Prostacyclin receptor-mediated ATP release from erythrocytes requires the voltage-dependent anion channel

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Sridharan M, Bowles EA, Richards JP, Krantic M, Davis KL, Dietrich KA, Stephenson AH, Ellsworth ML, Sprague RS. Prostacyclin receptor-mediated ATP release from erythrocytes requires the voltage-dependent anion channel. Am J Physiol Heart Circ Physiol 302: H553–H559, 2012. First published December 9, 2011; doi:10.1152/ajpheart.00998.2011.—Erythrocytes have been implicated as controllers of vascular caliber by virtue of their ability to release the vasodilator ATP in response to local physiological and pharmacological stimuli. The regulated release of ATP from erythrocytes requires activation of a signaling pathway involving G proteins (G, or G), adenyl cyclase, protein kinase A, and the cystic fibrosis transmembrane conductance regulator as well as the final conduit through which this highly charged anion exits the cell. Although pannexin 1 has been shown to be the final conduit for ATP release from human erythrocytes in response to reduced oxygen tension, it does not participate in transport of ATP following stimulation of the prostacyclin (IP) receptor in these cells, which suggests that an additional protein must be involved. Using antibodies directed against voltage-dependent anion channel (VDAC)1, we confirm that this protein is present in human erythrocyte membranes. To address the role of VDAC in ATP release, two structurally dissimilar VDAC inhibitors, Bcl-xL BH4–23 and TRO19622, were used. In response to the IP receptor agonists, iloprost and UT-15C, ATP release was inhibited by both VDAC inhibitors although neither iloprost-induced cAMP accumulation nor total intracellular ATP concentration were altered. Together, these findings support the hypothesis that VDAC is the ATP conduit in the IP receptor-mediated signaling pathway in human erythrocytes. In addition, neither the pannexin inhibitor carbenoxolone nor Bcl-xL BH4–23 attenuated ATP release in response to incubation of erythrocytes with the β-adrenergic receptor agonist isoproterenol, suggesting the presence of yet another channel for ATP release from human erythrocytes.

Regulated release of ATP plays a role in complex signaling pathways within different organ systems. Erythrocytes have been implicated as controllers of vascular caliber by virtue of their ability to release the vasodilator, ATP, in response to local physiological stimuli (46, 47, 49) as well as when exposed to pharmacological agents (4, 29), and prostacyclin (PGI2) analogs (41). It has been demonstrated previously that exposure of the erythrocyte to low O2 tension or mechanical deformation activates the heterotrimeric G protein Gs, whereas stimulation of erythrocyte β-adrenergic or PGI2 (IP) receptors activates the heterotrimeric G protein Gs (27–29). Stimulation of either G protein activates a signal transduction pathway that includes adenyl cyclase, PKA, and the CFTR and, ultimately, results in release of ATP (40, 42, 43, 45, 50). Although these components of the signaling pathways for ATP release from erythrocytes have been well characterized, the identity of the final ATP conduit appears to depend on the initiating stimulus.

ATP can be released from cells via three primary mechanisms: exocytosis, channels or transporters, or via cell lysis (30). The erythrocyte lacks the protein machinery to form vesicles (52), and hemolysis does not serve as a regulated form of ATP release. Therefore, ATP release from the erythrocyte must occur through channels or transporters. Previously, it was demonstrated that pannexin 1, a protein known to form a channel capable of serving as an ATP conduit in other cell types, is involved in the release of ATP from erythrocytes in response to exposure of the cells to lowered oxygen tension (50). However, treating human erythrocytes with three structurally dissimilar inhibitors of pannexin 1 did not alter iloprost-induced ATP release (50). This finding suggested that a channel or transporter other than pannexin 1 must serve as a conduit for iloprost-induced ATP release from erythrocytes.

Here, we investigated the hypothesis that the voltage-dependent anion channel (VDAC) serves as the conduit for IP receptor-mediated ATP release. VDAC is a 30–35 kDa protein that is predominately found in the outer membrane of mitochondria and is known to be responsible for most of the metabolite flux across that membrane, including the movement of ATP (35, 39). In addition to being present in mitochondria, VDAC is also present in plasma membranes of mammalian cells, including erythrocytes (12, 39), and has been suggested to serve as a conduit for ATP release (26). In this study, we confirmed that VDAC is a component of human erythrocyte membranes. In addition, we determined that two dissimilar VDAC inhibitors, Bcl-xL BH4–23 and TRO19622, attenuate ATP release in response to IP receptor activation with either of two PGI2 analogs, iloprost or UT-15C. These studies support the role of VDAC as the conduit for ATP released from the erythrocyte in response to pharmacological activation of the IP receptor.

Materials and methods

Isolation of erythrocytes. Human blood was obtained from healthy volunteers by venipuncture using a syringe containing heparin (500
H554
VDAC IS AN ATP CONDUIT IN HUMAN ERYTHROCYTES

units). Blood was collected from 14 females and 9 males with an average age of 34 ± 3 years (range 19 to 61 years). Rabbit blood was obtained from male New Zealand white rabbits anesthetized with ketamine (12.5 mg/kg) and xylazine (1.5 mg/kg) intramuscularly, followed by pentobarbital sodium (10 mg/kg) administered via a cannula placed in an ear vein. A catheter was subsequently placed in a carotid artery, and heparin (500 units) was administered. After 10 min, the animals were exsanguinated.

After collection, whole blood was centrifuged at 500 g at 4°C for 10 min and the plasma, buffy coat, and uppermost erythrocytes were removed by aspiration and discarded. The remaining erythrocytes were washed three times in wash buffer containing (in mM) 21.0 tris(hydroxymethyl)aminomethane, 4.7 KCl, 2.0 CaCl2, 140.5 NaCl, 1.2 MgSO4, and 5.5 glucose and 0.5% bovine albumin fraction V (final pH 7.4). Wright stains of erythrocytes prepared in this fashion revealed less than 1 leukocyte per 50 high power fields (~8–10 leukocytes/mm²). Previous studies demonstrate that these erythrocyte preparations are also devoid of platelet contamination (17). Cells were prepared on the day of use. The protocols for blood removal from humans and rabbits were approved by the Institutional Review Board of Saint Louis University and the Institutional Animal Care and Use Committee, respectively. Human subjects gave written informed consent.

All studies evaluating IP receptor-mediated increases in cAMP and ATP release were conducted using erythrocytes from healthy humans. Erythrocytes from both healthy humans and rabbits were used in studies in which the presence of VDAC in cell membranes was investigated.

Measurement of ATP. ATP was measured by the luciferin-luciferase technique (51). A 200 µl sample of erythrocyte suspension was injected into a cuvette containing 100 µl of firefly lantern extract (10 mg/ml, FLE 250; Sigma) and 100 µl of a solution of synthetic D-luciferin (50 mg/100 ml; Sigma). The light emitted was detected using a luminometer (Turner Designs). A standard curve was obtained for each experiment. Cell counts were obtained from the suspension of erythrocytes, and amounts of ATP measured were normalized to 4 × 10⁶ cells/ml.

Measurement of total intracellular ATP of erythrocytes. A known number of erythrocytes were lysed in distilled water and diluted 8,000-fold. ATP was measured as described above, and the values were normalized to ATP concentration per erythrocyte.

Measurement of free hemoglobin. To exclude the presence of significant hemolysis in studies where the release of ATP was measured, samples were centrifuged at 500 g at 4°C for 10 min and the presence of free hemoglobin in the supernatant was determined by light absorption at a wavelength of 405 nm. If increases in free hemoglobin were detected, the studies were not included.

Purification of erythrocyte membranes and Western analysis. Washed (human or rabbit) erythrocytes were diluted 1:100 with ice-cold hypotonic buffer containing (in mM) 5 Tris-HCl and 2 EDTA (pH 7.4) and stirred vigorously at 4°C for 20 min. The lysate was centrifuged at 23,000 g for 15 min at 4°C. The supernatant was removed and discarded. The pellet containing the erythrocyte membranes was washed two times with ice-cold buffer and centrifuged. The membranes were resuspended in ice cold buffer and frozen at −80°C. Membrane protein concentrations were determined using BCA Protein Assay (Pierce). Purified erythrocyte membranes were solubilized in SDS buffer of 0.277 M SDS, 60% glycerol, 0.25 M Tris-HCl (pH 6.8), 0.004% bromophenol blue, and 0.400 M dithiothreitol, boiled, loaded onto a precast gradient (4–20%) gel (Lonza), and subjected to electrophoresis. The proteins were transferred to a polyvinylidene difluoride membrane in buffer containing 25 mM Tris, 192 mM glycine, and 10% methanol. Membranes were blocked overnight with 5% nonfat dry milk in PBS containing 0.1% Tween 20 and then immunoblotted with one of three antibodies directed against VDAC. One antibody was directed against insect VDAC (Heliothis virescens), which has 66% sequence homology with human VDAC (generated and characterized at St. Louis University, rabbit poly-
for the Western blot studies) from which erythrocyte samples were obtained. For each set of experiments, no sample from an individual was used twice. However, some individuals were studied in more than one experimental protocol. The time for maximal ATP release was \(11/1000\)1, \(12/1000\)1, and \(11/1000\)1 min for Ilo, UT, and isoproterenol, respectively and these times were not altered by incubation with any inhibitors.

RESULTS

Determination of VDAC expression in human and rabbit erythrocyte membranes. VDAC protein was identified as a component of purified erythrocyte membranes from healthy humans \((n = 5)\) and rabbits \((n = 5)\) (Fig. 1). The membranes were probed with three distinct antibodies directed against VDAC (larval antibody) or VDAC1 (AB1 and AB4). In all cases a single band was identified at a molecular mass of \(31\) kDa (Fig. 1). In addition, the \(H.\) \(virescens\)-derived antibody recognized a protein at this molecular mass in larval preparations, and the antibodies directed against human VDAC recognized the protein in mouse mitochondrial preparations (Fig. 1).

Effect of BCL on IP receptor agonist-induced ATP release. Isolated human erythrocytes release ATP when incubated with the IP receptor agonists Ilo \((1\mu M, n = 7\); Fig. 2A) or UT \((1\mu M, n = 6\); Fig. 2B). Preincubation with BCL \((5 \times 10^{-2} \mu g/ml)\) resulted in inhibition of ATP release stimulated by both agonists with no effect on baseline ATP levels. The decreases in ATP release were not accompanied by a reduction in total ATP content of the erythrocytes; therefore, the decreased release cannot be attributed to depletion of ATP within the cells (Table 1).

Effect of BCL on Ilo-induced increases in cAMP accumulation. Increases in cAMP are required for \(G_{i}\)-coupled receptor-mediated ATP release from human erythrocytes (4, 41). To establish that BCL did not interfere with this component of the signaling pathway, we measured Ilo-induced increases in cAMP in the presence and absence of the inhibitor. BCL pretreatment was not associated with any change in cAMP levels produced by incubation of erythrocytes with \(1\mu M\) Ilo \((n = 6\); Fig. 3).

Effect of a second chemically dissimilar VDAC inhibitor, Tro, on IP receptor agonist-induced ATP release. In studies identical to those using BCL, ATP release from human erythrocytes stimulated by Ilo \((1\mu M, n = 8\); Fig. 4A) and UT \((1\mu M, n = 6\); Fig. 4B) was attenuated by pretreatment with Tro \(10\mu M\). Again total ATP content of erythrocytes was not different in Tro-treated cells (Table 1).

Effect of BCL and the pannexin 1 inhibitor, Carb, on isoproterenol-induced ATP release. To determine whether pannexin 1 or VDAC is involved in \(\beta\)-adrenergic receptor-mediated ATP release from the erythrocyte, erythrocytes were pretreated with BCL or Carb followed by isoproterenol \((1\mu M)\). Preincubation of human erythrocytes with BCL did not alter ATP release in response to the addition of the \(\beta\)-adrenergic

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**Fig. 1.** Identification of voltage-dependent anion channel (VDAC) in human and rabbit erythrocyte membranes. Solubilized erythrocyte membranes of humans (H) and rabbits (R) were resolved on precast gradient (4–20%) gels and probed with antibodies generated against human or \(H.\) \(virescens\) VDAC. Crude \(H.\) \(virescens\) larva preparations (L) and isolated mouse cardiac mitochondria (M) were used as positive controls. The gels pictured are representative of 5 human and 5 rabbit membrane preparations. Gel A (A), larval antibody; gel B (B), AB1; gel C (C), AB4.

**Fig. 2.** Effect of Bcl-xL BH44 –23 (BCL; 5 \times 10^{-2} \mu g/ml) on ATP release from human erythrocytes incubated with iloprost \((1\mu M, n = 7\); A) or UT-15C \((1\mu M, n = 6\); B). Erythrocytes were incubated with BCL or its vehicle, DMF, for 25 min before addition of the prostacyclin analog. Values are means ± SE. ‡Different from respective baseline and iloprost or UT-15C in the presence of BCL. \((P < 0.01)\; n,\) the number of different individuals studied. RBCs, red blood cells.
erogenic receptor agonist, isoproterenol \( (n = 6, \text{Fig. 5A}) \). In addition, isoproterenol-induced ATP release was not inhibited by the pannexin 1 inhibitor Carb \( (n = 5; \text{Fig. 5B}) \).

**DISCUSSION**

The mechanisms responsible for regulated release of ATP from cells, including erythrocytes, have been the subject of much interest in recent years. Although mature erythrocytes lack a nucleus, mitochondria, and other intracellular organelles, they do contain the glycolytic machinery required to make ATP \( (21–22) \). In addition to being important for the maintenance of erythrocyte flexibility and ionic equilibrium, ATP is released from erythrocytes through regulated signaling pathways \( (24, 25, 49) \). It is well-established that erythrocytes release ATP in response to exposure to low oxygen \( (O_2) \) tension \( (9) \), mechanical deformation \( (43, 45) \), \( \beta \)-adrenergic receptor agonist \( (29) \), or prostacyclin \( (IP) \) receptor agonists \( (41) \).

Exposure of the erythrocyte to low \( O_2 \) tension and mechanical deformation \( (43, 45) \), \( \beta \)-adrenergic receptor agonist \( (29) \), or prostacyclin \( (IP) \) receptor agonist \( (41) \). Exposure of the erythrocyte to low \( O_2 \) tension and mechanical deformation activates the heterotrimeric \( G \) protein \( G_i \) \( (27, 28) \), whereas \( \beta \)-adrenergic receptor and IP receptor stimulation activates the heterotrimeric \( G \) protein \( G_s \) \( (29, 41) \). Stimulation of either \( G \) protein activates signal transduction pathways that include adenyl cyclase, \( PKA \), and the \( CFTR \) \( (41–43, 45, 50) \). There have been a number of recent studies focused on identifying the conduits for ATP release from cells that are associated with specific signaling pathways. Some proteins that have been implicated as candidates for ATP conduits include gap junction proteins such as connexins and pannexins \( (8, 18, 31, 38) \), ATP binding cassette proteins such as MDR and MRP \( (1–3, 32, 33) \) maxi-anion channels \( (19) \), and VDAC \( (26) \).

We and others have reported that pannexin 1 is the conduit for ATP release in response to activation of the IP receptor \( (30a, 50) \). Here we demonstrate that ATP release from the erythrocyte in response to simulation of the IP receptor occurs via a VDAC.

VDAC was classically described as a protein only present in mitochondria where it makes up \( \sim 20\% \) of the outer membrane in eukaryotic cells \( (34) \). In conjunction with other proteins in the inner outer mitochondrial membrane, VDAC serves as the mitochondrial gatekeeper responsible for regulating the release of cytochrome \( c \) and the exchange of ATP and ADP \( (34) \). In addition to its mitochondrial localization, VDAC has been recently identified in the plasma membrane in many different cell types, including erythrocytes \( (12, 39) \). The role of the plasma membrane VDAC has been highly debated. In human umbilical vein endothelial cells, it was demonstrated that VDAC serves as a receptor for kringle 5 on the cell surface \( (15) \). Other studies have suggested that plasma membrane VDAC may play a role in redox regulation \( (6, 7) \). However, it has also been suggested that, similar to its role in the mitochondria, VDAC localized to the plasma membrane plays a role in regulating the release of ATP from cells \( (26) \). This hypothesis is supported by the finding that transfecting NIH 3T3 fibroblasts with the plasmalemmal form of VDAC 1 \( (pl-VDAC 1) \) led to increased ATP release from these cells in

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### Table 1. Effect of voltage-dependent anion channel inhibitors on intracellular ATP levels (in mM/cell) in human erythrocytes

<table>
<thead>
<tr>
<th></th>
<th>Bcl-xL, BH4&lt;sub&gt;42,23&lt;/sub&gt;</th>
<th>TRO19622</th>
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<tbody>
<tr>
<td>( n )</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.12 ± 0.31</td>
<td>3.35 ± 0.39</td>
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<tr>
<td>Inhibitor</td>
<td>3.16 ± 0.25</td>
<td>3.43 ± 0.39</td>
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Values are means ± SE.
response to changing the culture medium, a technique thought to reflect mechanical stimulation of the cells (26). The same study also demonstrated that nasal and tracheal epithelial cells and fibroblasts isolated from VDAC 1 knockout mice released less ATP in response to hypotonic stress compared with cells isolated from the wild-type counterparts (26).

Although VDAC is present in the plasma membrane of erythrocytes, a physiological role for this protein remains uncharacterized. Here, we confirm that VDAC 1 is present in human erythrocyte membranes as well as membranes of rabbit erythrocytes. In addition, we demonstrate for the first time that treating human erythrocytes with BCL, a compound known to inhibit VDAC (39), attenuates IP receptor-mediated ATP release (Fig. 2). Importantly, treating erythrocytes with BCL did not decrease intracellular ATP (Table 1), demonstrating that the reduced ATP release cannot be attributed to attenuation of ATP synthesis. In addition, BCL did not inhibit Ilo-induced cAMP accumulation (Fig. 3), demonstrating that BCL does not inhibit a component of the ATP release signal transduction pathway upstream of the activation of adenylyl cyclase. To further support the hypothesis that VDAC serves as an ATP conduit, we pretreated cells with a second purported inhibitor of VDAC, Tro. Although BCL is a well-established inhibitor of VDAC activity (39), less is known about the specificity and mechanism of action of Tro. Tro is a lipid soluble compound with a cholesterol-like structure that binds to the peripheral benzodiazepine receptor (TSPO). TSPO is known to closely associate with VDAC in mitochondria as part of the mitochondrial permeability transition pore complex (mPTP) (54). Importantly, it has been demonstrated previously that TSPO is present in erythrocyte membranes (11). A previous study using motor neurons to investigate the effects of Tro on cell survival reported that Tro binds TSPO and, consequently, inhibits the activity of VDAC (10). In our studies, Tro attenuated both Ilo- and UT-induced ATP release from the erythrocyte further supporting the role of VDAC as the conduit for ATP release in response to IP activation.

In addition to receptor-mediated activation of Gs via stimulation of IP receptors, erythrocytes also release ATP in response to stimulation of Gs-coupled β-adrenergic receptors by agonists, including isoproterenol. To determine whether pan-nexin 1 or VDAC are involved in isoproterenol-induced ATP release, we examined the effect of Carb and BCL on isoproterenol-induced ATP release. Similar to the studies with Ilo (50), Carb did not attenuate isoproterenol-induced ATP release. Interestingly, BCL also had no effect on isoproterenol-induced ATP release. This finding demonstrates that BCL is not a nonselective inhibitor of ATP release from erythrocytes, and it suggests that ATP release in response to isoproterenol requires a conduit distinct from that involved in Ilo-induced ATP release. This interesting finding is not entirely unexpected in light of studies demonstrating that components of the Gs-mediated signal transduction pathway for ATP release from the erythrocyte are compartmentalized (4, 5); that is, it has been shown that activation of one pathway for ATP release from erythrocytes (IP receptor-mediated pathway) involves components that are independent of the components involved in another pathway (β-adrenergic pathway) (4, 5). Thus, although the stimulation of either IP or β-adrenergic receptors leads to the activation of Gs, the phosphodiesterases and protein kinases involved in regulating the cAMP levels of these respective pathways are different. Phosphodiesterase 3 and protein kinase A and C are involved in the ATP release pathway associated with the prostacyclin receptor, whereas phosphodiesterases 2 and 4 and protein kinase A are involved in the pathway associated with the β-adrenergic receptor (4, 5). Therefore, our study also demonstrated that nasal and tracheal epithelial cells and fibroblasts isolated from VDAC 1 knockout mice released less ATP in response to hypotonic stress compared with cells isolated from the wild-type counterparts (26).

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finding that VDAC is involved in Ilo but not isoproterenol-induced ATP release is consistent with the selective compartmentalization involved in these signaling pathways within the erythrocyte. Recently, it was reported that ATP released from erythrocytes in response to isoproterenol, when augmented by the addition of forskolin and papaverine, was attenuated by carbenoxolone (23). However, under the conditions of that study, the nonselective activation of adenylyl cyclase by forskolin and inhibition of multiple phosphodiesterases with papaverine would result in the stimulation of multiple ATP release pathways in human erythrocytes. Thus the results of this study do not implicate pannexin 1 as an ATP conduit in the isoproterenol signaling pathway.

Although our work supports the hypothesis that VDAC is an ATP conduit in the IP receptor signaling pathway in human erythrocytes, an earlier study suggested that VDAC in the plasma membrane might not play such a role (37). It was reported that, using gene knockout and gene silencing techniques, VDAC did not serve as the maxi-anion channel that had been purported to be an ATP conduit in swelling-induced ATP release from mouse mammary C127 cells and swelling-, ischemia-, or hypoxia-induced ATP release from neonatal rat cardiomyocytes (37). It had long been assumed that the maxi-anion channel activity associated with ATP release in many cell types was due to the activity of VDAC based on the similarity of biophysical properties between these two channels. However, based on their studies, the authors concluded that the maxi-anion channel activity in mouse fibroblasts did not correlate with the presence of any of the three different isoforms of VDAC. These findings contradict the long held hypothesis that the maxi-anion channel represents a plasma membrane VDAC protein. Although this study did not support the hypothesis that the VDAC protein and the maxi-anion channel were one in the same, two points must be considered. The first is that although it provided evidence that the maxi-anion channel activity recorded could not be due to VDAC, the study did not suggest that VDAC could not also be an ATP conduit in cell membranes. Another important factor to consider is that the stimulus for ATP release was cell-swelling, a very different type of stimulus than receptor-mediated activation of a signaling pathway for ATP release. We demonstrated previously that in human erythrocytes, different stimuli for ATP release require different ATP conduits; therefore, the findings of Sabirov et al. (37) could also reflect the existence of additional yet to be identified conduits for ATP from these cells.

In conclusion, we confirm that VDAC 1 is present in human erythrocyte membranes. In addition we show that VDAC serves as the conduit for IP receptor agonist-induced ATP release from these cells (Fig. 6). In addition, we provide evidence suggesting the presence of a yet to be identified conduit for ATP release that is a component of a signaling pathway associated with activation of β-adrenergic receptors in human erythrocytes. The finding that different conduits for ATP release are components of discrete erythrocyte signaling pathways suggests that the selective activation of these pathways is important in vascular regulation. Moreover, the understanding of these pathways could make the erythrocyte a unique therapeutic target for the development of new strategies for the treatment of vascular disease.

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DISCLOSURES

Dr. Sprague has a grant from United Therapeutics, who manufacture UT-15C. The company had no input into experimental design, interpretation of results, or the conclusions of this work. United Therapeutics is acknowledged for grant support in the manuscript.

AUTHOR CONTRIBUTIONS


REFERENCES


