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Role of KCNE1-Dependent K^+ Fluxes in Mouse Proximal Tubule

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Abstract. The electrochemical gradient for K^+ across the luminal membrane of the proximal tubule favors K^+ fluxes to the lumen. Here it was demonstrated by immunohistochemistry that KCNE1 and KCNQ1, which form together the slowly activated component of the delayed rectifying K^+ current in the heart, also colocalize in the luminal membrane of proximal tubule in mouse kidney. Micropuncture experiments revealed a reduced K^+ concentration in late proximal and early distal tubular fluid as well as a reduced K^+ delivery to these sites in KCNE1 knockout (−/−), compared with wild-type (+/+) mice. These observations would be consistent with KCNE1-dependent K^+ fluxes to the lumen in proximal tubule. Electrophysiological studies in isolated perfused proximal tubules indicated that this K^+ flux is essential to counteract membrane depolarization due to electrogenic Na^+-coupled transport of glucose or amino acids. Clearance studies revealed an enhanced fractional urinary excretion of fluid, Na^+, Cl^-, and glucose in KCNE1 −/− compared with KCNE1 +/+ mice that may relate to an attenuated transport in proximal tubule and contribute to volume depletion in these mice, as indicated by higher hematocrit values.

In the proximal tubule, electrogenic Na^+-coupled entry of substrates such as amino acids and glucose across the luminal cell membrane as well as bicarbonate exit across the basolateral cell membrane are driven by the potential differences across the luminal and basolateral cell membrane, which is maintained by K^+ flux through K^+ channels (1,2). The molecular nature of the luminal K^+ channels is not known. The *KCNE1* (previously called *IsK*) gene was cloned in 1988 from rat kidney and was thought to encode a slowly activating K^+ channel as deduced from expression studies in the *Xenopus* oocyte (3). The KCNE1 protein was subsequently shown to be expressed in the luminal membrane of rat proximal tubules (4).

It was shown that KCNE1 does not form a channel by itself but participates in a complex with a pore-forming subunit to underlie specific K^+ channels. In the heart and in the inner ear, the KCNE1 partner has been identified as the product of the *KCNQ1* (or *KVLQT1*) gene (5–7). In humans, mutations in the genes encoding either KCNQ1 or KCNE1 are associated with long QT (LQT) syndrome, a familial disorder that predisposes cardiac sudden death and may be associated with deafness (8).

The pore-forming partner(s) of KCNE1 and the role of the respective channel complex in the kidney are not known. In proximal tubules, transport of amino acids and glucose is coupled to Na^+ influx, thereby depolarizing the luminal membrane. This depolarization activates a slow K^+ conductance that repolarizes the cell membrane (9–12). The voltage dependence of the KCNE1/KCNQ1 current suggested its contribution to this repolarization process (4).

The aim of this study was to elucidate the role of KCNE1 in proximal tubule function with the use of the KCNE1 knockout mouse as a model (13). In wild-type mice, we found KCNE1 in the brush-border membrane of proximal tubules colocalizing with KCNQ1. Micropuncture experiments were performed to compare K^+ transport in the proximal and up to the early distal tubule of KCNE1 −/− and +/+ mice *in vivo.* Electrophysiological studies in *in vitro* perfused proximal tubules were performed to investigate the role of KCNE1 during electrogenic Na^+-coupled transport. Finally, whole kidney excretion rates were evaluated in these mice to assess for a whole kidney phenotype.

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Materials and Methods

All animal experimentation was conducted in accord with the *NIH Guide for the Care and Use of Laboratory Animals.* Experiments were performed in KCNE1 knockout (−/−) and littermate wild-type (+/+) mice (129Sv/J) (13).

Immunohistochemistry

Mice were anesthetized with ketamine (100 mg/kg body wt intraperitoneally; Sigma, Deisenhofen, Germany) and xylazine (4 mg/kg body wt intraperitoneally; Bayer, Leverkusen, Germany) and perfused via the left ventricle with heparinized 0.9% NaCl solution (15 ml; 37°C) and, subsequently, 4% paraformaldehyde solution (15 ml; 37°C [pH 7.4]). To reduce venous resistance, the vena cava inferior was cut open distal of the renal veins. The kidneys were harvested and kept in the above-mentioned formaldehyde solution for 12 h at 4°C to 8°C, at which point they were embedded in paraffin and 2.5-μm slices were cut. After removal of the paraffin, the slices were incubated for 20 min in 10% goat serum and for 1 h with either a polyclonal antibody (1:1000) raised against the whole KCNE1 protein (14) or with an affinity-purified polyclonal antibody raised against a N-terminal peptide of KCNQ1 (CPADLGPRPRVSLDPRVSIY) (15). Specificity of the employed antibodies had been demonstrated by Western blotting for KCNE1 (14) and KCNQ1 (15), respectively. Cy3 goat anti-rabbit (Dianova, Hamburg, Germany) was used as a secondary antibody and HOE33342 to stain the nuclei. Immunofluorescence was examined with confocal laser microscopy ($\times$40 objective; excitation for Cy3: 543 nm, emission: $>$560 nm; LSM 510, Zeiss, Jena, Germany).

Clearance and Micropuncture Experiments under Anesthesia

Mice were anesthetized with thiobutabarbital (100 mg/kg body wt intraperitoneally; RBI, Natick, Massachusetts) and ketamine (100 mg/kg body wt intramuscularly), as described previously (16). Body temperature was maintained at 37.5°C by placing the animals on an operating table with a servo-controlled heating plate. The trachea was cannulated, and 100% oxygen was blown toward the tracheal tube throughout the experiment. The femoral artery was cannulated for BP measurement and blood sample withdrawal. The jugular vein was cannulated for continuous maintenance infusion of 2.25 g/dl bovine serum albumin in 0.9% NaCl at a rate of 0.5 ml/hr. For assessment of two-kidney and single-nephron filtration rates, [^{3}H] inulin was added to this infusion to deliver 20 μCi/h. Urine was collected by use of a bladder catheter. The left kidney was approached from a flank incision, freed of adherent fat and connective tissue, placed in a lucite cup, and covered with warm mineral oil. The above preparation took about 60 min, and the mice were allowed another 60 min to stabilize before micropuncture experiments were started.

During timed urine collection, the last proximal or first distal tubular loop on the kidney surface was identified by injection of small amounts of stained artificial tubular fluid into random proximal tubules by use of a micropipette (1 to 3 μm tip). After the dye was cleared from the respective tubular segment, timed fluid collections were made in 6 to 14 tubules per experiment with oil-filled pipettes over 3 to 5 min. Blood samples were collected immediately before starting and after finishing urine collection and micropuncture experiments. Experiments did not extend beyond 2 h.

In an additional set of clearance experiments that did not use micropuncture, the effect of amiloride on whole kidney function was assessed. Whereas the above-described functional studies were performed at the Department of Pharmacology, University of Tuebingen, this additional set of experiments was performed at the Department of Physiology, University of Freiburg. Mice were anesthetized with a mixture of ketamine/xylazine, as described above. A polyethylene catheter was inserted into the left femoral vein for application of fluorescein isothiocyanate (FITC)–labeled inulin (1% in 0.9% NaCl solution; 2 μl/g body wt bolus followed by 0.15 μl/min per g body wt; Sigma, Deisenhofen, Germany) and determination of GFR (17). The right femoral artery was catheterized for collection of blood. An impedance device (R. Busche, Physiologisches Institut Freiburg, Germany) was fixed at the tail to control BP throughout the experimental periods. After surgery, mice were allowed to stabilize for 30 min. After control measurements of GFR and renal electrolyte excretion for 30 min, amiloride (5 mg/kg body wt bolus intraperitoneally) was applied, and renal function was reassessed in a second 30-min period. The total blood volume taken during the experiment did not exceed 100 μl, and every blood sample was readily replaced by the same amount of 0.9% NaCl solution.

Electrophysiological Studies in Isolated Perfused Proximal Tubules

The experiments were performed in proximal straight tubules isolated from KCNE1 $-/-$ or $+/+$ mice. Segments of 0.5 to 1.0 mm length were dissected and perfused, following principally the method of Burg *et al.* (18). Modifications of the technique concerning track system, pipette arrangement, and the methods for the determination of the potential difference across the basolateral cell membrane (PD_{bl}) have been described in previous publications (19,20). The luminal perfusion rate was $>$10 nl/min. The bath (volume 1 ml) was perfused continuously at a rate of 20 ml/min. The bath and luminal perfusates were composed of the following (all numbers mmol/L): 120 NaCl, 5 KCl, 20 $NaHCO_3$, 1.3 $CaCl_2$, 1 $MgCl_2$, and 2 Na_2HPO_4. In the bath (in mmol/L), 1 glucose, 2 glutamine, and 1 Na-lactate and, in the lumen, 5 mannitol were added. Where indicated (in mmol/L), 2 mannitol were replaced by 1 glucose and 1 phenylalanine in the luminal perfusate. The solutions were constantly gassed with a mixture of 95% O_2 and 5% CO_2, resulting in a pH of 7.4. Steel tubing was used for perfusate supply to the bath to avoid loss of CO_2. Before the perfusate arrived at the bath, it passed a heat exchanger, where the temperature was raised to 38°C.

PD_{bl} was measured by a high-impedance electrometer (FD223, WPI, Science Trading, Frankfurt, Germany) connected with the electrode via an Ag/AgCl half cell. The electrodes used for recording of PD_{bl} were pulled from filament capillaries (1.5 mm outer diameter, 1.0 mm inner diameter, Hilgenberg, Malsfeld, Germany) with a Narishige PE 2 vertical puller, which was adjusted to deliver electrodes with a resistance between 100 and 200 MΩ. They were filled with 1 mol/l KCl solution immediately before use. An Ag/AgCl reference electrode was used connected with the bath via a 1 mol/l KCl-agar bridge. Experimental solutions were tested for liquid junction potentials.

A voltage recording was accepted only when the penetration of the cell membrane resulted in an instantaneous deflection of the reading. Furthermore, PD_{bl} had to be more negative than -50 mV and stable ($\pm$2 mV) for at least 1 min. Withdrawal of the electrode was to be followed by an immediate return of the electrode reading to the baseline value ($\pm$2 mV). The resistance of each electrode was checked by short current pulses delivered by a Grass S44 stimulator (Grass Instruments, Quincy, Massachusetts). The electrode holder was mounted on a Leitz micromanipulator (E. Leitz, Wetzlar, Germany). For penetrating the membrane, the electrode was advanced by a slight fingertip push against the guidance lever of the micromanipulator. The impalements were observed with an inverted microscope (Zeiss ICM, Carl Zeiss, Oberkochen, Germany) at a magnification of $\times$400.

Entry of positive charge by electrogenic transport is expected to depolarize the basolateral cell membrane. The magnitude of the depolarization depends on the magnitude of the induced current on the one hand and on the resistances of cell membranes and shunt on the other.

Analyses

Urinary flow rate was determined gravimetrically. Plasma and urine were analyzed for Na^+ and K^+ concentration by flame photometry (ELEX 6361 or FCM 6341, Eppendorf, Hamburg) and for Cl^- by electrometric titration (Chloridometer 6610, Eppendorf, Hamburg). Two kidney and single nephron GFR were determined by FITC-inulin or ^{3}H-inulin clearance, as indicated. Tubular fluid volumes were determined from column length in a constant-bore capillary. Concentrations of Na^+, K^+, or Cl^- in tubular fluid were determined as described previously (21), by use of a micro flame photometer that was developed and built by Rolf Englert and Klaus Stieler (Department of Pharmacology, University of Tübingen) on the basis of the original concept by Hampel (Frankfurt/Main, Germany) or a micro adaptation of the electrometric titration method. Concentrations of ^{3}H-inulin in plasma, tubular collections, and urine were measured by liquid-phase scintillation counting, whereas FITC-inulin concentrations in urine and plasma were determined by use of a spectrofluorometer (Shimadzu, Kyoto, Japan).

Statistical Analyses

Unpaired *t* test has been performed to analyze for statistical differences in KCNE1 −/− compared with KCNE1 +/+ mice. After establishing by ANOVA analysis for data derived from micropuncture experiments that there were no significant differences between mice within an experimental group (KCNE1 −/− or KCNE1 +/+), data of single nephrons of experimental groups were pooled and subsequently compared for significant differences between experimental groups. $P < 0.05$ has been considered to be statistically significant.

Results

Immunohistochemistry

KCNE1-specific staining was prominent in the brush-border membrane of the proximal convoluted and straight tubule of KCNE1 +/+ mice. No such staining was observed in KCNE1 −/− mice (Figure 1). The antibody directed against an N-terminal cytoplasmic epitope of KCNQ1 also labeled the brush-border membrane of proximal tubular cells: although initial segments of the proximal tubule (identified by close localization to glomeruli or direct connection with the parietal epithelium of the urinary pole of the glomerulus) appeared negative, further downstream, aspects of proximal convoluted as well as proximal straight tubules showed significant staining for KCNQ1 (Figure 2), which indicates that KCNQ1 colocalizes with KCNE1 at least at these sites. Because the expression of KCNE1 also appeared lower in the initial segments compared with the further downstream segments of the proximal tubule, a colocalization of KCNE1 and KCNQ1 in the initial proximal tubule at relatively low levels of expression cannot be excluded. No clear-cut signal for KCNQ1 could be detected downstream to the proximal tubule (not shown). The antibody for KCNE1, in addition, stained aspects of cortical collecting duct cells in KCNE1 +/+ mice, but the specificity of this finding was questioned by the observation of similar stainings in KCNE1 −/− mice (not shown). In KCNE1 −/− mice, KCNQ1 was still expressed in the brush-border membrane of the proximal tubule (not shown).

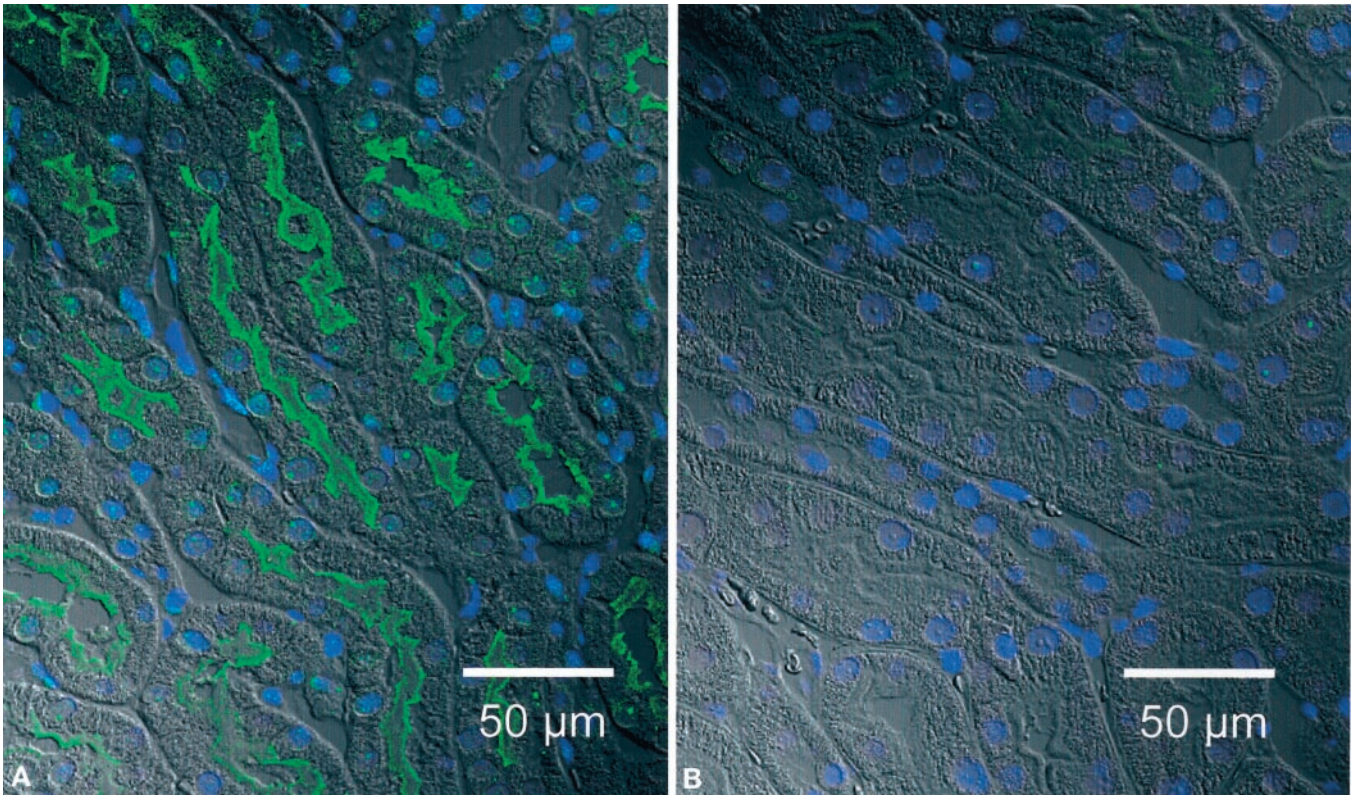

Figure 1. Immunolocalization of KCNE1 in brush border of mouse proximal tubule. (A) KCNE +/+ mouse; (B) KCNE −/− mouse. Double staining with an antibody against KCNE1 (green) and HOE33342 to stain the nuclei (blue).

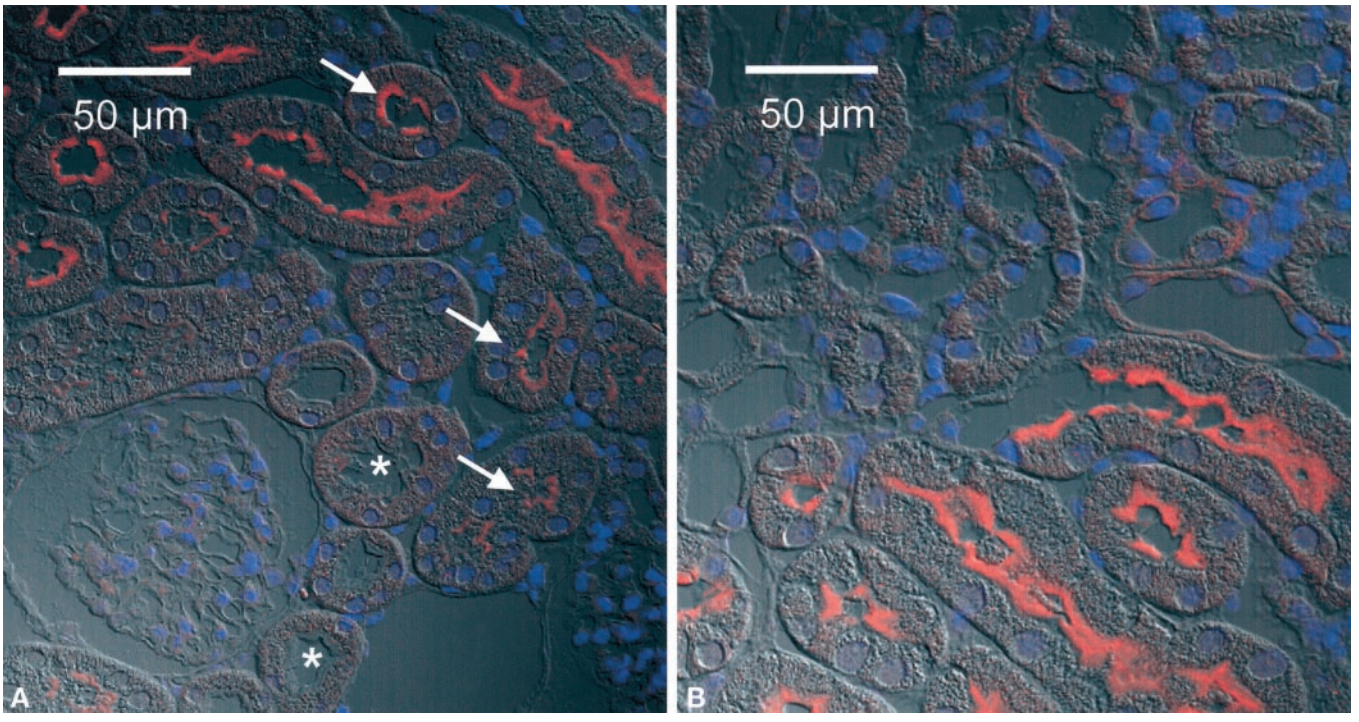

Figure 2. Immunolocalization of KCNQ1 in brush border of the KCNE1 +/+ mouse proximal tubule. Double staining with an antibody against KCNQ1 (red) and HOE33342 to stain the nuclei (blue). Although initial segments of the proximal tubule (identified by direct connection with the parietal epithelium of the urinary pole of the glomerulus or close localization to glomeruli [* in A]) appeared negative, further downstream, aspects of proximal convoluted (arrow in A), as well as proximal straight tubules (B), showed significant staining for KCNQ1.

Clearance and Micropuncture Studies Under Anesthesia

During micropuncture experiments, KCNE1 −/− exhibited a higher arterial hematocrit, but no significant differences in plasma concentrations of Na^+, K^+, or Cl^-, arterial BP, heart rate, or two kidney GFR (Table 1) were found. KCNE1 −/− mice exhibited a minor but significant decrease in plasma glucose concentration. Similar to the data on two kidney GFR, micropuncture experiments revealed that single-nephron GFR was not different between groups (Table 2). As depicted in Figures 3 and Table 2, however, the absolute and fractional delivery of K^+ to the last proximal (LP) or first distal (FD) tubular loop on kidney surface were reduced in KCNE1 −/− mice. These changes in KCNE1 −/− mice were accompanied

Table 1. Systemic and whole kidney data in micropuncture experiments[a]

Parameter	KCNE1 +/+ (n = 7)	KCNE1 −/− (n = 7)
Body weight (g)	34 ± 1	32 ± 1
MAP (mmHg)	120 ± 2	124 ± 3
Heart rate (1/min)	558 ± 13	537 ± 16
Hematocrit (%)	47 ± 1	50 ± 1*
Plasma [Na^+] (mM)	140 ± 1	140 ± 2
Plasma [Cl^-] (mM)	124 ± 3	123 ± 2
Plasma [K^+] (mM)	4.2 ± 0.1	4.2 ± 0.1
Plasma [gluc] (mM)	122 ± 4	105 ± 6*
GFR (μl/min per g body wt)	10.7 ± 1.2	9.6 ± 0.9
UV (nl/min per g body wt)	92 ± 13	126 ± 13
UNaV (nmol/min per g body wt)	10 ± 2	23 ± 5*
UCIV (nmol/min per g body wt)	16 ± 3	30 ± 4*
UKV (nmol/min per g body wt)	13 ± 2	14 ± 2
UglucV (ng/min per g body wt)	95 ± 7	142 ± 14*

[a] MAP, mean arterial blood pressure; gluc, glucose; UV, UNaV, UCIV, UKV, or UglucV, urinary excretion of fluid, Na^+, Cl^-, K^+, or glucose. * $P < 0.05$ *versus* KCNE1 +/+.

by a significant reduction in K^+ concentration in LP and FD tubular fluid (Figure 3). In comparison, the absolute delivery to the FD tubule as well as the fractional delivery of Cl^- to the LP and FD tubule were significantly increased in KCNE1 −/− mice (Table 2). No significant differences could be detected in the absolute or fractional delivery to LP or FD tubule of fluid or Na^+ between groups (Table 2).

Whereas two-kidney excretion of K^+ and urinary flow rate were not significantly different, urinary excretion of Na^+, Cl^-, and glucose were modestly but significantly increased in KCNE1 −/− *versus* +/+ mice (Table 1). Similarly, although no change was observed in whole kidney fractional excretion of K^+, fractional urinary excretion of fluid, Na^+, Cl^-, and glucose were significantly enhanced in KCNE1 −/− mice (Figure 4).

Clearance Experiments under Anesthesia: Effect of Amiloride

Under control conditions, plasma K^+ concentration in KCNE1 +/+ and −/− mice appeared to be higher and arterial BP lower in this set of experiments, compared with micropuncture experiments. Furthermore, plasma K^+ concentration was modestly decreased in KCNE1 −/− *versus* +/+ mice under control conditions, which had not been observed in micropuncture experiments (Table 3). Reasons for these differences between the two series of experiments remain unclear but may include differences in the age of the mice (as indicated by different body weights) or the experimental conditions (*e.g.*, mode of anesthesia or infusion protocol). Similar to the micropuncture experiments, KCNE1 −/− mice in this series exhibited higher values for arterial hematocrit under control conditions, whereas plasma Na^+ concentration, urinary flow rate, GFR, or the excretion of K^+ were not significantly different between groups. In comparison and similar to micropuncture experiments, the absolute and fractional excretion of Na^+ was significantly higher in KCNE1 −/− mice under control conditions (Table 3 and Figure 5).

Application of amiloride did not significantly alter plasma K^+ concentration in KCNE1 +/+ or −/− mice, but the modest difference observed between groups under control conditions before application of amiloride had dissipated. Furthermore, in KCNE1 +/+ mice the application of amiloride as expected significantly enhanced absolute and fractional urinary excretion of Na^+, whereas the absolute and fractional urinary excretion of K^+ was diminished (Table 3 and Figure 5). In KCNE1 −/− mice, amiloride elicited comparable changes in the renal excretion of Na^+ and K^+ (Table 3 and Figure 5).

Electrophysiological Studies in Isolated Perfused Proximal Tubules

In the absence of luminal substrate for Na^+-coupled transport, the potential difference across the PD_{bl} of isolated perfused straight proximal tubules approached −69 mV in KCNE1 +/+ mice and −61 mV in KCNE1 −/− mice ($P < 0.05$; Figure 6). The addition of phenylalanine plus glucose (each 1 mmol/L) to the luminal fluid depolarized the basolateral membrane by +1.8 mV in KCNE1 +/+ mice and by +10.6 mV in KCNE1 −/− mice ($P < 0.05$; Figures 6 and 7). Thus, the addition of substrates for Na^+-coupled transport led to a significantly stronger depolarization in KCNE1 −/− mice compared with KCNE1 +/+ mice. To explore whether inhibition of luminal K^+ channels could mimic the phenotype of KCNE1 −/− mice, isolated tubules of KCNE1 +/+ mice were exposed to the K^+-channel blocker Ba^{2+} (2 mmol/L). Before the addition of Ba^{2+}, addition of phenylalanine plus glucose (each 1 mmol/L) decreased PD_{bl} by +3.8 mV (Figure 6). The luminal application of Ba^{2+} depolarized the basolateral cell membrane by +6.9 ± 1.8 mV ($P < 0.05$; $n = 7$). In the presence of Ba^{2+}, the addition of phenylalanine plus glucose (each 1 mmol/L) depolarized the cell membrane by +10.6 mV (Figure 6), an effect virtually identical to the respective value in KCNE −/− mice and significantly different from the respective value in KCNE +/+ mice in the absence of Ba^{2+}.

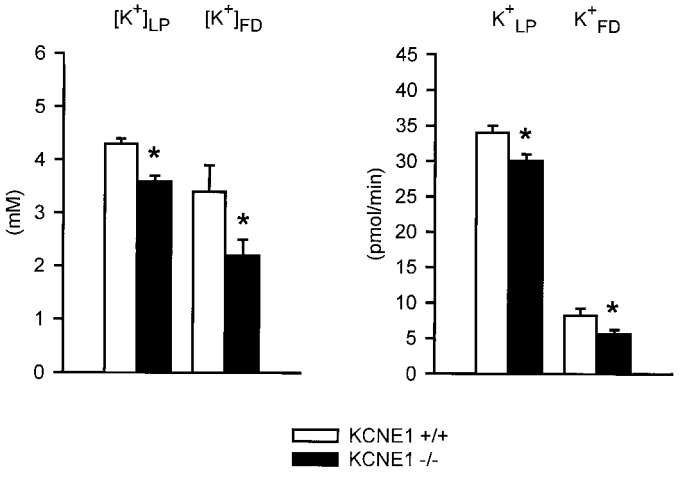

Figure 3. Concentration and delivery of K^+ in the last proximal (LP) and first distal (FD) tubular loop on kidney surface in KCNE1 −/− and +/+ mice. Data are mean ± SEM; $n = 45$ and 33 nephrons for collection in LP or FD tubule in KCNE1 +/+ and $n = 37$ and 25 in KCNE1 −/−, respectively. *$P < 0.05$ *versus* KCNE1 +/+.

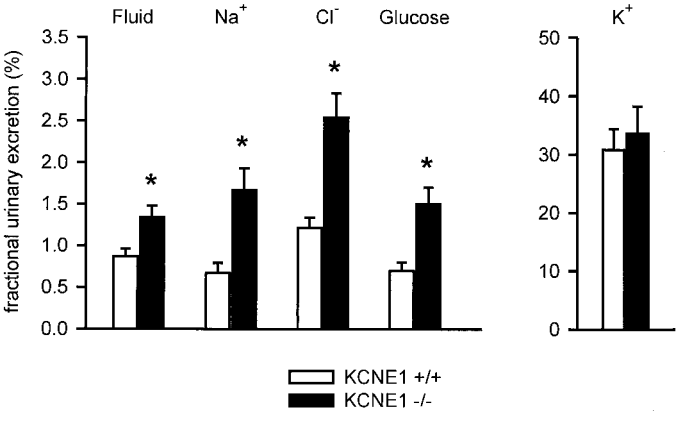

Figure 4. Fractional urinary excretion of fluid, Na^+, Cl^-, K^+, and glucose in KCNE1 −/− and +/+ mice. Data are mean ± SEM; $n = 7$ each group. *$P < 0.05$ *versus* KCNE1 +/+.

Table 2. Micropuncture data[a]

Parameter	KCNE1 +/+	KCNE1 −/−
SNGFR proximal/distal (nl/min)	13 ± 1/12 ± 1	13 ± 1/12 ± 1
V-LP/-FD (nl/min)	7.9 ± 0.3/2.8 ± 0.2	8.5 ± 0.5/3.2 ± 0.4
FR-V-LP/-FD (%)	65.5 ± 0.9/25.2 ± 1.6	67.5 ± 2.0/24.9 ± 2.2
Na-LP/-FD (pmol/min)	875 ± 42/63 ± 6	861 ± 43/82 ± 16
FR-Na-LP/-FD (%)	51.2 ± 1.6/4.0 ± 0.3	50.4 ± 1.6/4.7 ± 0.8
FR-K-LP/-FD (%)	66.0 ± 2.2/17.1 ± 1.9	57.4 ± 1.9*/10.6 ± 0.9*
Cl-LP/-FD (pmol/min)	793 ± 33/39 ± 6	878 ± 47/65 ± 12*
FR-Cl-LP/-FD (%)	53.2 ± 1.6/2.8 ± 0.3	59 ± 1.8*/4.6 ± 0.8*

[a] SNGFR, single-nephron glomerular filtration rate; V-, Na-, or CI-LP/-FD, absolute delivery of fluid, Na^+, or Cl^- to last proximal (LP) or first distal (FD) tubular loop on kidney surface; FR, fractional delivery; K, K^+. $N = 45$ and 33 nephrons for collection in LP or FD tubule in KCNE1 +/+ and $n = 37$ and 25 in KCNE1 −/−, respectively. * $P < 0.05$ *versus* KCNE1 +/+.

Thus, the luminal application of Ba^{2+} in KCNE1 +/+ mice fully mimicked the phenotype of KCNE1 −/− mice.

An increase of bath K^+ concentration from 5 to 20 mmol/L depolarized the basolateral cell membrane by 24.3 ± 1.7 mV ($n = 8$) in KCNE1 +/+ mice and by 19.7 ± 1.8 mV ($n = 8$) in KCNE1 −/− mice, the difference being not statistically significant. In the presence of phenylalanine plus glucose in the lumen, however, the same maneuver depolarized the basolateral cell membrane by 23.9 ± 1.4 mV ($n = 8$) in KCNE1 +/+ mice and by 13.3 ± 0.9 mV ($n = 8$; $P < 0.05$ *versus* KCNE1 +/+) in KCNE1 −/− mice. The depolarization after the addition of substrate thus leads to a significant decrease of the fractional K^+ conductance in KCNE1 −/− mice but not in KCNE1 +/+ mice.

Discussion

In accordance with previous experiments performed in the rat kidney (4), this study demonstrated the expression of KCNE1 in the brush border of proximal convoluted and straight tubule in mouse kidney. Further experiments revealed that KCNQ1 colocalizes with KCNE1 in the mid- to late-proximal convoluted tubule, as well as in the proximal straight tubule of mouse kidney (S2 and S3 segment). Thus, similar to the heart and inner ear (5,6,7), these findings support the notion that KCNE1 and KCNQ1 may form a K^+ channel complex at these sites of the tubular system.

The function of KCNE1 or a respective K^+ channel complex in the apical membrane of the proximal tubule was unknown. These micropuncture experiments in KCNE1 −/− and +/+ mice indicate that the absence of KCNE1 in the proximal tubule lowers the luminal K^+ concentration in the late proximal and early distal tubule as well as the delivery of K^+ to these sites because of an enhanced net reabsorption of K^+ in upstream tubular segments. Considering the presented evidence that KCNE1 is localized to the brush border of proximal tubule in wild-type mice as well as the proposed electrochemical gradient for K^+ across the luminal membrane at this site favoring potassium fluxes to the lumen (1), one may assume that KCNE1-dependent K^+ fluxes to the lumen occur in proximal tubules of wild-type mice. The absence of these K^+ fluxes would be expected to result in a net increase in K^+ reabsorption consistent with this observation in KCNE1 −/− mice. Notably, the mechanism(s) by which K^+ being filtered or secreted is reabsorbed in the proximal tubule is (are) still not clear but may involve predominantly paracellular pathways (22).

Table 3. Systemic and whole kidney data: response to amiloride[a]

Parameter	KCNE1 +/+ ($n = 8$) control/+ amiloride	KCNE1 −/− ($n = 8$) control/+ amiloride
Body weight (g)	24 ± 1	23 ± 1
MAP (mmHg)	75 ± 4/75 ± 4	93 ± 12/96 ± 10
Hematocrit (%)	45 ± 1/45 ± 1	48 ± 1/51 ± 1*
Plasma [Na^+] (mM)	140 ± 3/138 ± 3	137 ± 3/136 ± 3
Plasma [K^+] (mM)	6.6 ± 0.4/6.4 ± 0.3	5.5 ± 0.3*/6.0 ± 0.2
GFR (μl/min per g body wt)	11 ± 1/11 ± 1	12 ± 1/12 ± 1
UV (nl/min per g body wt)	113 ± 28/70 ± 14	109 ± 27/97 ± 14
FE fluid (%)	1.1 ± 0.3/0.6 ± 0.1	1.0 ± 0.2/0.8 ± 0.1
UNaV (nmol/min per g body wt)	2.3 ± 1.0/21.1 ± 5.2#	9.1 ± 2.2*/28.8 ± 4.8#
UKV (nmol/min per g body wt)	7.6 ± 1.5/1.8 ± 0.3#	12.2 ± 2.5/2.8 ± 0.5#

[a] bw, body weight; FE, fractional excretion; * $P < 0.05$ *versus* KCNE1 +/+; # $P < 0.05$ *versus* control period.

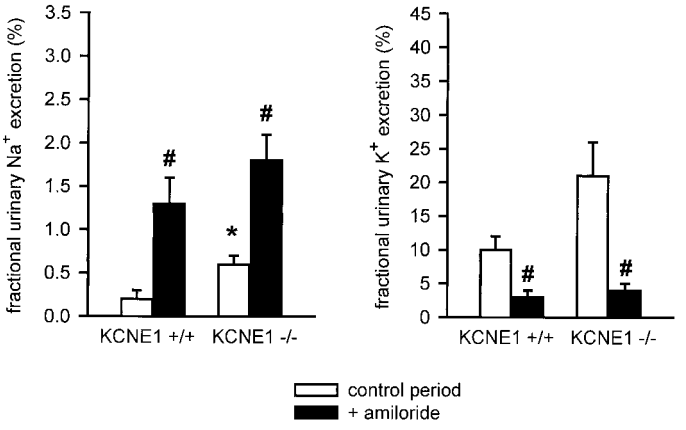

Figure 5. Fractional urinary excretion of Na^+ and K^+ under control conditions and in response to systemic application of amiloride in KCNE1 −/− and +/+ mice. Data are mean ± SEM, $n = 8$ each group. *$P < 0.05$ *versus* KCNE1 +/+. #$P < 0.05$ *versus* control period.

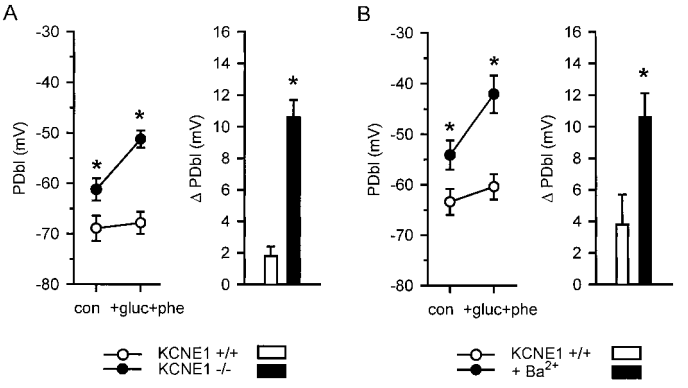

Figure 6. Effect of luminal glucose (gluc) and phenylalanine (phe) (each 1 mmol/l) on the potential difference across the basolateral cell membrane (PD_{bl}) in KCNE1 +/+ and −/− mice (A) as well as in KCNE1 +/+ mice during luminal perfusion with the K^+ channel blocker Ba^{2+} (2 mmol/L) (B). Δ, absolute change. Data are mean ± SEM; $n = 7$ to 8 per group. *$P < 0.05$ *versus* KCNE1 +/+.

These electrophysiological studies indicate that KCNE1-dependent K^+ movement from the cell to the lumen contributes to the maintenance of the electrical driving force for Na^+-coupled transport in the proximal tubule. Entry of positively charged Na^+ coupled to neutral organic substrates such as amino acids or glucose depolarizes the luminal cell membrane and because of the induction of a circular current also the basolateral cell membrane. The presence of KCNQ1/KCNE1 in the luminal cell membrane shunts part of the current entering and thus decreases the circular current, which depolarizes the basolateral cell membrane. Accordingly, the depolarization of the luminal and basolateral cell membranes are significantly decreased in KCNE1 +/+ mice, compared with KCNE1 −/− mice. Notably, KCNQ1/KCNE1 is probably activated by depolarization of the cell membrane, whereas the basolateral cell membrane K^+ conductance rather decreases upon depolarization. The inwardly rectifying properties of basolateral K^+ conductance has been demonstrated by cable analysis of frog proximal tubules (10) and is reflected by the decline of fractional K^+ conductance upon phenylalanine/glucose-induced depolarization shown here. Thus, in the absence of KCNE1, any depolarization will decrease the K^+ conductance, further aggravating the depolarization.

If the ability to repolarize the membrane potential in proximal tubule in response to Na^+-coupled transport were attenuated in KCNE1 −/− mice, what would be the expected consequence on kidney function? The concept would predict that the lack of KCNE1 augments the substrate-induced depo-

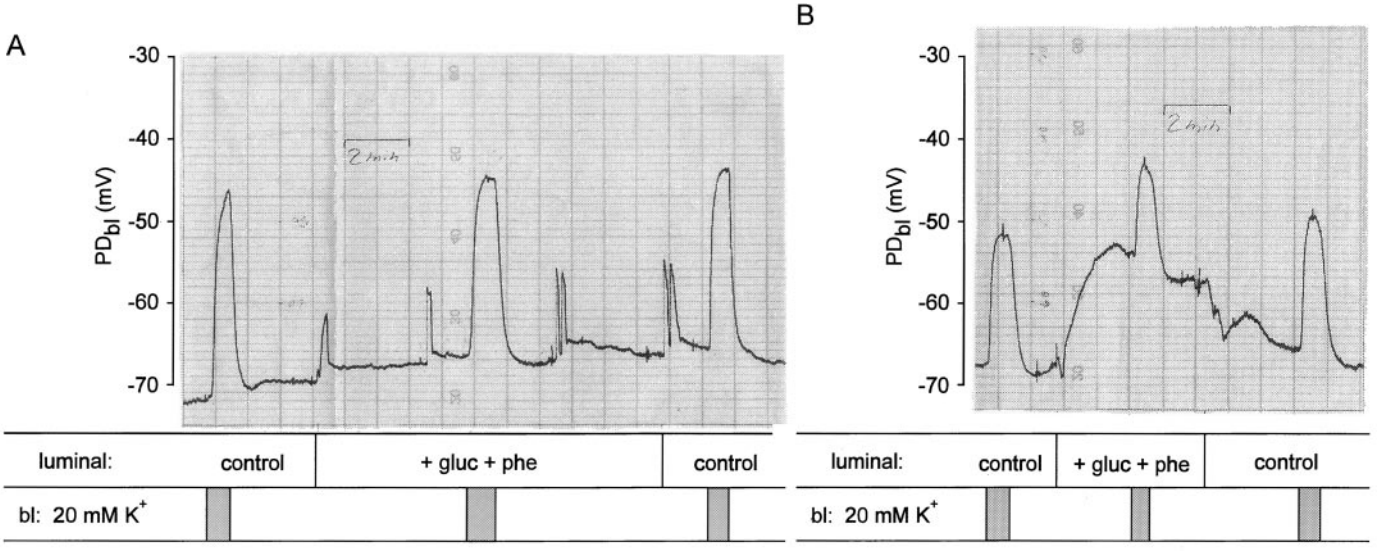

Figure 7. Effect of luminal gluc and phe (each 1 mmol/l) on PD_{bl} in KCNE1 +/+ mice (A) and KCNE1 −/− mice (B). In A, the short minor depolarizations were due to changing the perfusate. For the first three depolarizations, the perfusate contained glucose and phenylalanine; for the fourth, the perfusate was switched to control. Original tracings representative of at least seven similar experiments. bl, basolateral.

larization in the proximal tubule, which would decrease the electrical driving force for electrogenic Na^+-coupled transport. This should lead to a mild impairment of proximal tubular reabsorption of substrates, including glucose. KCNE1 −/− mice indeed exhibited a small but significant increase in absolute and fractional urinary excretion of glucose. The latter was associated with a likewise small but significant increase in urinary excretion of fluid, Na^+, and Cl^-, whereas the K^+ excretion was unaltered in mice deficient for KCNE1. Obviously, the minor decline of K^+ delivery to the early distal tubule was compensated by enhanced secretion in later nephron segments. Further experiments that employed amiloride showed that KCNE1 deficiency did not affect the absolute amount and fraction of Na^+ being reabsorbed or the amount and fraction of K^+ being secreted in amiloride-sensitive nephron segments, which indicates that nephron segments other than the late distal tubule or cortical collecting duct contributed to a lower fluid and electrolyte reabsorption in the kidney of KCNE1 −/− mice.

It seems reasonable to hypothesize that the observed minor rise in fractional excretion of fluid, Na^+, or Cl^- in KCNE1 −/− mice (+0.5%, 1.0%, or 1.3%, respectively, of filtered amounts) could be related at least in part to changes in the proximal tubule, *e.g.*, a distinct depolarization may attenuate Na^+ reabsorption and subsequently fluid and Cl^- reabsorption. In fact, micropuncture experiments demonstrated a minor but significant increase in the fractional delivery of Cl^- to the late proximal and early distal tubule (+6% and 1.8% of filtered Cl^-, respectively). In comparison, however, a significant difference in the delivery of fluid and Na^+ to these sites could not be detected between KCNE1 +/+ and KCNE1 −/− mice. Although these findings could mean that differences in Na^+ and fluid reabsorption in KCNE1 −/− mice are located downstream to the early distal tubule, it seems also possible that potentially present differences in proximal tubular reabsorption were just too small to be detected. For the latter, one has especially to consider that, although urine collections represent the result of whole kidney function for every mouse, collection of tubular fluid in the last proximal or early distal tubular loop on the kidney surface is subjected to some variation in the length of the studied tubular segment. Thus, the ability to detect small differences is limited.

An increase in urinary excretion of fluid and electrolytes may have contributed to volume depletion in KCNE1 −/− mice, as indicated by higher arterial hematocrit values. Under steady-state conditions, fluid and electrolyte intake has to match the respective loss. Because fluid and electrolyte intake did not differ between KCNE1 −/− and KCNE1 +/+ mice (data not shown), the observed higher fluid and NaCl excretion in KCNE1-deficient mice is likely to be due to the experimental conditions. It is suggested that the relatively high infusion rate employed in clearance and micropuncture studies may have offset some fluid- and NaCl-retaining mechanisms that are activated in KCNE1 −/− mice, thus unmasking the influence of KCNE1 deficiency on whole kidney excretion rates.

In summary, evidence has been provided that KCNE1 and KCNQ1 colocalize in the apical membrane of proximal tubule in mouse kidney. Results from *in vivo* micropuncture experiments were in accordance with KCNE1-dependent K^+ fluxes to the lumen in proximal tubule of wild-type mouse kidney. Electrophysiological studies indicated that this K^+ flux is essential to counteract membrane depolarization during electrogenic transport in the proximal tubule of Na^+ with glucose or amino acids. Finally, it was suggested that a resulting defect in proximal tubule reabsorption of glucose and electrolyte may contribute to volume depletion in KCNE1-deficient mice.

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