Distal Renal Tubular Acidosis in Mice Lacking the AE1 (Band3) Cl\(^{-}/\)HCO\(_3\)\(^{-}\) Exchanger (slc4a1)

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Mutations in the human gene that encodes the AE1 Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger (SLC4A1) cause autosomal recessive and dominant forms of distal renal tubular acidosis (dRTA). A mouse model that lacks AE1/slc4a1 (slc4a1\(^{-/-}\)) exhibited dRTA characterized by spontaneous hyperchloremic metabolic acidosis with low net acid excretion and, inappropriately, alkaline urine without bicarbonaturia. Basolateral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange activity in acid-secretory intercalated cells of isolated superfused slc4a1\(^{-/-}\) medullary collecting duct was reduced, but alternate bicarbonate transport pathways were upregulated. Homozygous mice had nephrocalcinosis associated with hypercalciuria, hyperphosphaturia, and hypocitraturia. A severe urinary concentration defect in slc4a1\(^{-/-}\) mice was accompanied by dysregulated expression and localization of the aquaporin-2 water channel. Mice that were heterozygous for the AE1-deficient allele had no apparent defect. Thus, the slc4a1\(^{-/-}\) mouse is the first genetic model of complete dRTA and demonstrates that the AE1/slc4a1 Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger is required for maintenance of normal acid-base homeostasis by distal renal regeneration of bicarbonate in the mouse as well as in humans.


Normal kidney function is critical for maintenance and control of systemic acid-base homeostasis, as highlighted by several inborn and acquired diseases (1–5). Three main activities contribute to this role: Proximal tubular reabsorption of filtered bicarbonate, proximal tubular synthesis and excretion of ammonia, and collecting duct excretion of protons and regeneration of bicarbonate. Type A acid-secretory intercalated cells (A-IC) in the collecting duct express the apical vacuolar H\(^{+}\)-ATPase and a basolateral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger kAE1 (kidney band 3, SLC4A1) that respectively mediate proton extrusion into urine and bicarbonate release into blood (2,6). Bicarbonate is formed in the A-IC by the cytosolic carbonic anhydrase. The concerted action of these transport proteins and enzymes in A-IC is important for acid secretion and for parallel de novo generation of bicarbonate and/or its reabsorption from the luminal fluid.

AE1 is expressed in red blood cells and in renal A-IC. The kidney-specific kAE1 gene product lacks the N-terminal 79 amino acids (aa) that are present in mouse erythroid AE1 (eAE1), as a result of alternative promoter usage (7,8). Human kAE1 lacks the N-terminal 65 aa that are present in human eAE1 (6). The N-terminal extension of eAE1 polypeptide is important for interaction with erythroid-specific cytoskeletal and membrane proteins. One group of human AE1 mutations causes dominantly transmitted erythroid-specific abnormalities such as hereditary spherocytic anemia, Southeast Asian ovalocytosis (SAO), and other “hereditary stomatocytoses with cation leak” (9) accompanied by normal renal function. A distinct set of AE1 mutations can cause distal renal tubular acidosis (dRTA) without apparent erythroid abnormality (1,10–14).

dRTA-associated AE1 mutations are predominantly autosomal dominant among white individuals (13,15,16), with disease characterized by hyperchloremic metabolic acidosis, variable hypokalemia, osteomalacia, childhood growth retardation, nephrocalcinosis, and nephrolithiasis progressing to renal insufficiency (5,15,17). At least two pathophysiologic mechanisms have been proposed to explain autosomal dominant dRTA as a result of AE1 mutations (1,9): A dominant negative intracellular retention phenotype (18) and a dominant or co-dominant mistargeting to the apical membrane (19,20). A distinct set of mutations found among Southeast Asians and Melanesians is transmitted in autosomal or compound recessive patterns (11,14). The recessive disease is often of earlier onset and accompanied by a urinary concentrating defect, with affected infants susceptible to severe dehydration (1,3,5,15,17,21–23). Rare recessive mutations cause combined early-onset hemolytic anemia and dRTA manifest as hydrops fetalis (24). The severity of these symptoms emphasizes the importance of AE1 for normal kidney function. In this study, we examined the renal phenotype of AE1-deficient mice to gain additional insight into the physiologic function of kAE1, the pathogenesis of dRTA.
as a result of AE1 gene mutations, and the mode of inheritance of AE1 mutation–associated dRTA.

Materials and Methods

Animals
Generation and breeding of mice that were deficient for anion exchanger 1 (Slc4a1 −/−) on a C57BL/6 background was previously described (25). Mice were maintained on a standard rodent chow (Kliba AG, Kaiseraugst, Switzerland) and had access to drinking water ad libitum.

Food and water consumption was monitored, and urine and stool output was quantified in metabolic cages, with urine collection under mineral oil. After an adaptation period to the metabolic cage environment, experiments were performed over periods of 1 to 3 d. During days 1 and 2, mice were kept under control conditions (tap water with 2% sucrose). On day 3, 0.28 M NH₄Cl was added to the drinking water as an oral acid challenge (26,27). All animal studies were according to Swiss Animal Welfare Laws and approved by the Local Veterinary Authority (Kantonales Veterinäramt) Zurich.

Blood and Urine Analysis
Blood and urine samples that were obtained from metabolic cage experiments were injected into a blood gas analyzer (ABL 505, Radiometer Copenhagen, Copenhagen, Denmark) for determination of pH, Pco₂, calculated [HCO₃⁻], K⁺, Na⁺, Ca²⁺, and Mg²⁺ (blood), and HCO₃⁻ (urine). pH of urine that was collected under oil was measured using a pH microelectrode (Metrohm, Herisau, Switzerland). Urine was diluted in distilled water for anion determinations by ion chromatography (Metrohm ion chromatograph) on a SUPP 5-250 column eluted with 3.2 mM Na₂CO₃/1 mM NaHCO₃. Urine was diluted in 4 mM tartaric acid for determination of K⁺, Na⁺, Ca²⁺, and Mg²⁺ on a METROSEP C-2-150 column eluted with 4 mM tartaric acid/1 mM dipicolinic acid. Serum creatinine concentration was measured using a kit based on the F-Daox method (Wako Chemicals, Neuss, Germany). Urine creatinine was analyzed by the Jaffé method (28,29).

Urinary ammonium concentration was determined according to the Berthelot Protocol (30). Urinary citrate concentration was measured with the kit from R-Biopharm (Murten, Switzerland). Titratable acids were determined according to Chan (31). Briefly, equal volumes of urine and 0.1 N HCl were mixed and boiled for 2 min. After 10 min of cooling to 37°C, the mix was titrated with 0.1 N NaOH to pH 7.4. A blank sample (distilled water) was identically treated. The difference in volumes of NaOH required to titrate the sample and the blank, multiplied by the normality of NaOH times 1000, revealed the concentration of titratable acids in mmol/L. Osmolarity of blood and urine was determined using a Roebling freezing point osmometer (Auer Bittmann Soulié, Dietikon, Switzerland). GFR, fractional excretion of ions, anion gap in blood and urine, and net acid excretion were calculated from the measured values.

Statistical Analyses
All data were tested for significance using the ANOVA and t test. Only data with P < 0.05 were considered as significantly different.

Results
The renal phenotype of AE1-deficient mice was not analyzed in early studies because of high perinatal and postnatal lethality (Supplementary Figure 2) (25,32). Crossing of the few surviving Ae1−/− male homozygote mice with Ae1+/− females resulted in AE1-deficient Ae1−/− progeny that exhibited approximately 16% postweaning survival to the age of 10 to 14 wk. AE1-deficient neonates were smaller than their littermates and required 5 to 6 wk of age before they could be successfully weaned. Surviving AE1-deficient mice grew more slowly than heterozygous or wild-type animals, with Ae1−/− male weight at 12 wk of 22.6 ± 1.1 g (n = 8) versus Ae1+/+ weight of 26.3 ± 1.7 g (n = 9).

At age 5 to 7 d, the Ae1−/− mouse hematocrit measured on two separate occasions was 7.1 ± 1.0% (n = 9) and 13.3 ± 1.0% (n = 18), with reticulocytosis of >70%. In six additional mice of mean age 14 wk, hematocrit was 10.9 ± 0.5%. These severely anemic values are nonetheless slightly higher than those originally reported (25).

Twelve-week-old mice were used to assess consequences of the congenital absence of AE1 on renal acid-base transport and systemic acid-base homeostasis. Immunoblotting with an antibody directed against an epitope that was common to the erythrocyte and kidney isoforms confirmed the complete absence of AE1 from kidneys of AE1-deficient mice (Supplementary Figure 1). Kidney function and systemic acid-base balance were evaluated under basal conditions and during a 24-h oral acid load. Under control conditions (Table 1, Figure 1) wild-type and slc4a1+/− mice exhibited no apparent difference in blood or urine parameters. In contrast, slc4a1−/− mice had severe metabolic acidosis with low blood pH and bicarbonate levels and slightly increased blood chloride and phosphate levels (Table 1). Elevated serum urea levels pointed to mild renal insufficiency in AE1-deficient mice, and elevated serum osmolality suggested mild systemic dehydration. The urine of AE1-deficient mice was alkaline, with reduced excretion of net acid and of titratable acids (Table 1, Figure 1). Twenty-four-hour urine volume of Ae1−/− mice was two- to three-fold higher than for wild-type and heterozygous mice, with correspondingly lower osmolality. In Ae1−/− mice, hypercalcemia and hypocitraturia were also evident in basal conditions.

Challenge with an oral acid load (NH₄Cl added to drinking water) induced metabolic acidosis in wild-type and heterozygous mice, as evident from reduced blood pH and bicarbonate levels, hyperchloremia, and decreased urine pH with increased net acid excretion (Table 2, Figure 1). In AE1-deficient mice, this NH₄Cl-loading protocol disproportionately exacerbated the basal metabolic acidosis, decreasing blood pH to 6.82 ± 0.02 (compared with 7.21 ± 0.01 in wild-type and 7.20 ± 0.01 in heterozygous mice; n = 7 to 9). The severely decreased blood bicarbonate concentration in AE1-deficient mice was accompanied by a less than proportionate reduction in Pco₂, consistent with a partial respiratory compensation (Tables 1 and 2, Figure 1).

Despite the severe metabolic acidosis in slc4a1−/− mice, urine pH remained more alkaline and net acid excretion remained much lower than in wild-type littermates.

NH₄Cl loading had to be terminated after 24 h. Longer
periods of acid loading led to death of AE1-deficient mice from apparent dehydration (weight loss) and massive hemolysis (dark urine as a result of hemoglobulinuria; Supplementary Figure 3). The severe dehydration in acid-loaded AE1-deficient mice was reflected in elevated serum osmolality and elevated serum concentrations of sodium and chloride. It is interesting that heterozygous mice failed to display a wild-type urinary concentration response to the oral acid load.

The acid-base parameters of wild-type and heterozygous mice did not differ in their responses to acid loading. Whereas haploinsufficiency of AE1 did not affect basal systemic acid-base status, complete absence of AE1 led to hyperchloremic metabolic acidosis and to lethal intolerance of an oral acid load easily borne by heterozygotes.

Intercalated cells (IC) were maintained in the collecting ducts of 9-wk-old Ae1−/− mice. The proportion of cortical collecting duct (CCD) IC that were type A-IC (measured as pendrin-negative, H+-ATPase-positive cells) was 48% in 516 A-IC cells from two Ae1−/− kidneys and 48% in 715 A-IC from one wild-type kidney. The proportion of A-IC was also indistin-

Table 1. Control conditionsa

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ae1+/+</th>
<th>Ae1+/−</th>
<th>Ae1−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
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<td></td>
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</tr>
<tr>
<td>pH</td>
<td>7.27 ± 0.02</td>
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<td>HCO3⁻</td>
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<td>Pco₂</td>
<td>48.8 ± 1.7</td>
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<td>4.4 ± 0.1c</td>
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<td>147.3 ± 1.1</td>
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<td>Cl⁻</td>
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<td>1.6 ± 0.1</td>
<td>2.4 ± 0.3c</td>
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<td>10.8 ± 0.2</td>
<td>15.1 ± 1.1</td>
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<td>310.2 ± 1.5</td>
<td>336.0 ± 9.1c</td>
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<td>creatinine</td>
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<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>urea</td>
<td>6.4 ± 1.6</td>
<td>ND</td>
<td>26.8 ± 3.8b</td>
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<td>6.77 ± 0.12</td>
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<td>2336 ± 279.1</td>
<td>1085.1 ± 103.1b</td>
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<td>creatinine</td>
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<td>HCO³⁻/creatinine</td>
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<td>0.1 ± 0.0</td>
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</tr>
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<td>K⁺/creatinine</td>
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<td>2.9 ± 0.4c</td>
<td>6.9 ± 1.1</td>
</tr>
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<td>Na⁺/creatinine</td>
<td>3.5 ± 0.5</td>
<td>1.2 ± 0.2c</td>
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<td>Cl⁻/creatinine</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.6</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>P₁/creatinine</td>
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<td>0.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
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<td>NH₄⁺/creatinine</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.4 ± 0.2</td>
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<tr>
<td>Ca²⁺/creatinine</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.11 ± 0.02c</td>
</tr>
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<td>Mg²⁺/creatinine</td>
<td>0.44 ± 0.09</td>
<td>0.19 ± 0.04</td>
<td>0.66 ± 0.19</td>
</tr>
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<td>citrate/creatinine</td>
<td>0.71 ± 0.09</td>
<td>ND</td>
<td>0.25 ± 0.04b</td>
</tr>
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<td>creatinine clearance</td>
<td>1.03 ± 0.17</td>
<td>ND</td>
<td>1.11 ± 0.22</td>
</tr>
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<td>FE Na⁺ (%)</td>
<td>0.26 ± 0.05</td>
<td>ND</td>
<td>0.68 ± 0.15c</td>
</tr>
<tr>
<td>FE K⁺ (%)</td>
<td>21.25 ± 4.11</td>
<td>ND</td>
<td>10.31 ± 2.23</td>
</tr>
<tr>
<td>FE Cl⁻ (%)</td>
<td>0.31 ± 0.08</td>
<td>ND</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>FE Ca²⁺ (%)</td>
<td>0.76 ± 0.09</td>
<td>ND</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>FE Mg²⁺ (%)</td>
<td>5.62 ± 2.76</td>
<td>ND</td>
<td>6.88 ± 1.16</td>
</tr>
<tr>
<td>FE P₁ (%)</td>
<td>2.09 ± 0.42</td>
<td>ND</td>
<td>1.81 ± 0.44</td>
</tr>
<tr>
<td>urinary anion gap</td>
<td>177.8 ± 89.3</td>
<td>21.4 ± 36.3</td>
<td>36.8 ± 24.2</td>
</tr>
<tr>
<td>titratable acids</td>
<td>38.7 ± 4.6</td>
<td>28.0 ± 6.1</td>
<td>5.5 ± 6.6b</td>
</tr>
<tr>
<td>NEA</td>
<td>120.0 ± 10.2</td>
<td>94.1 ± 14.6</td>
<td>49.1 ± 12.4b</td>
</tr>
<tr>
<td>NEA/creatinine</td>
<td>1.9 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

Súpport of basal blood and urine data. Urine was collected during 24 h in metabolic cages, and blood was sampled at the end of the collection period. Concentrations of electrolytes, titratable acids, and urea are expressed in units of mmol/L, creatinine in mg/dl, Pco₂ in mmHg, osmolarity in mOsmol/L, creatinine clearance in ml/min; n = 8 to 10 animals per genotype. ND, not determined; NEA, net acid excretion.

aP < 0.001 versus wild-type.
bP < 0.05 versus wild-type.
was severely exacerbated after NH$_4$Cl loading (control conditions revealed metabolic acidosis. This acidosis ethyl ester (BCECF)-loaded tubules as the initial rate of intracellular 7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescin, acetoxymethyl ester of freshly isolated outer medullary collecting ducts from wild-type and AE1-deficient mice. Activity was measured in 2'-AIC in all conditions but did acidify in response to the oral NH$_4$Cl load. Blood pH (B) and [HCO$_3$]$^-$ concentration (C) in wild-type and heterozygous mice were indistinguishable in all conditions. However, blood pH and [HCO$_3$]$^-$ in AE1-deficient mice under control conditions revealed metabolic acidosis. This acidosis was severely exacerbated after NH$_4$Cl loading (n = 8 mice for each genotype and time point). *P < 0.001 versus control conditions in the same genotype; *P < 0.001 versus wild-type.

Among candidate anion exchangers that might contribute to this apparent compensation are Ae2/Slc4a2 (38–40), Ae3/slc4a3, Ae4/Slc4a9 (41), Slc26a7 (37), and (if atypically induced in A-IC) pendrin/Slc26a4 (42–45). Renal levels of Ae2 and Ae3 transcript variants (Figure 3A) as well as AE4 mRNA abundance (data not shown) were not elevated in slc4a1–/– mice (Figure 3A). Unchanged renal mRNA levels of AE2 variant transcripts were confirmed by RNase protection assay (data not shown). AE4 polypeptide levels were also unchanged in slc4a1–/– kidney, consistent with previously reported lack of sensitivity to acid loading in wild-type mice (41) (Figure 3B). However, levels of pendrin polypeptide were markedly decreased, consistent with the previously reported sensitivity of wild-type mouse kidney pendrin to acid loading (42,44,46). In addition, no consistent changes were found in slc4a1–/– kidney levels of mRNA encoding A-IC gene products Slc26a7 (37), aquaporin-6 (AQP6) (47), the K$^+$/Cl$^-$ co-transporter KCC4 (48), and the renal anion exchanger Slc26a11 (49), whose nephron segment localization is unreported.

Basal 24-h urine volume in AE1-deficient mice was three- to four-fold higher than the wild-type and heterozygote values. Urine osmolality was correspondingly approximately 50% lower than in wild-type and heterozygous mice (Tables 1 and 2, Figure 4). The accompanying elevation in plasma osmolality in slc4a1–/– mice suggested a urinary concentrating defect. NH$_4$Cl loading led to loss of 14% of initial body weight within 24 h and to greatly increased plasma osmolarity without the accompanying increase in urine osmolality seen in wild-type mice (Figure 4). Basal creatinine clearance was similar in all genotypes but decreased greatly in AE1-deficient mice during acidosis (Tables 1 and 2), likely reflecting systemic volume depletion and dehydration.

We therefore examined expression and localization of AQP2 under control conditions. In AE1-null mice, AQP2 abundance was increased slightly in cortex and substantially in medulla, consistent with the observed systemic dehydration. Most inner medullary AQP2 was confined to cytoplasmic vesicular structures, in contrast to the predominantly luminal membrane localization in wild-type mice. In contrast, the less dramatically increased AQP2 of OMCD was normally localized to the luminal membrane in both slc4a1–/– and slc4a1+/+ mice (Figure 5). Gross kidney morphology revealed reduced inner medullary mass, resembling that of chronic hydronephrosis. Thus, the urinary concentrating defect of AE1-null mice is associated with defective inner medullary AQP2 trafficking in the setting of inner medullary atrophy, with a compensatory increase in abundance of outer medullary AQP2.

Distal renal tubular acidosis is often associated with nephrocalcinosis and nephrolithiasis (3,15,23,50). The elevated fractional excretion of calcium in AE1-null mice is (as in humans) a risk factor for nephrocalcinosis (Tables 1 and 2, Figure 6). Urinary citrate is thought to protect against nephrocalcinosis, (OMCD) from 4 mice versus AE1 –/– −0.123 ± 0.005 units pH/min [n = 246 type A-IC in seven OMCD from four mice; Figure 2]. However, acidification by slc4a1–/– A-IC was insensitive to diBA(5)C4 and showed reduced DIDS sensitivity (inhibition by approximately 20%), suggesting upregulation of a pharmacologically distinct anion exchange activity.

Figure 1. Urine and blood gas analysis. Urinary pH and blood gas values of wild-type (Ae1+/+), heterozygous (Ae1+/−) and AE1-deficient (Ae1−/−) mice during metabolic cage experiments under control conditions for 2 d and after oral acid loading with 0.28 M NH$_4$Cl for a third day. (A) Urinary pH in AE1-deficient mice was more alkaline than for other genotypes in all conditions but did acidify in response to the oral NH$_4$Cl load. Blood pH (B) and [HCO$_3$]$^-$ concentration (C) in wild-type and heterozygous mice were indistinguishable in all conditions. However, blood pH and [HCO$_3$]$^-$ in AE1-deficient mice under control conditions revealed metabolic acidosis. This acidosis was severely exacerbated after NH$_4$Cl loading (n = 8 mice for each genotype and time point). *P < 0.001 versus control conditions in the same genotype; *P < 0.001 versus wild-type.
and its reduced excretion in acidosis is attributed to upregulation of the NaDC1 Na⁺/citrate co-transporter (51). The hypocitraturia of AE1-deficient mice (Table 1, Figure 6) constitutes an incomplete dRTA, with oral acid loading required to unmask a mild phenotype (1,3,15,17). The AE1-deficient mouse model contrasts with the severe and often early-onset complete dRTA in patients with mutations in the orthologous human gene (1,6,53).

Discussion

Mutations in the human SLC4A1/AE1 gene cause familial dRTA (1,15,17). The attendant hyperchloremic metabolic acidosis often presents as childhood growth retardation with increased risk for dehydration, accompanied by hypokalemia and nephrocalcinosis (1,3,15,17). The AE1-deficient mouse model examined here reproduces many features of the human disease and therefore represents the first mouse model of an inborn form of complete dRTA. A mouse that lacks the IC-specific vacuolar H⁺-ATPase B1 subunit (Atp6v1b1−/−) shows only incomplete dRTA, with oral acid loading required to unmask a modest urinary acidification defect (52). This mild phenotype contrasts with the severe and often early-onset complete dRTA of patients with mutations in the orthologous human ATP6V1B1 gene (1,6,53).

Table 2. 24-h NH₄Cl loading

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ae1+/+</th>
<th>Ae1+/-</th>
<th>Ae1−/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.21 ± 0.01</td>
<td>7.20 ± 0.01</td>
<td>6.82 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>HCO₃⁻</td>
<td>20.6 ± 0.7</td>
<td>18.8 ± 0.7</td>
<td>7.1 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>PCO₂</td>
<td>53.3 ± 1.8</td>
<td>49.6 ± 1.5</td>
<td>44.4 ± 3.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>K⁺</td>
<td>3.7 ± 0.2</td>
<td>4.0 ± 0.1</td>
<td>4.9 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na⁺</td>
<td>147.9 ± 1.0</td>
<td>148.1 ± 1.0</td>
<td>156.4 ± 2.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>117.0 ± 1.8</td>
<td>119.9 ± 2.0</td>
<td>133.0 ± 4.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>P₁</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>3.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>anion gap</td>
<td>10.1 ± 1.8</td>
<td>9.5 ± 2.3</td>
<td>16.3 ± 2.4&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>osmolarity</td>
<td>304.1 ± 1.2</td>
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<td>346.8 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>creatinine</td>
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<td>0.10 ± 0.02</td>
<td>0.15 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>urea</td>
<td>10.8 ± 1.6</td>
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<td>29.6 ± 5.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.07 ± 0.06</td>
<td>6.10 ± 0.10</td>
<td>6.47 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>osmolarity</td>
<td>3302.5 ± 178.4</td>
<td>2388 ± 97.1</td>
<td>1176.5 ± 69.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>creatinine</td>
<td>83.1 ± 5.8</td>
<td>86.1 ± 13.1</td>
<td>33.2 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO₃⁻/creatinine</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>K⁺/creatinine</td>
<td>3.5 ± 0.6</td>
<td>3.1 ± 0.7</td>
<td>5.2 ± 1.0</td>
</tr>
<tr>
<td>Na⁺/creatinine</td>
<td>1.8 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>Cl⁻/creatinine</td>
<td>7.6 ± 0.5</td>
<td>7.3 ± 1.0</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>P₁/creatinine</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>NH₄⁺/creatinine</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Ca²⁺/creatinine</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Mg²⁺/creatinine</td>
<td>0.19 ± 0.04</td>
<td>0.20 ± 0.08</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>creatinine clearance</td>
<td>1.46 ± 0.40</td>
<td>0.80 ± 0.31</td>
<td>0.47 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FE Na⁺ (%)</td>
<td>0.10 ± 0.03</td>
<td>0.09 ± 0.05</td>
<td>0.34 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FE K⁺ (%)</td>
<td>6.78 ± 2.06</td>
<td>3.99 ± 1.05</td>
<td>16.72 ± 4.06</td>
</tr>
<tr>
<td>FE Cl⁻ (%)</td>
<td>0.44 ± 0.10</td>
<td>0.58 ± 0.22</td>
<td>0.76 ± 0.15</td>
</tr>
<tr>
<td>FE Ca²⁺ (%)</td>
<td>0.15 ± 0.06</td>
<td>0.24 ± 0.11</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td>FE Mg²⁺ (%)</td>
<td>4.42 ± 1.69</td>
<td>2.41 ± 1.78</td>
<td>3.80 ± 1.08</td>
</tr>
<tr>
<td>FE P₁ (%)</td>
<td>1.98 ± 0.43</td>
<td>3.02 ± 1.01</td>
<td>3.52 ± 0.62</td>
</tr>
<tr>
<td>urinary anion gap</td>
<td>−202.3 ± 69.7</td>
<td>−223.2 ± 57.6</td>
<td>80.6 ± 61.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEA</td>
<td>205.7 ± 9.5</td>
<td>176.6 ± 10.2</td>
<td>85.2 ± 5.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEA/Crea</td>
<td>2.6 ± 0.2</td>
<td>2.9 ± 0.5</td>
<td>2.7 ± 0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Summary of blood and urine data after 24 h of oral NH₄Cl loading. Urine was collected during 24 h in metabolic cages, and blood was sampled at the end of the collection period. Concentrations of electrolytes, titratable acids, and urea are expressed in units of mmol/L, creatinine in mg/dl, PCO₂ in mmHg, osmolarity in mOsmol/L, creatinine clearance in ml/min; <i>n</i> = 8 to 10 animals per genotype.

<sup>b</sup> <i>p</i> < 0.001 versus wild-type.

<sup>c</sup> <i>p</i> < 0.05 versus wild-type.
A-IC of the collecting duct are believed to complete the reabsorption of filtered bicarbonate in excess of the reabsorptive capacities of the proximal tubule and the thick ascending limb (2). The absence of bicarbonaturia in the face of the metabolic acidosis of AE1-deficient mice suggests (at least in the presence of reduced blood bicarbonate levels) that AE1 is not critical for reabsorption of filtered bicarbonate. Thus, the main function of A-IC may be rather to replenish body bicarbonate that is newly generated by cytosolic carbonic anhydrase and to release it into blood via AE1 and perhaps other basolateral anion exchangers.

Although of considerable magnitude, the residual activity of alternative chloride-bicarbonate exchange activity in A-IC of the slc4a1/H11002/H11002 mouse apparently does not suffice to support adequate bicarbonate release into the blood. The compensatory anion exchange activity was measured in unperfused OMCD tubules at isotonic bath of pH 7.4 and therefore may not optimally reflect conditions in the acidic, moderately hypertonic outer medulla of the AE1/H11002/H11002 mouse. The molecular identity, regulation, and physiologic function of the alternate anion exchanger(s) remain to be elucidated. Potential candidate anion exchangers reported previously in A-IC basolateral membrane include AE4/Slc4a9 and Slc26a7. Although AE4 has been local-
Slc26a7 anion exchanger has been reported in the basolateral membrane of A-IC of rat OMCD (37) and in an incompletely defined cell subset in human OMCD (55). Slc26a7-mediated Cl⁻/HCO₃⁻ exchange is activated by hypertonicity (37) (L. Jiang and S.L.A., unpublished observations, 2006), likely in part by mobilization from intracellular stores to the basolateral membrane (56). Slc26a7 is functionally absent in Brattleboro rats and rescued by their treatment with DDAVP (57). The reduced urinary osmolality of the slc4a1 knockout mouse suggests that any contribution of Slc26a7 to the residual Cl⁻/HCO₃⁻ activity of the superfused slc4a1 knockout OMCD might be similarly reduced. However, the localization and the transport activity of mouse Slc26a7 remain controversial, because the protein has been localized with different antibodies to the proximal tubule rather than to A-IC (58) and has been reported to mediate pH-gated Cl⁻ channel activity rather than Cl⁻/HCO₃⁻ exchange (59).

dRTA as a result of mutations in SLC4A1 can be inherited as an autosomal recessive or an autosomal dominant disease. dRTA in white patients is generally transmitted in an autosomal dominant pattern (13,15,16), whereas patients of Southeast Asian and Melanesian origin more often present recessive or compound heterozygote forms of the disease (11,12,60). Several mechanisms have been proposed to explain the dominant and recessive forms of dRTA. kAE1 forms functional homodimers or hetero-oligomers, and oligomerization of a dominant negative mutant AE1 with a wild-type polypeptide in the heterozygote A-IC likely explains some dominant disease. The mutant/wild-type heterodimer either fails to traffic to the cell surface or, in some cases, traffics normally but may not function normally at the cell surface (18,61,62). Another subset of kAE1 mutant polypeptides seems to cause dominant dRTA by inappropriate targeting to A-IC apical membrane, with resultant apical bicarbonate secretion likely short-circuiting luminal acid secretion (19,20,63). These interpretations of human dRTA are consistent with the normal renal function displayed by mice that are heterozygous for the Aε1 null allele. The data strengthen the hypothesis that haploinsufficiency of AE1 does not explain the
pathophysiology of dominant forms of human dRTA. The reason for the mild concentrating defect that is observed in the heterozygous animals during acid loading remains to be elucidated. This concentrating defect is the only abnormality yet detected in heterozygous mice.

AE1-deficient mice appear as a model for the hydrops fetalis, hemolytic anemia, and dRTA that are exhibited by children who completely lack eAE1 as a result of homozygosity for the autosomal recessive AE1 mutant V488M, Band 3 Coimbra (24). The severe disease in AE1-deficient mice also resembles the hydrops, anemia, and acidosis in Japanese black cattle that are homozygous for AE1 mutations that correspond to human AE1 646X (64). However, most autosomal recessive dRTA in humans is caused by AE1 mutations that are compatible with normal eAE1 abundance and near-normal eAE1 anion transport function in erythrocytes. These conditional loss-of-function mutations in human AE1 are functionally rescued in erythrocytes by the tissue-specific expression of the AE1-binding protein glycophorin A, which likely functions as both trafficking chaperonin and plasmalemmal subunit of eAE1 (10,14).

Patients who have inborn forms of dRTA are often prone to dehydration as a consequence of inappropriate renal water loss (3,15,17,21–23). AE1-deficient mice exhibited a urinary concentrating defect, evident as elevated serum osmolarity with inappropriately low urine osmolarity. These mice were unable to increase urine osmolarity and decrease urine output during 24-h NH4Cl loading, leading to severe dehydration as reflected in further elevation of serum osmolarity. This urinary concentrating defect is likely attributable to the intracellular retention of AQP2 water channel polypeptides in the epithelial cells of the inner medullary collecting duct. However, the increased luminal AQP2 expression and total AQP2 abundance in the outer medulla suggest intact regulation by the antidiuretic hormone vasopressin and likely represent compensatory mechanisms. AQP2 in rat inner medullary collecting duct is inhibited by activation of luminal calcium sensing receptor (65). The hypercalciuria of AE1-deficient mice may reduce luminal AQP2 expression via stimulation of luminal calcium sensing receptor. Thus, the mild diabetes insipidus of the AE1-deficient mouse kidney may be associated with defective AQP2 regulation that is spatially restricted to the inner medulla.

Nephrocalcinosis and nephrolithiasis can be associated with all forms of complete and incomplete dRTA and often constitute the initial clinical presentation of disease (3,15,23,50). The associated hypercalciuria may reflect osteomalacia or decreased function and/or expression of renal calcium transporting proteins in the presence of metabolic acidosis (66). AE1-deficient mice similarly exhibited hypercalciuria, hypochloriduria, and hyperphosphaturia, and nephrocalcinosis in a predominantly medullary distribution (3,22,51,63,67,68). We additionally noted higher serum urea levels in mice that lacked AE1, suggesting mild renal insufficiency. It remains to be clarified whether the renal failure, which likely has a prerenal component, is secondary to chronic metabolic acidosis, hydronephrosis, nephrocalcinosis, or potentially all of these. Conversely, the mild, chronic renal insufficiency may contribute to the metabolic acidosis.

**Conclusion**

AE1-deficient mice recapitulate key renal features of inherited forms of dRTA as found in patients with mutations in either AE1 or the ATP6V1B1 and ATP6V0A4 subunits of the vacuolar H+-ATPase. These mice will allow investigation of the underlying mechanisms of secondary manifestations of the disease such as nephrocalcinosis and dehydration as a result of AQP2 dysregulation. The mice will also allow study of the renal tubular adaptations to congenital dRTA, including upregulation of other bicarbonate transport proteins. AE1-deficient slc4a1−/− mice thus demonstrate that renal kAE1 is critical for systemic acid-base homeostasis. kAE1 mediates the essential step in bicarbonate release from acid-secretory IC into blood.

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Disclosures
None.

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