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Research Article

Previous studies using isolated tissues suggest that the colonic H, K-ATPase (cHKA), expressed in the colon and kidney, plays an important role in K+ conservation. To test the role of this pump in K+ homeostasis in vivo, we generated a cHKA-deficient mouse and analyzed its ability to retain K+ when fed a control or K+-free diet. When maintained on a control diet, homozygous mutant (cHKA-/-) mice exhibited no deficit in K+ homeostasis compared to wild-type (cHKA+/+ greater, similar mice. Although fecal K+ excretion in cHKA-/- mice was double that of cHKA+/+ mice, fecal K+ losses were low compared with urinary K+ excretion, which was similar in both groups. When maintained on a K+-free diet for 18 d, urinary K+ excretion dropped over 100-fold, and to similar levels, in both cHKA-/- and cHKA+/+ mice; fecal K+ excretion was reduced in both groups, but losses were fourfold greater in cHKA-/- than in cHKA+/+ mice. Because of the excess loss of K+ in the colon, cHKA-/- mice exhibited lower plasma and muscle K+ than cHKA+/+ mice. In addition, cHKA-/- mice lost twice as much body weight as cHKA+/+ mice. These results demonstrate that, during K+ deprivation, cHKA plays a critical role in the maintenance of K+ homeostasis in vivo.

Increased Sensitivity to K^+ Deprivation in Colonic H,K-ATPase-deficient Mice

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Abstract

Previous studies using isolated tissues suggest that the colonic H,K-ATPase (*cHKA*), expressed in the colon and kidney, plays an important role in K^+ conservation. To test the role of this pump in K^+ homeostasis in vivo, we generated a *cHKA*-deficient mouse and analyzed its ability to retain K^+ when fed a control or K^+-free diet. When maintained on a control diet, homozygous mutant ($cHKA^{-/-}$) mice exhibited no deficit in K^+ homeostasis compared to wild-type ($cHKA^{+/+}$) mice. Although fecal K^+ excretion in $cHKA^{-/-}$ mice was double that of $cHKA^{+/+}$ mice, fecal K^+ losses were low compared with urinary K^+ excretion, which was similar in both groups. When maintained on a K^+-free diet for 18 d, urinary K^+ excretion dropped over 100-fold, and to similar levels, in both $cHKA^{-/-}$ and $cHKA^{+/+}$ mice; fecal K^+ excretion was reduced in both groups, but losses were fourfold greater in $cHKA^{-/-}$ than in $cHKA^{+/+}$ mice. Because of the excess loss of K^+ in the colon, $cHKA^{-/-}$ mice exhibited lower plasma and muscle K^+ than $cHKA^{+/+}$ mice. In addition, $cHKA^{-/-}$ mice lost twice as much body weight as $cHKA^{+/+}$ mice. These results demonstrate that, during K^+ deprivation, *cHKA* plays a critical role in the maintenance of K^+ homeostasis in vivo. (*J. Clin. Invest.* 1998. 101:536–542.)
Key words: P-type ATPase • gene targeting • K^+ homeostasis • colon • kidney

Introduction

Long-term maintenance of K^+ balance depends on both the kidneys and the colon, which adjust K^+ excretion to dietary intake by regulating the rates of K^+ secretion and absorption (1, 2). K^+ absorption occurs in the renal distal tubule (3) and collecting duct (4, 5) and in the distal colon (6), and is particularly important during K^+ deprivation. The available data suggest that K^+ absorption in both kidney and colon is mediated by one or more H,K-ATPases (HKAs)[1] of the P-type family of ion-transport ATPases (2, 7–9). Two mammalian HKA isoforms, the gastric HKA and the colonic HKA (*cHKA*) have been characterized by molecular cloning (10, 11) and functional expression (12–15). *cHKA* is expressed at high levels in the distal colon (11) and is localized to the apical membranes of surface epithelial cells (15, 16), suggesting that it plays an important role in K^+ conservation by the colon. *cHKA* mRNA is also expressed in the kidney (11) and is sharply induced in the outer medullary collecting duct by K^+ deprivation (17–19). This induction correlates with increased K^+ absorption (4) and K^+-ATPase activity (20, 21) in this segment, which was attributed to an HKA.

Most of the studies implicating HKAs in K^+ conservation in the colon and kidney have been carried out in vitro, using isolated nephron segments (7, 8) or colon (2) from rat, rabbit, or guinea pig. There is little, if any, data regarding the importance of these pumps in K^+ homeostasis in vivo. To determine the role of the *cHKA* isoform, we have developed a mouse model carrying a disruption of the corresponding gene, and have analyzed the ability of wild-type and homozygous mutant animals to conserve K^+ in the colon and kidney under normal and K^+-depleted conditions.

Methods

Preparation of targeting construct and generation of mutant animals. A clone containing the 3′ region of the mouse *cHKA* gene was identified by screening a strain 129/SvJ phage genomic library with a rat *cHKA* cDNA probe (11). The clone was partially characterized by PCR, restriction mapping, and sequence analysis. A 6.7-kb HindIII fragment containing exons 15–23 was digested with EcoRI, which cut at a unique site located in exon 20, thereby yielding a 5-kb 5′ arm and a 1.7-kb 3′ arm. These arms were inserted on either side of the *neomycin resistance (neo)* gene in the targeting vector MJK-KO (gift of Dr. Steven Potter, Children's Hospital Medical Center, Cincinnati, OH), with the *cHKA* and *neo* genes in the same orientation, and the *herpes simplex virus thymidine* (*HSV-tk*) gene at the 3′ end of the *cHKA* sequences (Fig. 1 *A*).

Embryonic stem cells (ES) cells were electroporated with the linearized targeting construct (5 nM) and grown in the presence of both G418 (200 μg/ml), which selects for the presence of the *neo* gene, and gancyclovir (2 μM), which selects for the absence of the *HSV-tk* gene.

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1. *Abbreviations used in this paper: cHKA*, colonic H,K-ATPase; ES, embryonic stem cells; GFR, glomerular filtration rate; HKA, H,K-ATPase; *HSV-tk*, herpes simplex virus thymidine kinase; *neo*, neomycin resistance; RT, reverse transcriptase.

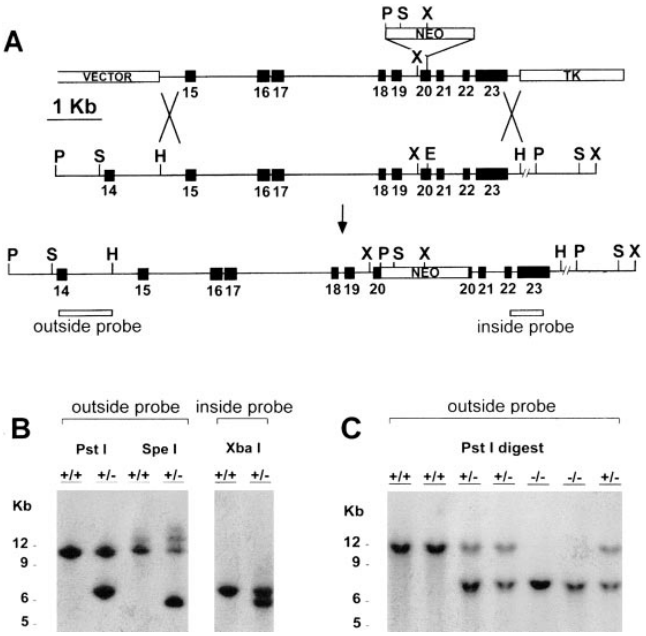

Figure 1. cHKA gene targeting strategy and Southern blot analysis. (*A*) Homologous recombination between the targeting construct (*top*) and the wild-type gene (*middle*) yields a mutant allele (*bottom*) in which the *neo* gene disrupts exon 20. The outside and inside probes and the restriction sites used for identification of the targeted allele are indicated. P = PstI, S = SpeI, H = HindIII, X = XbaI, NEO = *neo* gene, TK = *HSV-tk* gene. (*B*) Southern blot analysis of DNA from wild-type (+/+) and targeted (+/−) ES cells using the indicated probes reveal novel PstI (6.8 vs. 10.5 kb for +/+), SpeI (5.8 vs. 10.5 kb for +/+), and XbaI (6.8 vs. 5.8 kb for +/+) fragments in the targeted cells. (*C*) Southern blot analysis of tail DNA from offspring of a representative heterozygous cross show that live offspring of all three genotypes are produced.

After 6 d, gancyclovir was removed and cells were selected in the presence of G418 for four additional days. ES cells containing a disrupted *cHKA* allele were identified by Southern blot analysis using probes from outside and inside the homologous region that was present in the construct (Fig. 1). Chimeric mice were generated by injecting targeted ES cells into C57Bl/6J blastocysts and implanting the blastocysts into ICR pseudopregnant females. Breeding of chimeric males with Black Swiss females yielded an animal carrying the disrupted *cHKA* gene in its germline. This mouse was bred to establish a colony on a NSA background. Genotypes were determined by Southern blot or PCR analysis of genomic DNA isolated from tail biopsies.

Northern blot and reverse transcriptase-PCR (RT-PCR) analyses. Total RNA was isolated using Tri Reagent^R (Molecular Research Center, Inc., Cincinnati, OH) and Northern blots were prepared as described earlier (11). Hybridization analyses were performed at high stringency by the method of Church and Gilbert (22). All of the probes were generated by PCR. The rat *cHKA* probe was a mixture of three PCR products, spanning nucleotides −135–525, 2369–3000, and 3098–3678. The *neo* gene probe contained the entire coding sequence. The mouse GAPDH probe spanned nucleotides 865–1095.

RNA samples from $cHKA^{++}$ and $cHKA^{-/-}$ tissues were reverse transcribed using an oligo(dT) primer and SuperScript II RNase H$^-$ reverse transcriptase (GIBCO BRL, Gaithersburg, MD). PCR was performed using a forward primer corresponding to nucleotides 2368–2389 in exon 17 and two different reverse primers, one complementary to nucleotides 2980–3002 in exon 21, numbered according to the rat *cHKA* sequence (11), and the other complementary to sequences from the 3′ end of the *neo* gene. The PCR products were fractionated by agarose gel electrophoresis, visualized by staining with ethidium bromide, and then subcloned and sequenced.

Balance studies. Adult mice, 3–5 mo old, were housed in individual metabolic cages (O'Hara & Co., Tokyo, Japan) in which the animal resides above a tapered glass ball that separates the urine from the feces, and fed either a control diet containing 1% K^+ or a K^+-free diet containing $< 0.004\%$ K^+ (Harlan Teklad, Madison, WI), with free access to distilled water. The feces collected over three consecutive days were pooled, weighed, and resuspended overnight in 0.75 N nitric acid at 4°C. After centrifugation an aliquot of the supernatant was measured for K^+ and Na^+ content with a flame photometer (model 480; Corning Medical and Scientific, Medfield, MA). The volume of urine recovered during each 24-h period was measured and K^+ and Na^+ concentrations were determined by flame photometry.

Plasma analyses. Blood was collected from awake mice by tail bleeding into heparin-treated capillary tubes and immediately analyzed for gases, electrolyte concentrations, and pH using a Chiron Diagnostics analyzer (model 384; Norwood, MA). Aldosterone levels were determined with a solid-phase ^{125}I radioimmunoassay kit designed to work on unextracted plasma (Diagnostic Products Corp., Los Angeles, CA).

Measurement of glomerular filtration rate (GFR) and fractional K^+ excretion. Mice were anesthetized with an intraperitoneal injection of inactin (100 μg/g body weight) and ketamine (50 μg/g body weight) and maintained at 37°C. Polyethylene catheters were inserted into the femoral artery and vein and into the bladder. Fluorescein isothiocyanate–labeled inulin (1% in saline) was delivered to the animals as a bolus of 2 μl/g body weight followed by a constant infusion at a rate of 0.15 μl/min/g body weight throughout the remainder of the experiment. A 30-min recovery and equilibration period was allowed before the start of two consecutive 30-min clearance periods. Urine and a 40-μl blood sample were collected at the midpoint of each period. Each blood sample was replaced with an identical volume of blood obtained from a donor animal. Fluorescein isothiocyanate–labeled inulin concentrations in plasma and urine were measured with a Fluoroskan II fluorescent microplate reader (Labsystems Inc., Marlboro, MA). For each animal, GFR was averaged over the two clearance periods. Fractional K^+ excretion, in the same anesthetized mice for which GFR was analyzed, was calculated using the values determined for GFR, plasma K^+, and urinary K^+ excretion.

Determination of muscle K^+ content. Immediately after removal from the animal, leg skeletal muscle was homogenized in distilled water and the proteins were precipitated with 10% TCA. After centrifugation, the K^+ content of the supernatant was analyzed by flame photometry.

Statistical analyses. Data were expressed as means±SEM and statistical significance was assessed by using a mixed factorial ANOVA with repeated measures.

Results

Generation of mice carrying a disruption of the cHKA α-subunit gene. The *cHKA* gene was disrupted using the targeting strategy diagrammed in Fig. 1 *A*. 10 targeted ES cell clones were identified by Southern blot analysis of DNA from 100 G418- and gancyclovir-resistant colonies (Fig. 1 *B*). One of three targeted ES cell–lines that were used for blastocyst-mediated transgenesis led to germline transmission of the targeted *cHKA* allele. Breeding of heterozygous *cHKA* mice yielded live offspring of all three possible genotypes, as determined by Southern blot analysis of DNA from tail biopsies (Fig. 1 *C*). Genotype frequencies for 314 pups obtained from heterozygous matings were almost identical to the normal 1:2:1 Mendelian ratios (26% wild-type, 51% heterozygous, and 23% homozygous mutant), indicating the absence of embryonic

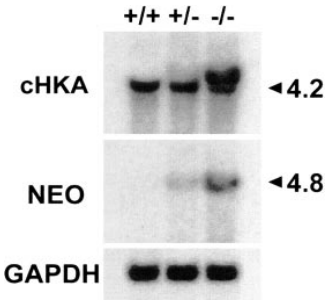

Figure 2. Northern blot analysis of colon RNA (15 μg) from *cHKA*$^{+/+}$, *cHKA*$^{+/-}$, and *cHKA*$^{-/-}$ mice. The blot was hybridized successively with a *cHKA* probe, a *neo* probe, and a *GAPDH* probe. Autoradiographic exposure times were 1 d.

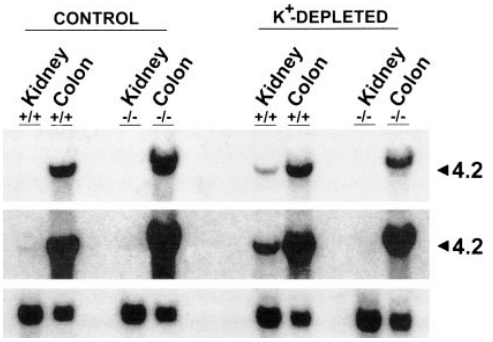

Figure 4. Northern blot analysis of *cHKA* mRNA in the colon and kidneys of control or K$^+$-depleted *cHKA*$^{+/+}$ and *cHKA*$^{-/-}$ mice. Total RNA (15 μg) was isolated from pooled tissues of five animals fed control or K$^+$-free diets for 18 d. Hybridization was performed with a *cHKA* probe; autoradiographic exposures were 20 (*top*) and 80 h (*middle*). The blot was stripped, hybridized with a GAPDH probe (*bottom*), and exposed for 1 d to confirm equal loading of RNA in each lane. Note that the amount of GAPDH mRNA differs between the kidney and colon, but is similar in the samples for each organ.

lethality. When maintained on a normal diet the *cHKA*$^{-/-}$ mice were indistinguishable from wild-type mice with respect to survival, body weight, and behavior. The fur of *cHKA*$^{-/-}$ mice sometimes appeared to be poorly groomed compared with *cHKA*$^{+/+}$ mice, but the significance of this observation remains to be determined.

Northern blot analysis revealed the expected 4.2-kb wild-type *cHKA* mRNA in the colon of *cHKA*$^{+/+}$ and *cHKA*$^{+/-}$ mice, whereas 4.1- and 4.8-kb transcripts were observed in the colon of *cHKA*$^{-/-}$ mice (Fig. 2, *top*). The relative intensities of the signals for the mutant transcripts in *cHKA*$^{+/-}$ and *cHKA*$^{-/-}$ mice suggest that the gene is upregulated in *cHKA*$^{-/-}$ colon, possibly in response to the deficit in K$^+$ absorption described below. Hybridization with a *neo* gene probe demonstrated that the *neo* gene was included in the relatively abundant 4.8-kb transcript, but not in the 4.1-kb transcript (Fig. 2, *middle*). This suggests that the smaller transcript arose by the excision of exon 20, and that the larger transcript arose by the use of the polyadenylation site in the *neo* gene. The latter event would eliminate 1.0 kb of sequence from the end of the *cHKA* mRNA while adding the 1.6-kb *neo* gene, thereby accounting for the 0.6-kb increase in size of the larger transcript. The existence of these transcripts, in which sequences encoding critical transmembrane domains were eliminated, was confirmed by RT-PCR analysis (Fig. 3). PCR amplification using primers from exons 19 and 21 yielded a 520-bp product in which the donor site of exon 19 was spliced to the acceptor site of exon 21, thereby deleting exon 20 and the *neo* gene and introducing a frameshift in the coding sequence. PCR amplification using primers from exon 17 and the 3′ end of the *neo* gene yielded a 1.75-kb product that included wild-type splicing patterns for exons 17–20 and terminated with the 1.6-kb *neo* gene.

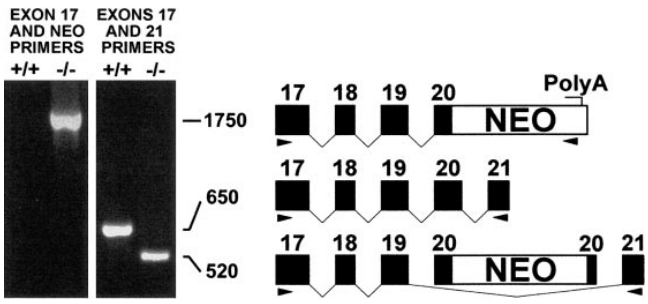

Figure 3. RT-PCR analysis of wild-type and mutant mRNAs. Total colon RNA from *cHKA*$^{+/+}$ and *cHKA*$^{-/-}$ mice was reverse transcribed using an oligo(dT) primer. Amplification of first-strand cDNAs using specific primers (*arrowheads*) from exon 17 and the 3′ end of the *neo* gene yielded a 1.75-kb product, and amplification with primers from exons 17 and 21 yielded 650- (wild-type) and 520-bp (mutant) products. Sequence analysis demonstrated that these products correspond to the splicing patterns shown on the right.

Effects of K$^+$ depletion on cHKA mRNA expression in the colon and kidney. Northern blot analyses using a *cHKA* probe were performed on total RNA from the colon and kidney of *cHKA*$^{+/+}$ and *cHKA*$^{-/-}$ mice maintained on control or K$^+$-free diets for 18 d (Fig. 4). The *cHKA* mRNA was abundant in the colon of *cHKA*$^{+/+}$ mice, but only trace levels were observed in *cHKA*$^{+/+}$ kidneys. The amount of 4.1-kb mutant mRNA was reduced in the colon of K$^+$-depleted *cHKA*$^{-/-}$ mice. The wild-type mRNA was sharply upregulated in the kidney, but not in the colon of K$^+$-depleted *cHKA*$^{+/+}$ mice. The mutant *cHKA* transcripts observed in the colon were not detected in the kidneys of *cHKA*$^{-/-}$ mice maintained on either the normal or the K$^+$-free diet (Fig. 4).

Urinary and fecal K$^+$ and Na$^+$ excretion. K$^+$ and Na$^+$ excretion in urine and feces was monitored during a 27-d period in which *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice were fed a control diet for the first 9 d and a K$^+$-free diet for the next 18 d.

The volume of urine per day was comparable in *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice during both the control diet period (1.93±0.27 and 2.00±0.16 ml/d, respectively) and during the K$^+$-free diet period (2.24±0.22 and 2.53±0.40 ml/d, respectively). As shown in Fig. 5 *A*, urinary K$^+$ excretion was similar in *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice maintained on the control diet (762±95 vs. 809±70 μmol/d). Urinary K$^+$ excretion in both *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice dropped by ~ 90% after 24 h of K$^+$ depletion, and then decreased very slowly throughout the K$^+$-free period. During the last week, K$^+$ excretion was 5.9±1.0 and 5.0±0.6 μmol/d for *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice, respectively (Fig. 5 *A*, *inset*). The difference was not statistically significant ($P < 0.10$), although daily K$^+$ excretion was consistently greater in *cHKA*$^{-/-}$ than in *cHKA*$^{+/+}$ mice. Urinary Na$^+$ excretion was similar for *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice fed the control diet (403±51 and 428±37 μmol/d, respectively), and decreased by approximately the same extent in both groups during the K$^+$ depletion period (236±18 and 273±22 μmol/d (Fig. 5 *B*).

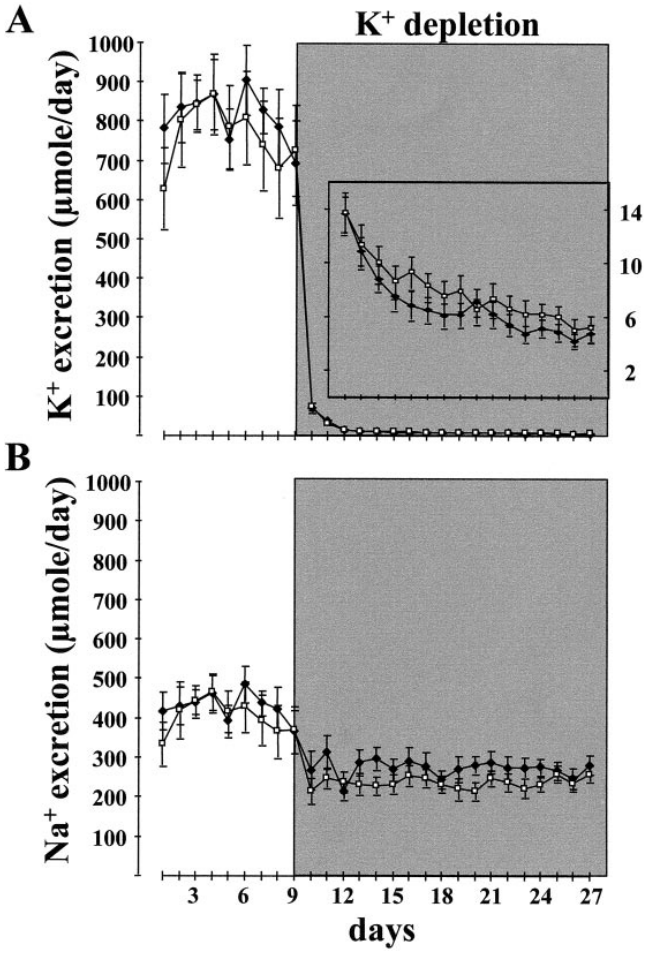

Figure 5. Urinary K^+ and Na^+ excretion in $cHKA^{+/+}$ (*filled diamonds*) and $cHKA^{-/-}$ (*open squares*) mice. Animals were fed a control diet for 9 d, followed by a K^+-free diet for 18 d (*shaded area*). Urinary volume and K^+ and Na^+ concentrations were determined daily for each animal. Results are expressed as total K^+ (*A*) or Na^+ (*B*) excreted per day. The inset in *A* shows K^+ excretion between days 12 and 27 on a magnified scale. The differences in K^+ or Na^+ excretion between $cHKA^{+/+}$ ($n = 14$) and $cHKA^{-/-}$ ($n = 16$) mice were not statistically significant.

The quantity of feces excreted by $cHKA^{-/-}$ and $cHKA^{+/+}$ mice was similar during the control diet period (1.49±0.07 and 1.56±0.08 g/3 d, respectively). This output dropped by ~ 35–40% in both groups of mice after being switched to the K^+-free diet (0.91±0.04 and 1.01±0.03 g/3 d, respectively). When maintained on the control diet, fecal K^+ excretion in $cHKA^{-/-}$ mice was twice that of $cHKA^{+/+}$ mice (117±5 vs. 53±6 µmol/3 d, respectively, $P < 0.001$). The difference was more pronounced during the K^+ depletion period, as K^+ excretion was reduced 4.5-fold in $cHKA^{-/-}$ mice (26.0±0.6 µmol/3 d) and 7-fold in $cHKA^{+/+}$ mice (7.6±1.5 µmol/3 d) ($P < 0.001$) (Fig. 6 *A*). Fecal excretion of Na^+ was approximately the same in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice fed the control diet (52±4 and 46±7 µmol/3 d, respectively) and was unchanged in each group by K^+ depletion (38±2 and 40±5 µmol/3 d, respectively) (Fig. 6 *B*).

Body and kidney weight. The body weights of $cHKA^{-/-}$ and $cHKA^{+/+}$ mice were not significantly different at the beginning of the experiments (34.9±1.2 and 34.7±0.7 g, respectively), and weight gains were comparable for both groups during the control diet period (37.2±1.5 and 37.3±1.2 g). In contrast, when maintained on the K^+-free diet, $cHKA^{-/-}$ mice lost weight more rapidly than $cHKA^{+/+}$ mice. After 18 d of K^+ depletion, the decrease in body weight was ~ 20% in $cHKA^{-/-}$ mice and 10% in $cHKA^{+/+}$ mice ($P < 0.03$) (Fig. 7 *A*).

Total kidney weights were comparable in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice fed a control diet (0.55±0.04 and 0.51±0.04 g, respectively). After 18 d on the K^+-free diet, the mean kidney weight of $cHKA^{-/-}$ mice was slightly greater than that of $cHKA^{+/+}$ mice (0.69±0.04 vs. 0.60±0.03 g, $P < 0.02$) (Fig. 7 *B*).

Plasma electrolytes, aldosterone levels, GFR, and muscle K^+ content. The K^+ status was assessed in mice fed control or K^+-free diets for 18 d by measuring plasma K^+ concentrations and K^+ content of skeletal muscle. Plasma aldosterone levels and GFR were also determined, as they are known to be altered during K^+ deprivation (23).

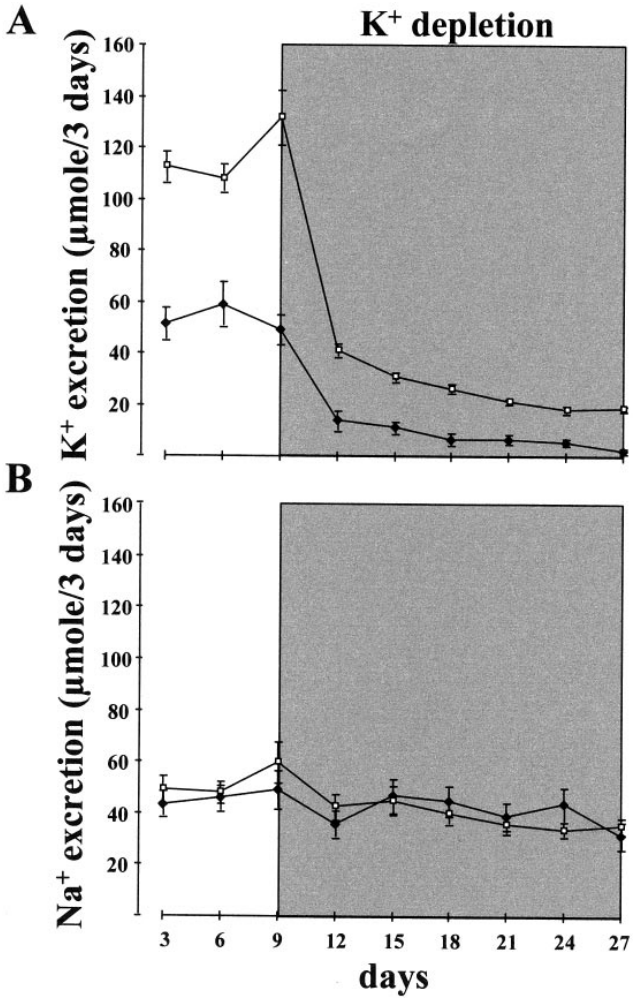

Figure 6. Fecal K^+ and Na^+ excretion in $cHKA^{+/+}$ (*filled diamonds*) and $cHKA^{-/-}$ (*open squares*) mice. Animals were fed a control diet for 9 d and a K^+-free diet for 18 d (*shaded area*). Feces from each animal were collected over consecutive 3-d periods and K^+ and Na^+ contents were measured. The results are expressed as total K^+ (*A*) or Na^+ (*B*) excreted per 3 d. Fecal K^+ excretion was higher in $cHKA^{-/-}$ ($n = 16$) than in $cHKA^{+/+}$ ($n = 14$) mice, both on control ($P < 0.001$) and on K^+-free ($P < 0.001$) diets.

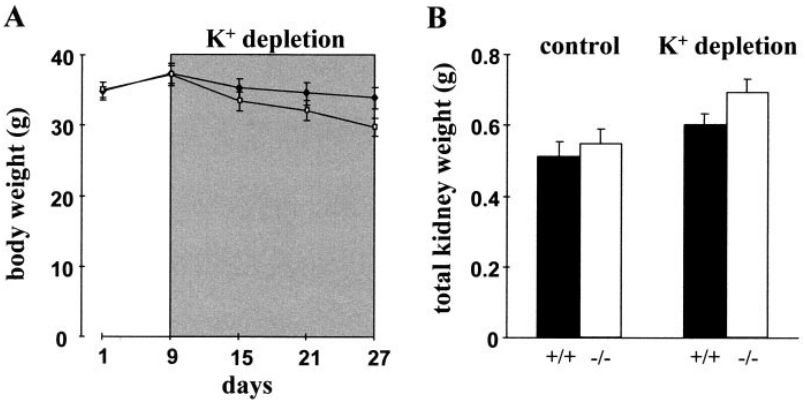

Figure 7. Body and kidney weights of $cHKA^{+/+}$ and $cHKA^{-/-}$ mice. (*A*) For each animal, body weight was monitored during control and K^+-free diet periods. Weight loss throughout the K^+ depletion period was twofold greater in $cHKA^{-/-}$ mice (*open squares*) compared to $cHKA^{+/+}$ mice (*filled diamonds*) ($P < 0.03$ at day 27). (*B*) Kidney weight was measured in animals fed a control diet or K^+-free diet for 18 d. Kidneys were significantly enlarged in $cHKA^{-/-}$ mice fed K^+-free versus control diets ($P < 0.02$). Kidneys of K^+-depleted $cHKA^{-/-}$ mice were also significantly larger than kidneys of K^+-depleted $cHKA^{+/+}$ mice ($P < 0.02$). The numbers of mice studied were 11 $cHKA^{+/+}$ and 10 $cHKA^{-/-}$ on control diet, and 14 $cHKA^{+/+}$ and 16 $cHKA^{-/-}$ on K^+-free diet.

No difference in plasma K^+ was observed between $cHKA^{-/-}$ and $cHKA^{+/+}$ mice maintained on a control diet (5.01±0.24 and 5.05±0.27 mmol/liter, respectively), however plasma K^+ in $cHKA^{-/-}$ mice was significantly reduced relative to that of $cHKA^{+/+}$ mice at the end of the K^+ depletion period (2.39±0.19 vs. 3.14±0.14 mM, respectively, $P < 0.02$) (Fig. 8 *A*). Plasma Na^+, HCO_3^-, and pH were similar in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice during the control diet period and did not change significantly during the K^+ depletion period (Fig. 8, *B–D*).

GFR was determined in anesthetized mice. It should be noted that four of the nine K^+-depleted $cHKA^{-/-}$ mice that were examined died during the surgery needed to perform GFR measurements, whereas there were no deaths among the eight K^+-depleted $cHKA^{+/+}$ mice that were analyzed. Cardiac arrhythmias, apparent in the pulse-rate recordings obtained with the femoral artery catheter, were noted in two of the five K^+-depleted $cHKA^{-/-}$ mice that survived during the procedure; the arrhythmias were severe in an animal with a plasma K^+ concentration of 1.57 mM and mild in an animal with a plasma K^+ concentration of 2.25 mM. Arrhythmias were not observed in any of the other groups of mice. GFR was comparable in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice maintained on the control diet (438±43 and 395±53 μl/min, respectively). After 18 d

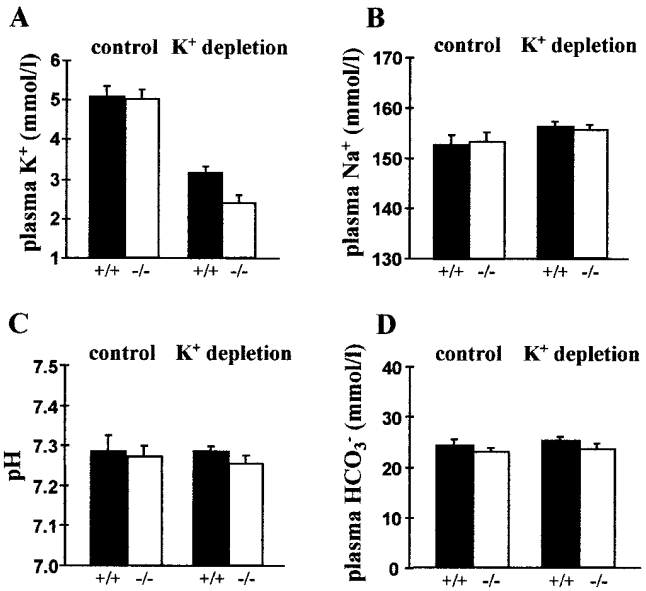

Figure 8. Plasma electrolytes and pH in $cHKA^{+/+}$ and $cHKA^{-/-}$ mice. Plasma K^+ (*A*), Na^+ (*B*), pH (*C*), and pCO_2 were measured on arterial blood collected from animals fed control or K^+-free diets for 18 d. Plasma HCO_3^- (*D*) was calculated from pH and pCO_2 values. Plasma K^+ was significantly reduced in both $cHKA^{+/+}$ and $cHKA^{-/-}$ mice fed a K^+-free diet when compared to mice on the control diet ($P < 0.005$); in addition, plasma K^+ was significantly lower in K^+-depleted $cHKA^{-/-}$ mice compared to K^+-depleted $cHKA^{+/+}$ mice ($P < 0.02$). No statistically significant differences in plasma Na^+, pH, or HCO_3^- were observed between any of the experimental groups. The numbers of mice studied were 11 $cHKA^{+/+}$ and 10 $cHKA^{-/-}$ on control diet, and 14 $cHKA^{+/+}$ and 16 $cHKA^{-/-}$ on K^+-free diet.

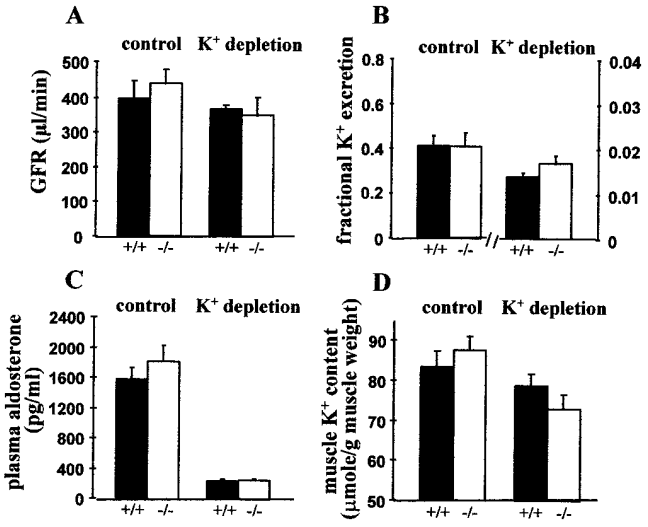

Figure 9. GFR, fractional K^+ excretion, plasma aldosterone, and muscle K^+ content in $cHKA^{+/+}$ and $cHKA^{-/-}$ mice fed control or K^+-free diets for 18 d. GFR (*A*) was not significantly reduced in either wild-type or knockout mice by K^+ depletion. Fractional K^+ excretion (*B*) was decreased similarly in both K^+-depleted $cHKA^{+/+}$ and K^+-depleted $cHKA^{-/-}$ mice ($P < 0.001$). Plasma aldosterone (*C*) was also reduced in both groups of animals by K^+ depletion ($P < 0.001$). Skeletal muscle K^+ content (*D*) expressed per gram of wet muscle weight was reduced significantly by K^+ depletion only in $cHKA^{-/-}$ mice ($P < 0.05$). The numbers of mice studied ($cHKA^{+/+}$ on control diet, $cHKA^{+/+}$ on K^+-free diet, $cHKA^{-/-}$ on control diet, $cHKA^{-/-}$ on K^+-free diet) were respectively: 10, 8, 9, and 5 for GFR and fractional K^+ excretion; 6, 9, 14, and 11 for aldosterone levels; 11, 9, 9, and 9 for muscle K^+.

of K^+ depletion, the mean values for GFR were slightly reduced in both groups (351±51 and 366±14 μl/min, respectively) (Fig. 9 *A*), but the differences were not statistically significant. Fractional K^+ excretion was the same in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice fed the control diet (0.41±0.06 and 0.41±0.04, respectively). Fractional K^+ excretion was sharply decreased, and to approximately the same extent in both groups at the end of the K^+ depletion period (0.017±0.002 in $cHKA^{-/-}$ and 0.014±0.001 in $cHKA^{+/+}$ (Fig. 9 *B*). Although the mean value for fractional K^+ excretion was 20% greater in the K^+-depleted $cHKA^{-/-}$ mice, the difference was not statistically significant.

Plasma aldosterone levels were comparable in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice fed the control diet (1,832±210 and 1,563±154 pg/ml, respectively) and sharply reduced to the same extent in both groups (255±20 and 231±19 pg/ml, respectively) at the end of the K^+ depletion period (Fig. 9 *C*).

Muscle K^+ content was similar in $cHKA^{-/-}$ and $cHKA^{+/+}$ mice fed the control diet (87.5±3.4 and 83.5±3.9 μmol/g muscle weight, respectively). At the end of the K^+ depletion period, muscle K^+ content was significantly reduced in $cHKA^{-/-}$ mice ($P < 0.05$) but not in $cHKA^{+/+}$ mice (72.7±3.8 and 78.8±2.9 μmol/g muscle weight, respectively) (Fig. 9 *D*).

Discussion

In this study we prepared a mouse line carrying a disruption of the *cHKA* α-subunit gene in order to investigate the role of *cHKA* in K^+ homeostasis in vivo. In the colon of $cHKA^{-/-}$ mice, mutant transcripts were formed either by aberrant splicing or by inclusion of the *neo* gene, which eliminates sequences encoding some of the transmembrane domains that are required for transport activity. Although wild-type *cHKA* mRNA was sharply induced in kidneys of K^+-depleted $cHKA^{+/+}$ mice, the aberrant *cHKA* transcripts could not be detected in kidneys of K^+-depleted $cHKA^{-/-}$ mice, demonstrating that insertion of the *neo* gene eliminates expression of the mRNA in the kidney. $cHKA^{-/-}$ mice were born at normal Mendelian ratios, and grew to adulthood with no obvious disease phenotype when maintained on a control diet. Thus, healthy adult *cHKA*-deficient mice were available for studies of the effects of K^+ deprivation.

The most significant finding of this study is that $cHKA^{-/-}$ mice are more sensitive than $cHKA^{+/+}$ mice to K^+ deprivation, thereby demonstrating, for the first time, that *cHKA* plays an important role in K^+ homeostasis in the living animal. When maintained on a K^+-free diet, $cHKA^{-/-}$ mice had lower plasma K^+, lower muscle K^+, and lost more body weight than $cHKA^{+/+}$ mice. Unlike the wild-type mice, $cHKA^{-/-}$ mice exhibited a high incidence of mortality as a result of the surgical procedures carried out at the end of the K^+ depletion period, which is probably indicative of a general weakness due to the more severe hypokalemia. These data provide an in vivo correlate to the results of previous in vitro studies, which strongly suggested that *cHKA* might play a role in K^+ conservation (2, 7, 8).

Data from many laboratories indicate that K^+ absorption in the kidneys is mediated by one or more HKAs (7, 8), and K^+ depletion has been shown to induce K^+ absorption in both the distal tubule (3) and collecting duct (4, 5, 24). *cHKA* mRNA levels are sharply upregulated in rat kidney by K^+ depletion (17–19, 25). In situ hybridization (17, 19) and RT-PCR analysis of RNA from isolated tubules (25) revealed that this induction occurs primarily in the outer medullary collecting duct, with lesser levels of induction in the cortical collecting duct. On the basis of these observations, it has been suggested that *cHKA* might be responsible for K^+ absorption in the collecting duct during K^+ deprivation (17–19, 25). As observed in the rat, K^+ depletion caused a sharp increase in *cHKA* mRNA in kidneys of $cHKA^{+/+}$ mice, consistent with the possibility that it functions in renal K^+ conservation in the mouse. However, when the animals were switched to a K^+-free diet, urinary K^+ excretion was rapidly reduced in both $cHKA^{+/+}$ and $cHKA^{-/-}$ mice, dropping by 95% within 24 h and by > 99% during the last week of K^+ deprivation. These data demonstrate that *cHKA* is not the major mechanism for K^+ conservation in the kidney during K^+ depletion, which is likely to be due to a shutdown of K^+ secretion. However, they do not rule out the possibility that *cHKA* is involved in renal K^+ conservation by further reducing urinary K^+ losses. Indeed, the mean values for daily K^+ excretion were consistently higher in $cHKA^{-/-}$ than in $cHKA^{+/+}$ mice during K^+ depletion (∼ 20% greater during the final week). The difference was not statistically significant ($P = 0.10$), but the data are suggestive, particularly in light of the fact that the $cHKA^{-/-}$ mice had a more severe hypokalemia.

The colon is known to participate in the maintenance of K^+ balance through both K^+ secretion and absorption (2), and there is strong evidence that *cHKA* mediates K^+ absorption (26–28). Our data clearly demonstrate that *cHKA* in colon plays a major role in reducing fecal K^+ excretion under both normal and K^+-depleted conditions. When animals were maintained on a control diet, $cHKA^{-/-}$ mice excreted over twice as much K^+ as $cHKA^{+/+}$ mice. Fecal K^+ losses were reduced in both groups when they were switched to the K^+-free diet, but $cHKA^{-/-}$ mice were unable to reduce fecal K^+ loss to the low levels observed in wild-type mice. Fecal K^+ loss in $cHKA^{-/-}$ mice during the K^+ depletion period was four times higher than that of $cHKA^{+/+}$ mice and exceeded the K^+ loss occurring in the urine. Because of the high K^+ loss in feces of $cHKA^{-/-}$ mice, total K^+ losses (urinary + fecal) for $cHKA^{-/-}$ mice was greater than that of $cHKA^{+/+}$ mice; during the final 6 d of the K^+ depletion period, total K^+ excretion in urine and feces of $cHKA^{+/+}$ and $cHKA^{-/-}$ mice was 33±5 and 70±7 μmol, respectively ($P < 0.001$).

There is evidence that two HKA isoforms may be expressed in the colon (29, 30). The data described here show that *cHKA*-deficient mice are not able to retain K^+ in the colon as effectively as $cHKA^{+/+}$ mice, clearly demonstrating that *cHKA* is required for normal K^+ recovery from intestinal fluid, and that full compensation for the deficit does not occur. However, these data do not rule out the possibility of a second pump, as there is substantial K^+ conservation in colon of K^+-depleted $cHKA^{-/-}$ mice. The decrease in fecal K^+ during K^+ depletion may be due to decreased secretion, as it is known that reduction in plasma aldosterone or K^+, both of which were observed in the K^+-depleted mice, can cause such a decrease (31). Additional studies using tissues from the $cHKA^{-/-}$ mouse will help to resolve the issue of whether a second HKA isoform is responsible for some of the K^+ conservation that occurs in the colon.

Our results with mice differ in two respects from previous results with rats. After 3–5 wk of K^+ deprivation, the kidneys of rats are markedly enlarged (32, 33). In contrast, mouse kidneys were only slightly larger after 3 wk of K^+ depletion, and

the effect was more pronounced in *cHKA*$^{-/-}$ than in *cHKA*$^{+/+}$ mice, possibly because the K^+-depleted state is more severe in *cHKA*$^{-/-}$ mice. Studies in the rat have also shown that diet-induced hypokalemia is accompanied by metabolic alkalosis (34), but in the mouse hypokalemia was not accompanied by alkalosis, as indicated by normal plasma pH and HCO_3^- in both K^+-depleted *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice. The normal acid-base status in both *cHKA*$^{-/-}$ and *cHKA*$^{+/+}$ mice during K^+ deprivation demonstrates that H^+ secretion by *cHKA* in wild-type mice does not cause an alkalotic state.

An interesting observation in *cHKA*$^{-/-}$ mice was the increased loss of body weight that took place from the beginning of the K^+ depletion period. *cHKA*$^{-/-}$ mice lost 20% of their body weight after 3 wk of K^+ depletion, compared with only 10% for *cHKA*$^{+/+}$ mice. The mean value for total urinary and fecal Na^+ excretion for *cHKA*$^{-/-}$ mice, which should provide a reasonable estimation of the relative amounts of food consumed by each group of animals, was $\sim$ 90% of the values obtained for *cHKA*$^{+/+}$ mice. It is unclear whether this small apparent difference in food consumption might account for the difference in rate of weight loss. Thus, the basis for the increased rate of body weight loss in the *cHKA*-deficient animals remains to be determined, even though its relation to the more severe K^+-depleted state of *cHKA*$^{-/-}$mice seems likely.

In summary, we have developed a *cHKA*-deficient mouse and have used it to demonstrate that *cHKA* plays an important role in the maintenance of K^+ homeostasis in vivo under K^+-deprived conditions. In the K^+-depleted *cHKA*-deficient mouse, the additional K^+ loss, beyond that occurring in the wild-type mouse, was primarily in the colon, and to a much lesser extent in the kidney. The excess K^+ loss leads to lower plasma and muscle K^+ and greater body weight loss in the *cHKA*-deficient mouse than in the wild-type mouse. The *cHKA*-deficient mouse model will help to resolve issues concerning HKAs in the kidney and colon, such as the number of distinct isoforms, the specific contribution of *cHKA* to K^+ and HCO_3^- reabsorption, and the cell-type and membrane location of *cHKA*.

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