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ATP Crossing the Cell Plasma Membrane Generates an Ionic Current in *Xenopus* Oocytes*

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Elena Bodas‡§, Jordi Aleu‡, Gemma Pujol, Mireia Martín-Satué, Jordi Marsal, and Carles Solsona¶

From the Laboratory of Molecular and Cellular Neurobiology, Department of Cell Biology and Pathology, Medical School, Hospital of Bellvitge, University of Barcelona, Campus of Bellvitge, Feixa Llarga s/n, L'Hospitalet de Llobregat, E-08907 Barcelona, Spain

The presence of ATP within cells is well established. However, ATP also operates as an intercellular signal via specific purinoceptors. Furthermore, nonsecretory cells can release ATP under certain experimental conditions. To measure ATP release and membrane currents from a single cell simultaneously, we used *Xenopus* oocytes. We simultaneously recorded membrane currents and luminescence. Here, we show that ATP release can be triggered in *Xenopus* oocytes by hyperpolarizing pulses. ATP release (3.2 ± 0.3 pmol/oocyte) generated a slow inward current (2.3 ± 0.1 μA). During hyperpolarizing pulses, the permeability for ATP^{4-} was more than 4000 times higher than that for Cl^-. The sensitivity to $GdCl_3$ (0.2 mM) of hyperpolarization-induced ionic current, ATP release and E-ATPase activity suggests their dependence on stretch-activated ion channels. The pharmacological profile of the current inhibition coincides with the inhibition of ecto-ATPase activity. This enzyme is highly conserved among species, and in humans, it has been cloned and characterized as CD39. The translation, in *Xenopus* oocytes, of human CD39 mRNA encoding enhances the ATP-supported current, indicating that CD39 is directly or indirectly responsible for the electrodiffusion of ATP.

Eukaryotic cells constantly form ATP, which has a large variety of functions. In addition, exocytosis of a variety of granules or vesicles results in ATP release, such as in the neuromuscular junction (1), in rat cerebral cortex synaptosomes (2), in celiac neurons (3), in isolated cholinergic nerve terminals from *Torpedo* electric organ (4), and chromaffin cells (5). Nonsecretory cells can also release ATP, as in skeletal muscle (6), heart cells (7), erythrocytes (8), *Torpedo* electric organ (9), astrocytes (10), smooth muscle (11), and airway epithelium (12). Because cells of this type contain few secretory granules or secretory vesicle-like structures, it has also been suggested that ATP may not be released through a membrane fusion pathway.

Indirect evidence has been obtained in support of the hypothesis of nonsecretory exocytotic release of ATP. In patch clamp experiments (13–15), it was found that cell membranes containing cystic fibrosis transmembrane regulator (CFTR)[1] or related proteins may be permeable to ATP. Direct measurement of ATP release by myosin functionalized tips of atomic force microscope reveals hot spots of ATP release on the surface of epithelial cells expressing CFTR (16). Other experimental reports (12, 17–20) do not support this view, suggesting that the diameter of the pore of CFTR is too small to allow ATP to cross the plasma membrane. However, in this type of experiment, it is impossible to measure ATP release and ionic currents simultaneously in an individual cell, and so it was impossible to establish a direct relationship between the current activation and the release of ATP.

We have developed a simple method to detect ATP release in oocytes subjected to a two-electrode voltage-clamp (Fig. 1*A*). We monitored, on line and simultaneously, the release of ATP and the currents flowing across the plasma membrane in a single cell.

EXPERIMENTAL PROCEDURES

Simultaneous Voltage-Clamp Recording and ATP Release—Procedures for preparation of *Xenopus laevis* oocytes and voltage-clamp recording were as described (21). ATP detection was performed at least 48 h after defolliculation. Oocytes were placed in a dark chamber at 20 °C and bathed in a recording solution containing 115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES/NaOH, pH 7.4. A vial of firefly lantern luciferase extract from *Photinus pyralis* (Sigma) was diluted in 2 ml of the recording solution containing 5 mg/ml D-luciferin (Sigma). The resulting suspension was centrifuged for 30 s in a benchtop centrifuge. The supernatant was desalted in a 10-ml 10DG Bio-Rad column equilibrated with recording solution containing 1.8 mM $MgCl_2$. The eluate was maintained at 4 °C and placed in the 250-μl recording chamber. A plastic optic fiber of 1 mm diameter was placed above the upper surface of an oocyte bathed in a luciferin-luciferase enriched solution, between the two recording electrodes, and the other end of the fiber was placed in front of a photomultiplier (Hamamatsu, R374). The resulting electric signal was filtered at 5 Hz in a Bessel filter (Frequency Devices) and collected by the Digidata-1200A converter (Axon Instruments). In some cases, a known dose of ATP was added through a third pipette, with a calibrated volume ejected by pressure (PLI-100, Medical Systems). In some experiments, six optic fibers were placed in front of the six planes of the recording cuvette.

Characterization of Ionic Currents—In some experiments, *Xenopus* oocyte plasma membrane capacitance was measured before and after applying a hyperpolarizing pulse from −60 to −180 mV. Cell membrane electrical capacitance was calculated by recording the capacitative transient associated with a small voltage step (from −60 to −90 mV), during

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‡ These authors contributed equally to this work.
§ Recipient of a grant from Fundació August Pi i Sunyer.
¶ To whom correspondence should be addressed. Tel.: 34-93-402-4279 or 34-93-403-5809; Fax: 34-93-403-5810; E-mail: solsona@bellvitge.bvg.ub.es.

[1] The abbreviations used are: CFTR, cystic fibrosis transmembrane regulator; DIDS 4,4′-diisothiocyanatostilbene-2′-disulfonic acid; SITS, 4-acetamido-4′isothiocyanatostilbene-2,2′-disulfonic acid; E-type ATPase, ecto-ATPase.

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which the inward current was not activated. Integration of the transient yielded the charge (Q) transferred during the voltage step (V), from which capacitance (C) was calculated as follows: $C = Q/V$.

The reversal potential was calculated while the current was still flowing. 100-ms pulses ranging from –220 to –150 mV, in 10-mV jumps, were imposed in the last 2 s of 20-s hyperpolarizing pulses. The currents were plotted against voltage, and extrapolation to zero was calculated. The reversal potential of the residual current, calculated at different potentials at the end of the 10-s hyperpolarizing pulse, was –22 ± 3 mV (n = 10), ranging from –15 to –23 mV.

Permeability of the membrane to ATP^{4-} was calculated according to the Goldman-Hodgkin-Katz equation, as follows,

$$\frac{P_{Cl^-}}{P_{ATP}} = \frac{|Z_{ATP}|}{|Z_{Cl^-}|} \cdot \frac{\dfrac{[ATP]_i}{1 - e^{EF/RT}}}{\dfrac{[Cl^-]_i - [Cl^-]_o \cdot e^{EF/RT}}{1 - e^{EF/RT}}} \quad \text{(Eq. 1)}$$

where F is Faraday's constant, R is the gas constant, T is the absolute temperature in Kelvin, Z is the valence of ATP or chloride, and E is the reversal potential. Internal Cl^- concentration was 62 mM, according to Dascal (50).

The number of ATP molecules (n) that crossed the oocyte plasma membrane was calculated as follows: $n = \int I(dt)/(e- \cdot |Z|)$, where $\int I(dt)$ is the electrical charge (in coulombs) that crossed the plasma membrane, thus generating the elicited current (I), $e-$ is the electrical charge of one electron (in coulombs) and $|Z|$ is the valence. The leak current was subtracted, and the charge was calculated as the area delimited by the current.

ATP Content of Xenopus Oocytes—ATP content was measured in groups of 10 oocytes, which were homogenized in the presence of trichloroacetic acid 5%, left to stand for 30 min at 4 °C and finally centrifuged in a benchtop centrifuge for 5 min. Trichloroacetic acid from the supernatant was removed by washing the suspension in ethyl-ether until the pH of the aqueous phase was above 4.5. The amount of ATP was determined by the luciferin-luciferase luminescent reaction.

Ecto-ATPase Activity—Ecto-ATPase (E-type ATPase) activity of defolliculated *Xenopus* oocytes was assayed according to (22), in groups of 10 oocytes incubated in a saline solution containing ATP (1 mM). To activate ATPase, $CaCl_2$ (1 mM) was added.

Expression of Human CD39 in Xenopus Oocytes—Total RNA was isolated from human B-lymphocytes using the Ultraspec™ RNA isolation system (Biotecx). A sample of 2.7 μg of total RNA served as template for cDNA synthesis by reverse transcriptase (MuLV, Perkin-Elmer). The sense primer (5′-TCCCCCGGGCTTATGGAAGATA-CAAAGGAGTCTAAC-3′) contained a sequence identical to nucleotides 65–91 of human CD39. The antisense primer (5′-TCCCCCGGGCTAT-ACCATATCTTTCCAGAAATATGA-3′) was complementary to nucleotides 1574–1600 of the CD39 coding sequence. After amplification, the PCR product (1530 bp) was subcloned into pCR2.1 plasmid (Invitrogen) and sequenced with the DNA sequencing kit (PE Bio systems). This construct (10 μg) was linearized with BamHI (Promega), and the resultant product was used for *in vitro* mRNA synthesis using the mCAP™ RNA capping kit (Stratagene). The mRNA obtained was injected (1–2 μg/μl) into oocytes 3 days before starting current recording.

RESULTS AND DISCUSSION

In defolliculated *Xenopus* oocytes, hyperpolarizing square pulses (Fig. 1*B*) generated a slow inward current of varying amplitude (2.3 ± 0.1 μA; τ = 1.8 ± 0.6 s; n = 191), depending on the oocyte batch. ATP release during the generation of this current, measured in 40 oocytes, was 3.2 ± 0.3 pmol of ATP/oocyte, in pulses from –60 to –200 mV. The amount of ATP released was proportional to the amplitude of the hyperpolarizing pulse. The pulses from –60 to –180 mV gave a significantly ($p < 0.001$) lower ATP peak (54 ± 9%; n = 10) and a significantly ($p < 0.001$) lower inward current (54 ± 8%; n = 12) than pulses from –60 to –200 mV. There were no significant differences between these values. Sometimes, for a few minutes after the end of the hyperpolarizing pulse, a small residual current continued.

We ruled out the exocytotic origin of ATP release from cortical granules (23) of oocytes because it was independent of the external calcium concentration (2.4 ± 0.2 pmol of ATP in the presence of 1.8 mM $CaCl_2$ and 2.6 ± 0.3 pmol in solutions to which $CaCl_2$ was not added; n = 4). Cortical granules fuse with the plasma membrane during fertilization, the oocyte membrane becomes depolarized and calcium enters through voltage-sensitive channels. The hyperpolarizing pulses did not open the voltage-dependent calcium channels at the range used in these experiments. Furthermore, the membrane capacitance of the oocytes (24) did not change significantly (203 ± 8 nanofarads before and 194 ± 7 nanofarads after applying the hyperpolarizing pulse; n = 8). However, the increase in capacitance due to the fusion of granules may have been below the sensitivity of the recording techniques (between 10–15 nanofarads). If we assume that ATP release is supported by the exocytosis of small granules with a diameter of 100 nm con-

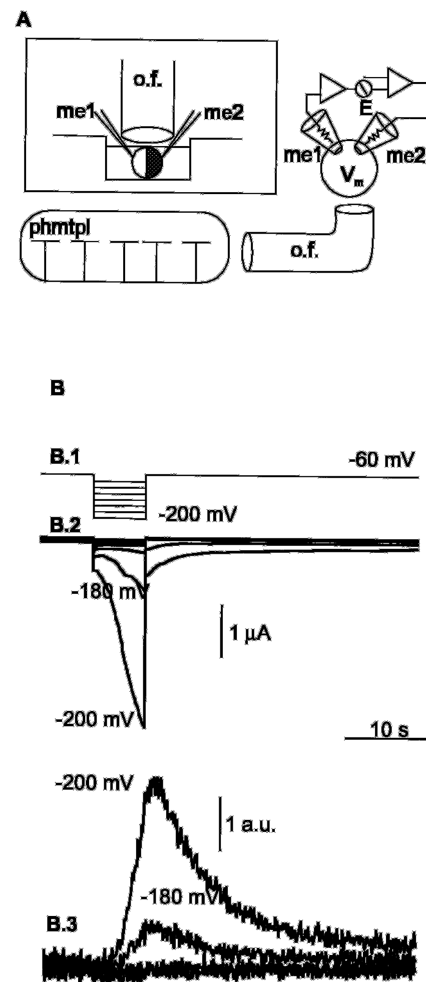

FIG. 1. **Voltage-dependent release of ATP from defolliculated *Xenopus* oocytes.** *A,* for simultaneous recording of ATP release and plasma membrane currents, we connected an optic fiber to a conventional two-electrode voltage-clamp set up for *Xenopus* oocytes. In a dark chamber, the oocyte was placed in a recording cuvette containing a luciferin-luciferase mixture. ATP released from the oocytes excited light emission by the chemiluminescent enzymes. Emitted photons were captured by the optic fiber, which was connected to a photomultiplier. *o.f.,* optic fiber; *me1* and *me2,* microelectrodes 1 and 2; *E,* voltage-clamp amplifier; V_m, resting membrane potential of oocyte; *phmtpl,* photomultiplier. *B, B.1,* hyperpolarizing pulses of 10 s, from –60 to –200 mV, in –20-mV steps, were applied to an oocyte; holding potential, –60 mV. *B.2,* currents elicited by the hyperpolarizing pulses were clearly recorded after applying voltages below –160 mV. When membrane potential returned to –60 mV, the currents disappeared very slowly. Currents presented in the figure were not corrected for leak. *B.3,* in voltages at which an inward current was generated, ATP release was clearly revealed by the emission of light from the mixture of luciferin-luciferase. Light was measured in arbitrary units (*a.u.*).

taining 100–200 mM ATP, such as synaptic vesicles, which are also present in the oocyte, it would induce an increase of 50–90 nanofarads, which would release 2–3 pmol of ATP. Such an increase would be detectable by the recording methods used. We conclude, therefore, that ATP release is not directly related to the exocytosis of secretory granules from the oocyte cytoplasm. However, we cannot rule out the possibility that a few membranous structures, such as secretory granules or caveolae, may be incorporated into the oocyte plasmalemma. These structures could contain a high density of a membrane protein that transport ATP out of the cell. This mechanism of incorporation of ion channels to the cell plasma membrane has been suggested for CFTR (25).

We also examined the ionic permeability associated with the slow inward current previously described in *Xenopus* (26, 27) and in *Rana perezi* (28) oocytes. Both groups concluded that the current was not generated by microruptures of the plasma membrane, which should cause an entry of Na^+. The inward current was still present when external NaCl was replaced by *N*-methyl-D-glucamine-Cl (26). We replaced 83% of external NaCl (15 mM) with urea (200 mM), and the current did not change significantly (–903 ± 33 nA in oocytes bathed in urea-containing medium, –833 ± 23 nA in oocytes bathed in physiological solution; $n = 4$). We ruled out the participation of K^+ in this current, considering that substitution of Na^+ by K^+ in the recording solution did not change the amplitude of the current (–1767 ± 80 nA in oocytes recorded in Na^+ substituted medium; –1835 ± 53 nA in oocytes bathed in physiological solution; $n = 5$). In addition, tetraethylammonium (10 mM) did not change the amplitude of the current (1.5 ± 0.1 μA in 10 mM tetraethylammonium-treated oocytes, 1.5 ± 0.6 μA in physiological solution; $n = 8$), nor was this current sustained by Cl^-; the substitution of Cl^- by other anions did not change the amplitude of the inward current, (2.1 ± 0.3 μA ($n = 5$) for sodium gluconate; 2.3 ± 0.3 μA ($n = 5$) for sodium thiocyanate; 1.9 ± 0.5 μA for SO_4^{2-}). The blocking drug of voltage-dependent chloride channels (anthracene-9-carboxylic acid) did not change the amplitude of the inward current (1.8 ± 0.1 μA in both anthracene-9-carboxylic acid-treated oocytes and oocytes bathed with physiological solution; $n = 4$).

Alternatively, it has been suggested that ATP can cross the plasma membrane through a hydrophobic channel, although the results are controversial (12–15, 17–20). The reversal potential varied considerably from one oocyte batch to another, ranging from 17 to 124 mV, with a mean of 101 ± 19 mV ($n = 10$). According to the Nernst equation, this reversal potential is in the range of the equilibrium potential for ATP^{4-}. Ionic ATP crossing the plasma membrane may have generated this current. This was supported by the finding that, using the Goldman-Hodgkin and Katz equation, the calculated permeability of ATP^{4-} was 4531 times higher than that of Cl^- (in the residual current ATP^{4-} is 4.5 times more permeable than Cl^-). In addition, the number of ATP molecules detected by the luciferin-luciferase reaction was of the same order of magnitude as the number calculated (see under "Experimental Procedures") from the current (Fig. 2).

The dependence of intracellular and extracellular ATP concentrations on ATP flux was also assayed. *Xenopus* oocytes contained 180 ± 30 pmol ATP per oocyte; $n = 7$. However, we were unable to determine what proportion of this ATP was present in the free form in the cytosol, ready to be released or entrapped into granules or vesicles, and what proportion was bound to proteins. Divalent cations (Ca^{2+} and Mg^{2+}) and pH determine the ionic derivatives that can be formed from the ATP molecule. The intracellular calcium concentration of *Xenopus* oocytes has been estimated to be 35 nM (29); in this range

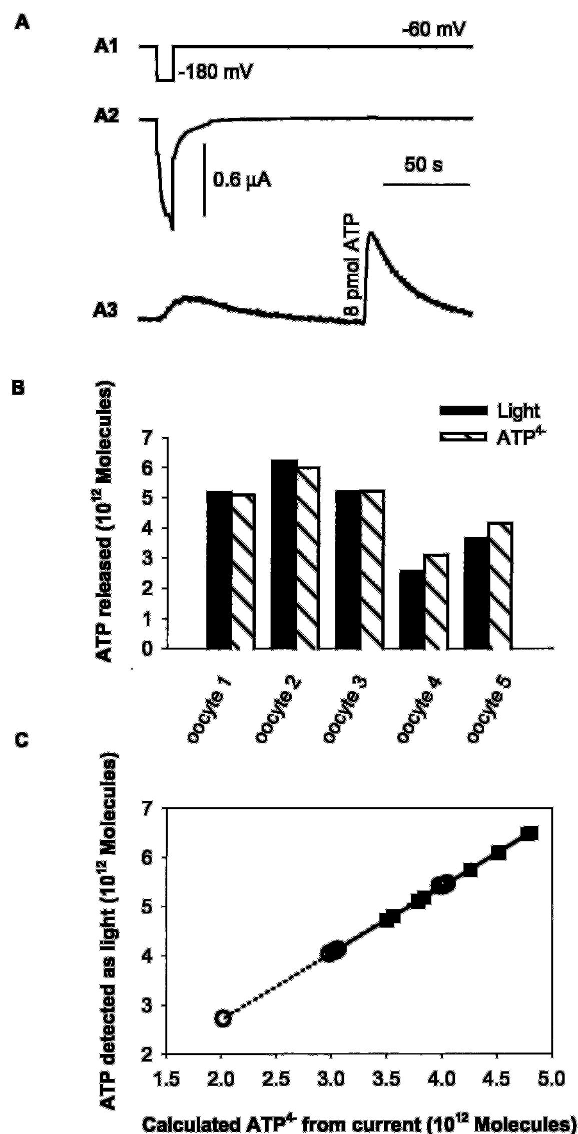

FIG. 2. **Release of ATP from defolliculated *Xenopus* oocytes.** *A,* to estimate the amount of ATP released during a hyperpolarizing pulse, we added a known dose of ATP near to the oocyte bathed in the luminescent mixture. *A1,* voltage command; *A2,* current trace; *A3,* light emitted by ATP release. A known quantity of an ATP solution was ejected to estimate the amount of ATP released by a single oocyte. *B,* comparison of the number of ATP molecules detected by the chemiluminescent reaction of luciferin-luciferase and the calculated number of molecules of ATP with four negative charges that can cross the plasma membrane and thus produce the current elicited by hyperpolarizing pulses. The number of chemiluminescently detected molecules fits with the calculated number of molecules of ATP^{4-} flowing through the plasma membrane. This figure also illustrates the variability of ATP release and currents in different oocytes. *C,* relationship between the number of molecules of ATP detected by the luciferin-luciferase reaction and the calculated number of ATP^{4-} molecules crossing the plasma membrane. *Black squares* and *continuous straight line* correspond to oocytes hyperpolarized from –60 to –200 mV. *White circles* and *discontinuous straight line* correspond to oocytes hyperpolarized from –60 to –180 mV.

of Ca^{2+} concentration and at pH 7.4, the ATP inside the cell must be in the form of ATP^{4-}. The extracellular level of ATP per oocyte was 404 ± 55 fmol/ml ($n = 3$). Nevertheless, the intracellular and extracellular concentrations of ATP fluctuate because of the activation of intracellular ATPases and extracellular ATP degradation by ectonucleotidases.

Decreasing the ATP content with cyanide treatment induced both the inhibition of ATP release and a decrease in the charge

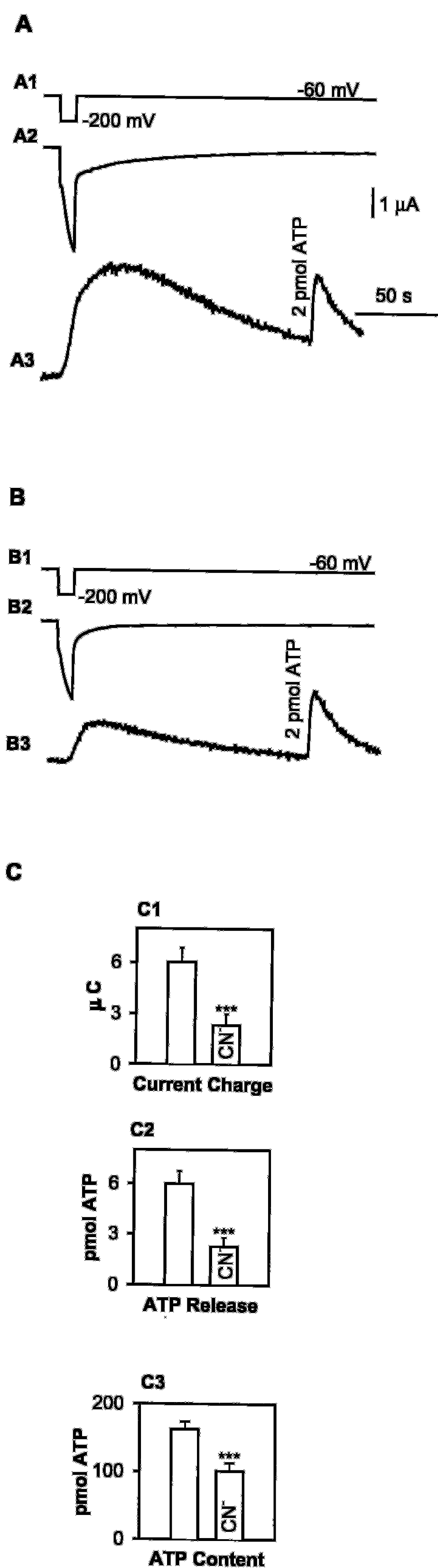

FIG. 3. **Effect of cyanide on ATP release from oocytes induced by hyperpolarization.** *A,* recording from an oocyte. *A1,* hyperpolarizing voltage command; *A2,* current (leak current was not subtracted); *A3,* light emitted by ATP release. *B,* recording from the same oocyte treated with 1 mM NaCN for 10 min. *B1,* voltage command; *B2,* current; *B3,* light emitted by ATP release. Note that a known dose of ATP was added before and after perfusing with NaCN, which indicates that the decrease in light emission was due to a reduction of ATP release rather than interference in the luminescent reaction. *C,* plot of results from five oocytes. *C1,* effect of 1 mM NaCN on the charge mobilized by the evoked current; *C2,* effect of 1 mM NaCN on the hyperpolarization-induced release of ATP; *C3,* changes in oocyte ATP content. *Bars* represent mean ± S.E. *White bars* correspond to nontreated oocytes. *CN^-* indicates oocytes treated with 1 mM NaCN. ***, $p < 0.001$.

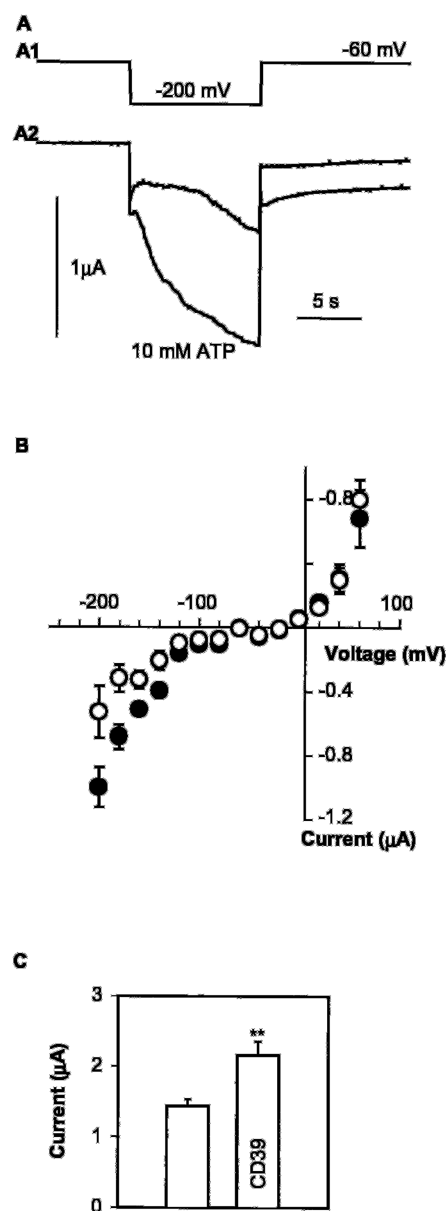

FIG. 4. **Current enhancement by extracellular ATP and by expression of human CD39.** *A,* effect of the extracellular ATP (10 mM) on the hyperpolarizing-elicited inward current. *A1,* hyperpolarizing voltage command; *A2,* current (10 mM extracellular ATP increased the hyperpolarization-elicited current). Leak current was not subtracted. *B,* plot of currents elicited by 10-s voltage pulses, in the absence (*open circles*) and presence (*filled circles*) of 10 mM ATP. In hyperpolarizing pulses, the presence of ATP in the recording medium increased the voltage-dependent currents. Each *data point* represents mean ± S.E. ($n = 5$). *C,* inward current elicited by hyperpolarizing pulses in water-injected oocytes (*open bar*) and in oocytes injected with mRNA for human CD39 (*CD39-labeled bar*). Each *bar* represents mean ± S.E. from 47 oocytes injected with water and 39 oocytes injected with mRNA of human CD39, from three different *Xenopus* donors .**, $p < 0.01$.

carried by the inward current elicited by hyperpolarizing pulses (Fig. 3). When ATP (10 mM) was added to the extracellular solution, the inward current increased when negative potentials were applied (Fig. 4, *A* and *B*). This effect might be related to an endogenous type P_{2x} purinoceptor, but *Xenopus*

Table I
Relationship between activation current, ecto-ATPase activity, and ATP release

Currents were evoked by hyperpolarizing pulses from −60 to −200 mV, before and after perfusing the oocyte with the test solutions. The inhibition of these agents was reversible after 15 min of washing, except in the case of SIDS, for which the inhibition was irreversible. The E-ATPase activity was determined in groups of 10 defolliculated oocytes. Most of the agents that inhibited E-type ATPase activity also inhibited the luminescent reaction of luciferin-luciferase: a known dose of ATP was applied to the bath, and no response was recorded. The inward current was inhibited in oocytes treated with SIDS and extensively washed. In these treated oocytes, we measured ATP release without any interference of SIDS with chemiluminescent enzymes; in this case, oocytes were not able to release ATP under hyperpolarising conditions. i.r., this condition interferes with the reaction.

Condition	Current inhibition		E-ATPase activity inhibition		ATP release inhibition (n = 4)
	%	n	%	n	
Anthracene-9-carboxylic acid, 2 mM	3 ± 3	4	4 ± 2	4	i.r.
Tetraethylammonium, 10 mM	2 ± 7	8	11 ± 4	4	0
Glibenclamide, 1 mM	5 ± 1	2	11 ± 4	4	0
$CaCl_2$, 12 mM	34 ± 7[a]	5	60 ± 6[b]	4	i.r.
Suramin, 50 μM	51 ± 6[b]	3	52 ± 4[a]	4	i.r.
SITS, 1 mM	67 ± 7[a]	5	95 ± 2[b]	4	100
DIDS, 1 mM	87 ± 7[a]	2	93 ± 2[b]	4	i.r.
DIDS, 0.1 mM	63 ± 1[c]	4	61 ± 4[b]	3	70 ± 1[c]
$GdCl_3$, 0.2 mM	76 ± 3[b]	4	100 ± 0[a]	3	74 ± 7[c]
$ZnCl_2$, 5 mM	86 ± 6[a]	5	100 ± 6[a]	4	i.r.

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

oocytes are completely devoid of P_{2x} purinoceptors 48 h after defolliculation (30). In our experimental conditions with 48–72 h defolliculated oocytes, clamped at −60 mV, ATP (10 mM) did not, as expected, evoke any current in the presence of P_{2x} purinoceptors (data not shown). The ectonucleotidase described in *Xenopus* oocytes (22) belongs to the group of E-type ATPases (ecto-ATPase or ecto-apyrase). These are structurally related to the CD39 lymphocyte antigen, which has recently been cloned (31). ATPases usually need Mg^{2+} as a cofactor, whereas E-type ATPases can hydrolyze ATP or ADP using either Ca^{2+} or Mg^{2+} as cofactor. The amount of ATP released from an oocyte under hyperpolarization pulses did not change when soluble apyrase (from potato) was added to the recording medium (data not shown). The hyperpolarization may have blocked the E-ATPase activity and enhanced the light signal by preventing ATP degradation. Indeed, it has been demonstrated that soluble luciferase does not pick up a transient phase of ATP release from platelets (32), except when luciferase was placed on the surface of activated platelets through a chimeric antibody-containing luciferase.

To test the role of E-ATPase in the current, the enzymatic activity and ATP release (Table I) we used a blocker of potassium channels, blockers of chloride channels of *Xenopus* oocytes and inhibitors of E-ATPase activity. Unfortunately, no specific inhibitors of this enzymatic activity are available. Suramin, an antimalarial agent with a K_i in the micromolar range for E-ATPase activity (33), abolishes either the slow inward current or the E-ATPase activity of *Xenopus* oocytes. Table I summarizes the relationship between the generation of the slow inward current and the E-ATPase activity. The degradation of ATP by E-ATPase (34 ± 2 pmol of P_i min^{-1} $oocyte^{-1}$; $n = 13$) was similar to that previously described (22). The E-ATPase activity and the inward current were insensitive to Cl^- and K^+ channel blockers, but they were both inhibited by suramin. Zn^{2+} and a high concentration of Ca^{2+} also inhibited the E-ATPase activity and the induced hyperpolarizing inward current. DIDS and SITS (1 mM), which are commonly used as Cl^- blockers, also inhibited the E-ATPase activity and the elicited current. The inhibition induced by DIDS (1 mM) was reversible, whereas that induced by SITS was irreversible. A lower concentration of DIDS (0.1 mM) partially inhibited the luciferin-luciferase reaction, in a such a way that it was possible to detect ATP release from stimulated oocytes. DIDS (0.1 mM) inhibited the current, the E-ATPase activity, and ATP release by the same order of magnitude (Table I). In addition, the lack of action of glibenclamide indicates that the protein that supports ATP release is not of the ABC type. In summary, the inhibition of E-ATPase always correlated with the inhibition of the inward current. However, it has recently been reported that Gd^{3+} (200 μM) inhibits ATP release from rat hepatoma cells (34). In those experiments, ATP release was induced by hypotonic solution, suggesting that it is sensitive to cell volume changes. It has also been demonstrated that gadolinium blocks stretch-activated ion channels (35). We show (Table I) that solutions containing Gd^{3+} (200 μM) inhibited the hyperpolarization-induced current, E-ATPase, and ATP release. Again, there is an association between current, E-ATPase activity and ATP release. We suggest that ATP release from oocytes can be triggered by the activation of stretch-sensitive ion channels, which in turn may be directly associated to E-ATPases. Indeed, mechanically gated channels are present in *Xenopus* oocytes (27).

Translation of the mRNA of human CD39 enhanced the inward current (Fig. 4*C*), thus demonstrating a direct relationship between E-type ATPase and the inward current. In 26 oocytes injected with a truncated form (nucleotides 640–750), the current was not enhanced. Indeed, it has been suggested (36) that E-type ATPases such as CD39 might be able to form membrane channels. The antigen CD39, like P_{2x} receptors and inwardly rectifying potassium channels, is an integral membrane protein, with two transmembrane domains and a large extracellular region that become active when tetramers are formed (36). This protein may participate in two apparently contradictory functions: the release of ATP and its degradation. However, two different activity states have been suggested (37) for E-ATPases, both of which can account for our results: the E-type molecule acts as an ionic channel or a membrane transporter of ATP in response to hydrolysis or binding of ATP. The E-type ATPases may, in resting conditions, be involved in some kind of transport process, by consuming extracellular ATP; the voltage or stretch stimulus would change the protein conformation, inducing ATP transport out of the cell. In physiological conditions, cells do not reach the low levels of voltage fixed in our experiments. The physiological stimulus for ATP release may be stretching.

Different cells, such as muscle fibers, neurons, or lining epithelial cells secrete ATP (see the Introduction). ATP release from epithelial cells may be relevant to genetic diseases. Cystic

fibrosis is a genetic disease caused by amino acid mutations in the sequence of the CFTR protein. CFTR is a membrane protein that is permeant for chloride anions (18, 38–41) and is located in the apical plasma membrane of the airway epithelium and other epithelial cells. The reduction of the chloride secretion in the respiratory pathways may explain the pathophysiology of cystic fibrosis patients (42). It has been suggested that in addition to its contribution to Cl^- cell permeability, CFTR can also support ATP permeability (15, 43–46). Airway epithelium releases ATP under mechanical stress (12) or under hyposmotic stress (47) in which cystic fibrosis patients epithelia fail to release ATP. Three cell mechanisms have been suggested to explain this ATP release (25). The first possibility is that CFTR may support ATP release directly, and it should therefore permeate either chloride or ATP ions. The second possibility is that ATP can be released through a different kind of protein or ionic channel, which should, in turn, be activated by CFTR. The third possibility is that cells incorporate the ATP-releasing protein by the exocytosis of granules of it in their membrane; CFTR would then be implicated in the regulation of exocytosis. Our results support the second possibility, according to which CD39 would be directly involved in ATP release, whereas CFTR would play a modulatory role. A recent report (48), measuring the release of ATP from single oocytes, pointed out that oocytes from some specific female donors expressing CFTR released ATP under certain restricted conditions, such as the presence of cAMP and changes in extracellular chloride concentrations. CFTR is a regulator of epithelial Na^+ channels (49). It is therefore possible that CFTR can regulate the protein configuration or the channel permeability of E-type ATPases as well. As mentioned before, E-type ATPases share a molecular configuration related to epithelial Na^+ channels; they have a large extracellular loop and only two transmembrane domains. Possibly, CFTR can interact with E-type ATPases triggering ATP release when activated by cAMP. However, our results do not rule out the third possibility mentioned above, in which granules containing a high concentration of E-ATPase are incorporated abruptly by exocytosis to the plasma membrane under the control of CFTR.

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