Cation transport activity of anion exchanger 1 mutations found in inherited distal renal tubular acidosis

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WALSH SB, BORGES F, GABILLAT N, UNWIN RJ, GUIZOUARN H. Cation transport activity of anion exchanger 1 mutations found in inherited distal renal tubular acidosis. Am J Physiol Renal Physiol 295: F343–F350, 2008.—Anion exchanger 1 (AE1) is encoded by SLC4A1 and mediates electroneutral anion exchange across cell membranes. It is the most abundant protein in the red cell membrane, but it is also found in the basolateral membrane of renal α-intercalated cells, where it is required for normal urinary acidification. Recently, four point mutations in red cell AE1 have been described that convert the anion exchanger to a cation conductance. SLC4A1 mutations can also cause type 1 hypokalemic distal renal tubular acidosis (dRTA). We investigated the properties of four dRTA-associated AE1 mutations (R589H, G609R, S613F, and G701D) by heterologous expression in Xenopus laevis oocytes. Although these AE1 mutants are functional anion exchangers, unlike the red cell disease mutants, we found that they also demonstrated a cation leak. We found a large cation leak in the G701D mutant. This mutant normally requires coexpression with glycophorin A for surface membrane expression in red blood cells and oocytes. However, we found that coexpressing wild-type kidney AE1 with G701D in oocytes still caused a cation leak, consistent with heterodimerized G701D reaching the cell membrane and retaining its cation conductance property. These findings have potential structural and functional implications for AE1 and indicate that while anion exchange and cation conductance properties are distinct, they can coexist.

ANION EXCHANGER 1 (AE1) is a multiple membrane-spanning protein that mediates electroneutral Cl⁻/HCO₃⁻ exchange across the cell membrane (32). In the red blood cell, AE1 has an NH₂-terminal cytoplasmic domain that acts as an anchorage site for the cell membrane cytoskeleton and a membrane-associated domain (predicted to span the membrane 12–14 times) that carries out anion exchange (9, 18). A kidney isoform of AE1 (kAE1) is present in the α-intercalated cells (α-IC) of the distal nephron. It differs from the erythroid form (eAE1) in lacking the 65 NH₂-terminal amino acids and is located in the basolateral membrane of the α-IC, where it plays a key role in normal urinary acidification linked to apical membrane electrogenic proton (H⁺) secretion (19). Mutations in AE1 protein can cause two distinct phenotypes: erythroid and renal.

Of the erythroid phenotypes, one group encodes polymorphisms of blood group antigens; another group is associated with autosomal dominant (AD) spherocytosis (in which red blood cells are characterized by increased osmotic fragility and reduced membrane surface area); and a third group consists of the hereditary stomatocytes (HSt) and Southeast Asian ovalocytosis (SAO). Both are AD red cell defects characterized by cold-induced cation leaks (3, 22, 31), in which the mutant protein is expressed normally at the cell surface, but has reduced or absent anion exchange activity (4, 5). Several AE1 mutations (L687P, D705Y, S731P, H734R) associated with HSt or spherocytosis appear to abolish the anion transport property of the protein, which is converted to a nonselective Na⁺ and K⁺ conductance (13).

The renal phenotype associated with AE1 mutations is distal renal tubular acidosis (dRTA), a disorder characterized by impaired urinary acidification and increased urinary losses of potassium (and sodium) (19). These mutations are distinct from the mutations causing HSt and SAO, in that anion exchange activity is preserved or only moderately reduced when expressed in Xenopus laevis oocytes. Studies in polarized cells show that the defect associated with these mutations is due to cytosolic retention or mistargeting of the AE1 protein. In the α-IC, kAE1 is synthesized in the endoplasmic reticulum (ER) before being trafficked to the basolateral membrane in oligomeric form (35). Known AD dRTA-associated mutations cause disease in heterozygotes by the mutant kAE1 “capturing” the wild-type kAE1 as a heterodimer, which is retained in the ER (6) or is missorted to the apical membrane (36). Autosomal recessive (AR) dRTA-associated mutations (G701D, S773P, ΔV850, and A858D) have been found only in Southeast Asia (5, 17, 36) and seem to exhibit different trafficking behavior. Polarized mammalian cells coexpressing wild-type AE1 with G701D or other AR mutants show “rescue” of the mutant and expression at the cell surface (6, 7).

We hypothesized that a dRTA-associated AE1 mutant might also have the property of a cation leak. Therefore, we decided to characterize the anion and cation transport properties of four dRTA-associated kAE1 mutations, selected on the basis of their proximity to the red cell disease mutations on the primary...
structure of the AE1 molecule, R589H, G609R, S613F, and G701D, using the X. laevis oocyte expression system.

MATERIALS AND METHODS

Plasmid and DNA construction. The pSP6Serythroid human AE1 (hAE1) plasmid was used to construct the kAE1, deleting the 65 NH2-terminal amino acids. The erythroid construct was amplified by PCR between the Val65 and the Tyr390 with a forward primer introducing a SacII restriction site in 5’ and a reverse primer overlapping the EcoRV site in the hAE1 sequence. The pSP6Serythroid hAE1 had the NH2-terminal part deleted by SacII-EcoRV digestion, with the SacII-EcoRV PCR product introduced in replacement. The obtained pSP6S5AE1 plasmid was checked by sequencing and used to make point mutations using the QuickChange Site-Directed Mutagenesis kit (Stratagene).

For point mutations, primers were used with the following codon substitutions: G669R: GGG/CGG, R589H: CGC/CAC, S613F: TCC/TTC, and G701D: GCC/GAC. Primers were purchased from Eurogentec (Seraing, Belgium); PCRs were performed using a Biometra “UNO Thermoblock” thermocycler (Göttingen, Germany). Sequences generated by PCR were sequenced in their entirety to ensure that no polymerase errors were introduced (Gexbyweb, Meylan, France).

HindIII-linearized pSP65AE1 wild-type and mutant plasmids were transfected using SP6 polymerase (Ambion transcription kit). The HindIII-linearized BSXG-glycophorin A (GPA) plasmid was transfected using T7 RNA polymerase (Ambion transcription kit). RNA concentrations were determined on a formamide/formaldehyde agarose gel in MOPS buffer.

X. laevis oocyte harvesting. X. laevis animals were placed in MS222 (0.2%) until completely anesthetized, according to the protocol approved by our animal ethics committee. The surgery consisted of the removal of five oocyte-containing ovarian lobes. After surgery, the animals were put back in cold water between 0 and 4°C to recover from the anesthesia, monitored for 3 h, and then replaced in the aquarium.

Oocyte injection. Collected oocytes were washed in modified Barth’s solution (MBS; composition (in mM): 85 NaCl, 1 KCl, 2.4 NaHCO3, 0.82 MgSO4, 0.33 Ca(NO3)2, 0.41 CaCl2, and 5 HEPES/NaOH, pH 7.35, 5% CO2-95% O2. Then, the oocytes were bathed in MBS without Cl– in the MBS was substituted with NaCl. Oocyte intracellular pH was measured when acidified oocytes were exposed to Cl–-free medium and corresponds to the initial slope of the alkalinization.

Electrophysiology experiments. Electrophysiological parameters were measured at room temperature (22°C) using a two-electrode voltage-clamp technique with a TEV 200 amplifier (Dagan, Minneapolis, MN) monitored by a computer through a Digidata 1200 A/D converter/PC clamp software (Axon Instruments, Foster City, CA), as previously described (13).

Western blotting. Oocyte membranes were prepared by homogenization of 20 oocytes (control or injected) in cold 20 mM Tris-HCl buffer, pH 7.4. 250 mM sucrose, and 5 mM protease inhibitor, Pefabloc (Roche, Paris, France), as previously described (21). The presence of kAE1 was detected by antibody Bric 170 directed against the COOH terminus of kAE1. The secondary antibody was anti-mouse IgG-peroxidase, which was detected by chemiluminescence using a Fujifilm Las-3000 Luminescence image analyzer.

RESULTS

Western blot results. Mutant kAE1 constructs were coexpressed in oocytes with the AE1 chaperonin glycophorin A (GPA), because without GPA there is absent membrane expression of G701D-kAE1 in oocytes (33). For other studied mutants, functional expression could be observed without GPA, as previously observed (2, 14, 30). To compare similar experimental conditions, we chose to coexpress all the dRTA mutants with GPA. Oocyte plasma membrane expression of wild-type and mutant kAE1 was confirmed by detecting bands of the appropriate molecular mass on Western blotting (Fig. 1). No signal was detected on lanes charged with membrane of oocytes expressing mutant G701D kAE1 alone without GPA (not shown). The Western blots were done at the same time as the functional studies.

Cl– influx experiments. To detect functional expression of the dRTA-associated mutants, 36Cl– influx experiments were carried out in oocytes coexpressing GPA and wild-type or

Intracellular pH measurements. Oocyte intracellular pH was measured using selective microelectrodes, as previously described (13). The ability of wild-type and mutant kAE1 to regulate intracellular pH (pH) was assessed by measuring pH of oocytes adapted in MBS without HCO3–, then incubated in the following medium: (in mM) 63.4 NaCl, 1 KCl, 24 NaHCO3, 0.82 MgSO4, 0.33 Ca(NO3)2, 0.41 CaCl2, and 5 HEPES/NaOH, pH 7.35, 5% CO2-95% O2. Then, the oocytes were bathed in MBS without Cl– in the MBS was substituted with NaCl.

Fig. 1. Representative Western blot of an oocyte membrane probed with anti-anion exchanger 1 (AE1) antibodies (BRIC170). From left to right: noninjected (A), wild-type kidney isoform of AE1 (kAE1)+glycophorin A (GPA; B), R589H+GPA (C), G609R+GPA (D), S613F+GPA (E), and G701D+GPA-expressing oocytes. Blot is representative of 3 experiments.

MW (kDa)

A
B
C
D
E
F

90

-
mutant kAE1. Cl\(^-\) influx in wild-type kAE1-expressing oocytes was \(>18\) times that measured in noninjected controls; Cl\(^-\) influx was well preserved in the dRTA mutants (see Fig. 2A), as previously reported (2, 30, 33).

**pHi measurements.** To confirm that Cl\(^-\) transport was dependent on Cl\(^-\)/HCO\(_3^-\) exchange activity in oocytes expressing wild-type or mutant AE1, we measured the change in pHi following acute exposure to a solution containing CO\(_2\)/HCO\(_3^-\) (in the presence of extracellular Cl\(^-\)), which caused an initial and rapid fall in pHi that stabilized as CO\(_2\) reached equilibrium across the cell membrane. On replacement of extracellular Cl\(^-\) with gluconate, pHi promptly recovered in the presence of functional anion exchange activity, due to HCO\(_3^-\) uptake in exchange for intracellular Cl\(^-\). Oocytes expressing wild-type kAE1 showed a fall in pHi in Cl\(^-\)-containing medium that recovered on replacement of extracellular Cl\(^-\) with the impermeant anion gluconate, as shown in Fig. 2B. This pattern was seen with all four mutants, indicating functional plasma membrane expression of Cl\(^-\)/HCO\(_3^-\) exchange. To compare the Cl\(^-\)/HCO\(_3^-\) exchange activity of wild-type with mutant kAE1, the initial rate of pHi recovery after acid loading was estimated as \(\Delta \text{pHi}/\text{min}\) (Fig. 2C). Mutant recovery rates were not significantly different from wild-type kAE1.

**Rubidium uptake experiments.** \(^86\)Rb\(^+\) was used as a measure of K\(^+\) flux, to demonstrate K\(^+\) permeability of the oocyte membrane. Rb\(^+\) uptake experiments were performed initially at 0°C, as previous work had demonstrated an AE1-mediated cation leak that was maximum at 0°C in HSt-associated AE1 mutants (4). The extracellular medium contained ouabain and bumetanide to inhibit any Rb movement through the Na\(^+\)–K\(^+\)–ATPase and the Na\(^+\)–K\(^+\)–2Cl\(^-\) cotransporter, respectively. Rb\(^+\) uptake (Fig. 3A) was low in both noninjected and wild-type kAE1-expressing oocytes. A large Rb\(^+\) uptake was recorded with one mutant, G701D. Compared with measurements done at 0°C, Rb\(^+\) uptake measured at room temperature (22°C) showed similar values for mutant kAE1-expressing oocytes; however, basal uptake (noninjected oocytes or expressing wild-type kAE1) is significantly raised by the increased temperature (Fig. 3B), obscuring any increase in Rb\(^+\) uptake induced by expression of the R589H, G609R, and S613F mutants.

**Pharmacological blockade.** The kAE1 mutants tested are known to have stilbene-sensitive anion transport (2, 30, 33), a finding supported by our observation of SITS-sensitive Cl\(^-\) influx in all mutants (data not shown). The Rb\(^+\) uptake of

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**Fig. 2.** A: representative experiment of \(^36\)Cl\(^-\) uptake at 22°C into noninjected (NI), wild-type kAE1- and mutant kAE1-expressing oocytes at day 3 postinjection. All injected oocytes are coexpressing GPA. Values are means ± SE of 8 oocytes. B and C: representative plots of intracellular pH (pHi) vs. time of an oocyte acidified with CO\(_2\) in the presence and then absence of extracellular Cl\(^-\). The oocytes are expressing wild-type kAE1 and the G609R mutation, respectively, with GPA. D: pHi recovery rate after an acid load of control oocytes (NI) or oocytes coexpressing GPA with wild-type or the different kAE1 point mutations. Values are means ± SE of 9 oocytes.
Fig. 3. Representative experiment of ouabain- and bumetanide-resistant Rb\(^{86}\) uptake is given at 0°C (A) and at 22°C (B) into NI, wild-type kAE1-, and mutant kAE1-expressing oocytes 3 days after injection. All injected oocytes are coexpressing GPA. Values are means ± SE of 8 oocytes. C: representative experiment of ouabain- and bumetanide-resistant Rb\(^{86}\) uptake at 0°C into NI, wild-type kAE1-, and mutant kAE1-expressing oocytes without (open bars) and with 10\(^{-4}\) M SITS (filled bars). All injected oocytes are coexpressing GPA, and measurements were done 3 days after injection. Values are means ± SE of 8 oocytes. D: dose-response curve of SITS-inhibition of ouabain- and bumetanide-resistant Rb\(^{86}\) uptake induced by G701D and GPA expression. The Rb\(^{86}\) uptake measured in control oocytes (NI) was subtracted. Values are means ± SE of 8 oocytes. Oocyte electrophysiology. The cation leak induced by the erythroid AE1 mutations has been characterized definitively as a nonselective cation conductance (13). Therefore, we investigated the conductive properties of the dRTA-associated kAE1 mutant G701D, as it exhibited the highest cation permeability. As shown in Fig. 5A, coexpression of G701D with GPA

Table 1. Intracellular cation content after incubation in NMDG

<table>
<thead>
<tr>
<th>Na(^{+}), μmol/g day wt</th>
<th>K(^{+}), μmol/g day wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Extracellular Na(^{+})</td>
<td>With NMDG</td>
</tr>
<tr>
<td>NI</td>
<td>71.0±11.8</td>
</tr>
<tr>
<td>kAE1 Wild-type</td>
<td>74.8±4.8</td>
</tr>
<tr>
<td>R589H</td>
<td>151.7±4.9*</td>
</tr>
<tr>
<td>S613F</td>
<td>127.1±8.7*</td>
</tr>
<tr>
<td>G701D</td>
<td>175.7±1.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 15 oocytes. NMDG, N-methyl-D-glucose; NI, noninjected; kAE1, kidney isoform of anion exchanger 1; U, undetectable. The intracellular cation measurements for non-injected, wild-type, and mutant kAE1- expressing oocytes after 3 days incubation in modified Barth’s solution (MBS) at 19°C with 0.5 mM ouabain and 5 μM bumetanide. Note the lack of K\(^{+}\) loss seen in intracellular cation content of oocytes incubated in NMDG medium compared with oocytes incubated in Na\(^{+}\)-containing MBS. In each column, cation content measurements were compared *Significant statistical difference (P < 0.05) between the relevant cation measurements compared with NI oocytes.
increased oocyte membrane conductance compared with non-injected oocytes, or those expressing wild-type kAE1 with GPA. The resting membrane potential was depolarized by 7 mV in oocytes expressing G701D compared with wild-type kAE1 (Table 2). The conductance in oocytes expressing G701D and GPA was sensitive to SITS (Fig. 5B).

Next, extracellular NaCl was replaced with KCl, Na+ with the impermeant cation NMDG, and extracellular Cl− with the impermeant anion gluconate (Table 2). These manipulations showed that the resting membrane potential of noninjected oocytes, or oocytes expressing wild-type kAE1, is largely sensitive to changes in extracellular K+ concentration (high extracellular K+ caused a 42-mV depolarization) and to a lesser extent extracellular Cl− (Cl− removal caused a 4-mV depolarization); however, in G701D-expressing oocytes the resting membrane potential is sensitive to both Na+ and K+ concentrations. In these oocytes, resting membrane potential was hyperpolarized (by 7 mV) in the absence of extracellular Na+, but it was depolarized (by 24 mV) in the presence of high extracellular K+.

Oocytes coexpressing AE1 and wild-type AE1. To investigate the effect of AE1 heterodimer formation on the cation leak property of the G701D mutant, Rb+ uptake experiments were done in oocytes coexpressing G701D with wild-type kAE1, but without GPA. Rb+ uptake was 57% of that observed with the same amount of G701D coexpressed with GPA (Fig. 6). This is consistent with a “rescue” effect of wild-type kAE1 on G701D (by forming heterodimers with G701D kAE1) and successful trafficking to the plasma membrane. Rb+ uptake (although reduced) indicates that surface membrane-expressed heterodimers of G701D (homodimers are not surface expressed).

Table 2. Measurements of resting membrane potential as a function of ionic composition of the extracellular medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>NI</th>
<th>kAE1 Wild-type</th>
<th>G701D</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl/KCl</td>
<td>−50.6 ± 3.3</td>
<td>−54.6 ± 2.5</td>
<td>−47.6 ± 2.5*</td>
</tr>
<tr>
<td>NMDGCl</td>
<td>−53.8 ± 2.9</td>
<td>−52.9 ± 0.9</td>
<td>−54.3 ± 3.6*</td>
</tr>
<tr>
<td>KCl</td>
<td>−7.9 ± 0.9*</td>
<td>−13.1 ± 0.5*</td>
<td>−23.3 ± 1.7‡</td>
</tr>
<tr>
<td>Gluconate</td>
<td>−40.8 ± 3.0*</td>
<td>−49.7 ± 0.8*</td>
<td>−44.4 ± 2.0‡</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 oocytes. Resting membrane potentials (Em in mV) of oocytes expressing either wild-type kAE1 or G701D with glycophorin A (GPA) incubated in different media are shown. Regular MBS with 85 mM NaCl and 1 mM KCl (NaCl/KCl); no extracellular NaCl substituted with NMDGCl and 1 mM KCl (NMDGCl); no extracellular NaCl and 85 mM KCl (KCl) and MBS, where NaCl and KCl were substituted with Na gluconate and K gluconate (gluconate). In each column, Em mean values were compared. *Significant statistical difference (P < 0.05) between Em of indicated oocytes, compared with equivalent oocytes in NaCl/KCl medium. †Significant statistical difference (P < 0.05) between Em of oocytes expressing wild-type kAE1 and G701D mutation in regular MBS. ‡Significant statistical difference (P < 0.05) between Em in KCl or in gluconate for G701D compared with wild-type kAE1-expressing oocytes.

Fig. 5. Current-voltage (IV) curves of NI oocytes (●) or those coexpressing GPA and wild-type kAE1 (●) or G701D mutation (○). The basal currents in noninjected oocytes are superimposed on the currents recorded in oocytes coexpressing GPA and wild-type kAE1. Currents were recorded at 500 ms of clamped potential in regular MBS. Values are means ± SE of 28 oocytes (G701D mutant), 15 oocytes (wild-type kAE1), or 8 oocytes (NI). B: current-voltage curves of oocytes coexpressing GPA and G701D mutations in the absence (●) or presence of 10−4 M SITS (○). Values are means ± SE of 5 paired oocytes and differences (below −40 mV and above 40 mV) were statistically significant with α = 0.05 (t-test) inset; current recordings of wild-type kAE1+GPA- and G701D+GPA-expressing oocytes (left and right, respectively).

Fig. 6. 86Rb+ uptake was measured in NI oocytes and oocytes expressing wild-type kAE1, G701D kAE1 alone, or coexpressing GPA and G701D or wild-type and G701D kAE1. A similar amount of wild-type or mutant kAE1 cRNA was injected (6 ng). For coinjection, 6 ng of G701D was injected with either 2.5 ng of GPA or 6 ng of wild-type kAE1. Values are means ± SE of 8 oocytes.
in the absence of GPA) and wild-type kAE1 still possess a cation transport property.

**DISCUSSION**

We have found that the four dRTA-associated kAE1 mutants we investigated are capable of inducing a monovalent cation leak when expressed in *X. laevis* oocytes, which is not present in oocytes expressing wild-type kAE1. The leak of Na⁺ and K⁺ in mutant-expressing oocytes depends on the electrochemical gradients for Na⁺ and K⁺, with Na⁺ influx counterbalancing K⁺ efflux. Electrophysiological experiments in G701D-expressing oocytes demonstrated an electrogenic leak sensitive to variations in extracellular Na⁺ and K⁺ concentrations, indicating the presence of a nonselective cation conductance property. Previously, it has been shown that four point mutations in erythroid AE1 converted the anion exchanger to a nonselective cation conductance (the alternative explanation of a coincidentally activated endogenous oocyte transporter not supported by the evidence) (13). We have now shown that four other point mutations associated with a renal (rather than an erythroid) phenotype also have a cation leak property, although in contrast to the four erythroid AE1 mutants studied previously, the renal AE1 mutants retain normal or near-normal anion exchange activity. Thus for the first time we have shown that these transport properties can coexist in human AE1, albeit with the measured anion flux (mediated via the anion exchanger mechanism) being much greater than the measured cation flux.

The four dRTA-associated mutations all occur on highly conserved sequences in the primary structure of kAE1: in the putative transmembrane helices 6 (R589H), 7 (G609R, and S613F) and 9, or the region adjacent to it (G701D) (9). These point mutations are not essential for anion exchange function, which is preserved. This is in accordance with previous observations showing that the transmembrane segments 6 and 7 seem to be unnecessary for anion transport, since noncomplementary fragments of AE1 lacking this segment could still induce anion transport when coexpressed in *X. laevis* oocytes (11). However, G701D lies in a region critical for anion exchange. This region is also the least well defined topologically; it is thought to be involved in a transport-related conformational change (TCC) and to be structurally labile (18). Certain residues appear to be important for anion transport in this region. E681 is thought to act as the proton binding site in H⁺ and SO₄²⁻ symport (15); even conservative site-directed mutagenesis at this site (E681D) abolishes anion exchange (23). Another crucial amino acid site for anion exchange appears to be H734 (24). It appears to interact with the negative charge of the E681 residue (23), apparently because of their close physical proximity (18). The erythroid mutants L687P, S731P, and H734R are closely associated with these sites, and they have been shown to abolish anion exchange (4, 13). Indeed, these mutations should be expected to disrupt the spatial position or charge of the 734 and 681 residues. G701D and the erythroid D705Y are also close to E681 in the primary sequence, and both mutations mediate large cation leaks. The intact anion exchange activity of the G701D mutant suggests that this residue is not essential for anion exchange function, perhaps because it is situated farther from the E681 residue in three-dimensional space.

There is growing evidence for the existence of (or ability to exhibit) ion channel activity in what until recently were considered to be exclusive symporters or antiporters (8) and that these transporters and ion channels can have structural similarities (10). Indeed, trout AE1 may act as an ion channel (12, 20), which illustrates how closely related the AE1 exchanger is to a channel. Therefore, point mutations that directly alter the conformation of the region involved in AE1’s TCC can elicit cation transport and (with at least one exception, G701D) abolish anion transport, whereas mutations distant from this region (R589H, G609R, and S613F) induce a small cation leak without affecting anion transport. These mutations are thought to be misfolded (35), and it is likely that such conformational changes are sufficient to provoke a cation leak, as AE1’s structure is already so closely related to that of an ion channel (cf. trout AE1).

Conformational change in the region of the TCC provides an explanation for the partial inhibition of the cation permeability observed in the G701D mutant by SITS, which also occurs in the erythroid mutants (L687P, D705Y, S731P, and H734R) (13). SITS binding and inhibition of AE1 depends on its conformation; indeed, the affinity chromatography of SITS is used to detect misfolding of AE1 mutants (29).

Up until now, cation leaky AE1 has been associated solely with red cell pathology; the cation leak described by Bruce et al. (4) in the mutants L687P, D705Y, S731P, H734R, and R760Q was postulated to be responsible for the dysmorphic red blood cells seen in patients with these mutations. Here, we have described four other point mutations of AE1 (R589H, S613F, G609R, and G701D) inducing a cation leak, which are associated with dRTA. There have been no reports of erythrocyte abnormalities or evidence of hemolytic anemia for these dRTA-associated AE1 mutations, except for G701D homozygotes (5, 33). It may be that the cation leak is compensated in heterozygote erythrocytes and therefore causes no red cell dysmorphism, whereas compensation is overcome in homozygotes due to a larger cation leak mediated by the two mutant alleles. Moreover, there is a recent report of hemolytic anemia and erythrocyte abnormalities in patients with G701D (and other Southeast Asian AE1 mutations) and dRTA, which appears to be related to the degree of acidemia (16); the red cell abnormalities resolve with alkali treatment, and the lack of acidemia in G701D heterozygotes could explain their normal red cell phenotype.

In contrast to red blood cells in which GPA acts as a chaperonin to address dRTA-associated AE1 mutations to the plasma membrane, the α-IC does not express GPA, and kAE1 is believed to be transported to the cell surface as an oligomer (36). We simulated this in the nonpolarized oocyte by coexpressing G701D kAE1 with wild-type kAE1 (without GPA) by using equal amounts of mRNA. This resulted in a smaller cation leak, approximately half the magnitude seen in the paired experiment using G701D and GPA (Fig. 6), which is consistent with cation leaky heterodimerized G701D and wild-type kAE1 at the cell membrane, nonleaky homodimerized wild-type kAE1 at the cell surface, and homodimerized G701D retained intracellularly. However, the cation transport property of G701D is still present when heterodimerized, suggesting that functional cation leaky kAE1 may be present in the α-IC membrane of G701D heterozygotes.
Is it possible that the mutations described have any pathophysiological significance for the α-IC cell, perhaps mediating K\(^+\) efflux into the urine? The AD mutants do show a cation leak when intracellular cation contents are measured at 19°C. Although largely retained intracellularly, R589H, S613F, and G609R have all been observed (in varying amounts) at the apical membrane in polarized cell models (6, 30, 36), but we acknowledge the likely differences between overexpressing cell models and native α-IC cells. Moreover, the small amount of AE1 expressed at the apical membrane in these cell models (especially R589H and S613F) and the relatively small cation leak seen with S613F and G609R (and the modest leak seen with R589H) make a significant pathophysiological role for these mutants in K\(^+\) wasting in dRTA unlikely. Indeed, hypokalemia is not a consistent feature in patients with European AE1-associated dRTA.

The G701D mutation is perhaps more likely to have some pathophysiological significance, since its cation leak is much higher than that in the AD mutants. If G701D were mistargeted to the apical membrane of α-IC, a K\(^+\) leak into urine could occur; if it were expressed on the basolateral membrane only, it may cause enough membrane depolarization to activate apical maxi-K channels (37) and facilitate flow-dependent K\(^+\) efflux into the urine? The AD mutants do show a cation leak when intracellular cation contents are measured at 19°C. Although largely retained intracellularly, R589H, S613F, and G609R have all been observed (in varying amounts) at the apical membrane in polarized cell models (6, 30, 36), but we acknowledge the likely differences between overexpressing cell models and native α-IC cells. Moreover, the small amount of AE1 expressed at the apical membrane in these cell models (especially R589H and S613F) and the relatively small cation leak seen with S613F and G609R (and the modest leak seen with R589H) make a significant pathophysiological role for these mutants in K\(^+\) wasting in dRTA unlikely. Indeed, hypokalemia is not a consistent feature in patients with European AE1-associated dRTA.

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