig. 4 B, □). Cell swelling activated outwardly rectifying whole-cell Cl\textsuperscript{−} currents in Intestine 407 cells, and this volume-sensitive outwardly rectifying (VSOR) Cl\textsuperscript{−} current was inhibited by NPPB, as reported previously (Kubo and Okada, 1992). The IC\textsubscript{50} value for Cl\textsuperscript{−} current inhibition was 16.0 ± 0.2 \textmu M (Fig. 4 B, ■), which is significantly different from that for ATP release inhibition (0.01 < P < 0.05).

These results suggest that swelling-induced ATP release is independent of the activity of volume-sensitive outwardly rectifying Cl\textsuperscript{−} channels in Intestine 407 cells.

Effects of Gadolinium on Swelling-induced ATP Release and Cl\textsuperscript{−} Currents
Recently, Taylor et al. (1998) showed that swelling-induced ATP release from human airway epithelial cells was in-
hibited by a trivalent lanthanide, Gd$^{3+}$, which is the most commonly used blocker of mechanogated ion channels (Hamill and McBride, 1996). Luciferin/luciferase ATP assay showed that ATP release from swollen human epithelial Intestine 407 cells was also sensitive to Gd$^{3+}$ (Fig. 5). The Gd$^{3+}$ effect was concentration dependent, with $IC_{50}$ of $\sim$10 $\mu$M. Since Intestine 407 cells are known to have Gd$^{3+}$-sensitive, stretch-activated, Ca$^{2+}$-permeable cation channels (Okada et al., 1990), there is a possibility that the Gd$^{3+}$ effect on ATP release had been mediated by the inhibiting effect on Ca$^{2+}$ influx through this stretch-activated cation channel. However, swelling-activated ATP release was not inhibited, but rather enhanced [from 1.18 $\pm$ 0.12 nM ($n = 11$) to 3.02 $\pm$ 0.27 nM ($n = 20$); $P = 0.000002$], by Ca$^{2+}$ removal from the extracellular solution. A similar enhancing effect of Ca$^{2+}$ removal was previously observed on cAMP-activated ATP release from CFTR-expressing cells and was explained by a cofactor role of extracellular Ca$^{2+}$ for ecto-ATPase activation (Prat et al., 1996).

Gd$^{3+}$ (30 $\mu$M) also inhibited swelling-activated ATP release in the Ca$^{2+}$-free conditions [to 1.80 $\pm$ 0.39 nM ($n = 19$); $P = 0.014$].

The effect of Gd$^{3+}$ on the volume-sensitive Cl$^{-}$ current was then examined in Intestine 407 cells. Gd$^{3+}$ (30 $\mu$M), which was applied 10–20 min before and during hypotonic challenge, failed to affect swelling-induced activation of the Cl$^{-}$ current (Fig. 6 A, left). Neither time- and voltage-dependent inactivation kinetics (Fig. 6 A, right) nor outwardly rectifying current–voltage relation (Fig. 6 B) was affected by Gd$^{3+}$.

These results clearly indicate that the activity of volume-sensitive outwardly rectifying Cl$^{-}$ channels is totally independent of the swelling-induced ATP release in Intestine 407 cells.

Effects of Antibodies on Swelling-induced ATP Release and Cl$^{-}$ Currents

To assess the molecular nature of swelling-induced ATP release pathway, we raised antibodies that can block ATP release upon osmotic swelling in Intestine 407 cells. As summarized in Fig. 7, pretreatment with the H2D2-5 antibodies at 12.5 $\mu$g/ml for 60 min markedly...
inhibited swelling-induced ATP release and slightly suppressed basal background ATP release. In contrast, mouse IgG failed to affect significantly the ATP release. Similar inhibiting effects were also obtained by pretreatment with the antibodies at 25 μg/ml for 30 min (data not shown, n = 13).

As shown in Fig. 8, VSO R Cl− currents were not significantly affected by pretreatment with H2D2-5 antibodies (25 μg/ml, 30–50 min). The mean peak current densities with pretreatment with the antibodies (n = 9) and the negative control IgG (n = 10) were 274.3 ± 32.6 and 281.4 ± 46.1 pA/pF (P = 0.45) at +60 mV and 166.1 ± 20.7 and 165.8 ± 28.0 pA/pF (P = 0.25) at −60 mV, respectively.

These results also show that the swelling-induced ATP release is not a prerequisite to activation of the volume-sensitive Cl− channel in Intestine 407 cells. Also, it is likely that the swelling-induced ATP release pathway is distinct from the pore of volume-sensitive outwardly rectifying Cl− channels in the human epithelial cell line.

**DISCUSSION**

Extracellular ATP at low micromolar concentrations is known to influence activities in a number of muscular and neuronal cells as well as platelets (Gordon, 1986). Recently, ATP release has been found to be induced by stimulation with cAMP (Schwiebert et al., 1995; Prat et al., 1996; Jiang et al., 1998), by removing extracellular Cl− (Rotoli et al., 1996), by mechanical perturbation (Grygorczyk and Hanrahan, 1997; Sprague et al., 1998; Pedersen et al., 1999), and by osmotic perturbation (Wang et al., 1996; Mitchell et al., 1998; Taylor et al., 1998) in nonneuronal, nonmuscular cells. CFTR was reported to be associated with ATP release induced by these maneuvers by some investigators (Schwiebert et al., 1995; Prat et al., 1996; Rotoli et al., 1996; Jiang et al., 1998).
current responses to step pulses (at *).

The pulse protocols were the same as those in Fig. 6. Gain of 

ulate P2-purinergic receptors (ATP present at the cell surface (Plesner, 1995). This 
cells express ecto-nucleotidases that rapidly hydrolyze 

This value would be an underestimate because most 

ular ATP is involved in the RVD after osmotic swelling: 

ergetic receptors (Yada et al., 1989), it is possible that 

Moatassim, 1993). Since Intestine 407 cells have puri-

receptor, suramin, inhibited RVD in both cell species (Wang et al., 1996; Roman et al., 1997; Tsumura et al., 1998).

Roman et al. (1997) and Wang et al. (1996) proposed 

the hypothesis that the released ATP is involved in activa-

tion or upregulation of volume-sensitive Cl\(^-\) channels, which are associated with Cl\(^-\) efflux during RVD, 

in rat hepatoma cells. However, addition of extracellu-

lar ATP did not activate inward Cl\(^-\) currents (Cl\(^-\) efflux), but inhibited outward Cl\(^-\) currents (Cl\(^-\) influx) 

under hypotonic conditions in human epithelial Intest-

ine 407 cells (Tsumura et al., 1996) and rat glioma C6 
cells (Jackson and Strange, 1995). Furthermore, the 

present study showed that Gd\(^{3+}\) (30 \(\mu\)M) virtually abol-

ished swelling-induced ATP release (Fig. 5) without af-

ecting activation of swelling-induced VSOR Cl\(^-\) cur-

rrents in Intestine 407 cells (Fig. 6). In addition, even 

when the ATP release had been blocked by anti-ATP 

release antibodies (Fig. 7), the activity of the VSOR Cl\(^-\) 

channel was normally induced upon osmotic swelling 
(Fig. 8). In Intestine 407 cells, therefore, it appears that 

ATP released upon osmotic swelling facilitates RVD by 

a mechanism other than activation of volume-sensitive, 

outwardly rectifying Cl\(^-\) channels or regulation of the 

volume-regulatory Cl\(^-\) efflux. Intestine 407 cells are 

known to respond to extracellular ATP with an increase in 

cytosolic Ca\(^{2+}\) and Ca\(^{2+}\)-activated K\(^+\) conductance (Yada et al., 1989). Ca\(^{2+}\)-activated K\(^+\) conductance was shown to be responsible for volume-regulatory K\(^+\) efflux during RVD of Intestine 407 cells (Hazama and Okada, 1988). Taken together, it can be speculated that 

swelling-induced ATP release brings about autocrine stimulation of Ca\(^{2+}\)-activated K\(^+\) efflux by stimulation of purinergic receptors, thereby facilitating RVD.

The route of swelling-induced ATP release is not yet identified, although some investigators have suggested that CFTR (Reisin et al., 1994; Schwiebert et al., 1995; Cantiello et al., 1998) and MDR1 (Abraham et al., 1996; Tominaga et al., 1995) are involved in the route. The involvement of CFTR can be excluded in the present study because Intestine 407 cells lack expression of CFTR (Hazama et al., 1998b) and failed to respond with ATP release to 
stimulation with 1 mM dibutyryl cyclic AMP (Tanaka, S., and Okada, Y., unpublished observations). In fact, a 

blocker of CFTR Cl\(^-\) channel, glibenclamide, failed to 

suppress swelling-induced ATP release in the present 

study (Fig. 3). Since Intestine 407 cells are known to ex-

press MDR1 (Tominaga et al., 1995), the involvement

![Figure 8](image)

**Figure 8.** Effects of pretreatment with anti-ATP release antibodies on whole-cell volume-sensitive Cl\(^-\) currents from swollen Intestine 407 cells. The representative records after 45 min treatment with 25 \(\mu\)g/ml mouse IgG (left) and H2D-25 antibodies (right). The pulse protocols were the same as those in Fig. 6. Gain of chart recorder was changed by 40%. (Bottom) Expanded traces of current responses to step pulses (at *).
of MDR1 in swelling-induced ATP release seems possible. However, the transporter function of P-glycoprotein, the product of the MDR1 gene, has recently been shown to be inhibited by glibenclamide (Golstein et al., 1999). Also, our recent studies have demonstrated that osmotic swelling induced ATP release to an essentially similar degree in both human epidermoid KB cells lacking MDR1 expression (KB-3-1) and a MDR1-transfected KB cell line (KB-G2) (Hazama, A., and Y. Okada, unpublished observations).

It seems unlikely that the pore of the volume-sensitive outwardly rectifying Cl− channel can provide a pathway of swelling-induced ATP release in human epithelial Intestine 407 cells because: (a) blockers of the Cl− channel, SITS, arachidonate and glibenclamide, did not abolish swelling-induced ATP release (Fig. 3), (b) a potent blocker of swelling-induced ATP release, Gd3+, did not inhibit the Cl− currents (Fig. 6), and (c) antibodies that inhibit swelling-induced ATP release never affected the Cl− currents (Fig. 8). However, the possibility remains that the ATP release represents an ATP efflux through ATP-permeable anion channels that can be activated by cell swelling, but is distinct from the volume-sensitive outwardly rectifying Cl− channel. Since the rate of swelling-induced ATP release seems very low (on the order of 10^{3} \text{ s}^{-1} \text{ per cell}) compared with the transporting rate of many types of ion channels (10^{6}–10^{8} \text{ s}^{-1} \text{ per channel}), it is also possible that the ATP release is mediated by transporter or exocytosis, but not by ATP-selective channels.

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