KCNJ10 (Kir4.1) is expressed in the basolateral membrane of the cortical thick ascending limb

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Zhang C, Wang L, Su XT, Lin DH, Wang WH. KCNJ10 (Kir4.1) is expressed in the basolateral membrane of the cortical thick ascending limb. Am J Physiol Renal Physiol 308: F1288–F1296, 2015. First published April 1, 2015; doi:10.1152/ajprenal.00687.2014.—The aim of the present study is to examine the role of KCNJ10 (Kir4.1) in contributing to the basolateral K conductance in the cortical thick ascending limb (cTAL) using KCNJ10+/- wild-type (WT) and KCNJ10-/- knockout (KO) mice. The patch-clamp experiments detected a 40- and an 80-pS K channel in the basolateral membrane of the cTAL. Moreover, the probability of finding the 40-pS K channel was significantly higher in the late part of the cTAL close to the distal convoluted tubule than those in the initial part. Immunostaining showed that KCNJ10 staining was detected in the basolateral membrane of the cTAL, but the expression was not uniformly distributed. The disruption of KCNJ10 completely eliminated the 40-pS K channel but not the 80-pS K channel, suggesting the role of KCNJ10 in forming the 40-pS K channel of the cTAL. Also, the disruption of KCNJ10 increased the probability of finding the 80-pS K channel in the cTAL, especially in the late part of the cTAL. Because the channel open probability of the 80-pS K channel in KO was similar to those of WT mice, the increase in the 80-pS K channel may be achieved by increasing K channel number. The whole cell recording further showed that K reversal potential measured with 5 mM K in the bath increased K channel number. The whole cell recording further showed that the probability of the 80-pS K channel in KO was similar to those of WT mice, but not the 80-pS K channel, suggesting the role of KCNJ10 in forming the 80-pS K channel.

KCNJ10 (Kir4.1) is an inwardly rectifying K channel and it interacts with Kcnj16 (Kir5.1) to form the 40-pS heterotramer expressed in the basolateral membrane of distal nephron segments (11, 17, 28). This conclusion was supported by previous observations that the 40-pS K channel was completely absent in the KCNJ10 knockout or in the Kcnj16 knockout animals (15, 29). Moreover, the finding that the disruption of KCNJ10 alone completely eliminated the 40-pS K channel in the distal convoluted tubule (DCT) strongly suggests that Kir4.1 is a pore-forming component of the Kir4.1/5.1 heterotramer. We and others previously detected an inwardly rectifying K channel in the basolateral membrane of the cortical thick ascending limb (cTAL) of both rat and CD1 mouse kidney with similar biophysical properties to those of the 40-pS K channel (5, 6, 14). However, it is not known whether a K channel of the same type is present in the cTAL of C57bl/6 mice. Furthermore, immunostaining has also neither excluded nor confirmed the expression of KCNJ10 in the cTAL of C57bl/6 mice (10, 13).

Loss-of-function mutations of KCNJ10 cause EAST/SeSAME syndrome in humans (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) (1, 2, 20, 24). The renal phenotype of the disease is reminiscent to Gitelman’s syndrome including hypomagnesemia, hypokalemia, and metabolic alkalosis, suggesting that the disruption of KCNJ10 mainly impairs the transport in the DCT. This is also suggested by our previous finding that the disruption of KCNJ10 significantly decreased Na-Cl cotransporter (NCC) expression (29). However, KCNJ10 has been shown to be expressed not only in the DCT but also in the TAL, connecting tubule (CNT), and initial cortical collecting duct in the human kidney (1). But, the loss-of-function mutations of KCNJ10 in humans did not show an abnormal transport of the TAL. Moreover, our initial experiments showed that the Na-K-Cl cotransporter 2 (NKCC2) expression was not affected by the disruption of KCNJ10. The lack of effect on NKCC2 in KCNJ10-/- mice could be either due to the absence of KCNJ10 expression in the TAL of C57bl/6 mice or due to a compensation mechanism in the cTAL. Thus, the first aim of the present study is to examine whether KCNJ10 is present in the cTAL of C57bl/6 mice with the use of immunostaining and electrophysiological methods. The second aim is to determine whether the function of KCNJ10 could be compensated by a K channel other than the 40-pS K channel in the cTAL, if its expression in the cTAL is confirmed in C57bl/6 mice.

METHODS

Animals. KCNJ10+/+, KCNJ10+/-, and KCNJ10-/- mice were obtained through mating KCNJ10+/- mice that were kindly provided by Dr. Paulo Kofuji at University of Minnesota to Dr. Lifton (10, 13). Primers used for genotyping were the following: Kcnj10, forward 5'-TGG ACG ACC TTC ATT GAC ATG CAG TGG-3' and reverse 5'-CTT TCA AGG GGC TGG TCT CAT CTA CCA CAT-3' (10). KCNJ10-/- mice were viable and had no obvious abnormality at birth compared with their wild-type (WT) littermates. However, their growth was retarded at postnatal days 6–7 (p6-7) days and the mice died within 2 wk (13). Thus, we carried out the experiments using p7-10 homozygous KCNJ10-/-, heterozygous KCNJ10+/-, and KCNJ10+/- mice. The animal protocol was approved by an independent Institutional Animal Care and Use Committee at both Yale University and New York Medical College.

Preparation of the cTAL. After mice were killed by cervical dislocation, we perfused the left kidney with 2 ml collagenase type 2 (1 mg/ml) containing L-15 medium (Life Technology). The collagenase-perfused kidney was removed and we only cut the renal cortex with a sharp razor. The renal cortex was further cut into small pieces that were then incubated in collagenase-containing L-15 media for...
30–40 min. After the collagenase treatment, the tissue was washed three times with L-15 medium and transferred to an ice-cold chamber for dissection. The method for dissecting cTAL was similar to those described previously for dissecting DCT (28). We dissected cTAL with at least 150- to 200-μm length and with either a glomerulus attachment or a clear DCT attachment. We defined the first 100 μm cTAL starting before glomerulus attachment as upper part of the cTAL and defined the rest of cTAL as lower part. The cTALs were adhered to a cover glass coated with polylysine and the cover glass was placed on a chamber mounted on an inverted microscope. The cTAL was superfused with a bath solution containing 140 mM NaCl, 5 mM KCl, 1.8 mM MgCl2, 1.8 mM CaCl2, and 10 mM HEPES (pH 7.4).

Patch-clamp experiment. For the single channel recording, an Axon 200B patch-clamp amplifier was used to record the channel current. Borosilicate glass (1.7-mm OD) was used to make the patch-clamp pipettes using a Narishige electrode puller. The pipette solution contained (in mM) 140 KCl, 1.8 MgCl2, and 10 HEPES (pH 7.4). The currents were low-pass filtered at 1 kHz and digitized by an Axon amplifier. The tip of the pipette was filled with pipette solution containing 140 mM KCl, 2 mM MgCl2, 1 mM EGTA, and 5 mM HEPES (pH 7.4). The pipette was then back-filled with amphotericin B (20 μg/0.1 ml) containing the pipette solution. The bath solution was either symmetrical 140 mM KCl (for the measurement of the whole cell K currents) or 140 mM NaCl + 5 mM KCl (for the measurement of K reversal potential). After a high-resistance seal (≥2 GΩ) was formed, the membrane capacitance was monitored until the whole cell patch configuration was formed. The currents were low-pass filtered at 1 kHz, digitized by an Axon interface with 4 kHz sampling rate (Digidata 1322). Data were analyzed using the pClamp software system 9.0 (Axon).

Immunostaining. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and the abdomens were cut open for perfusion of kidneys with 2 ml PBS containing heparin (40 U/ml) followed by 20 ml of 4% paraformaldehyde. After perfusion, the kidneys were removed and subjected to postfixation with 4% paraformaldehyde for 12 h. The kidneys were dehydrated and cut into 8-μm slices with Leica 1900 cryostat (Leica). The tissue slices were dried at 42°C for 1 h. The slides were washed with 1× PBS for 15 min, and permeabilized with 0.3% Triton dissolved in 1× PBS buffer containing 1% BSA and 0.1% lysine (pH 7.4) for 15 min.

Fig. 1. Kcnj10 is expressed in the basolateral membrane of the cortical thick ascending limb (cTAL). A: channel recording showing the 40-pS K channel activity in the basolateral membrane of the cTAL of Kcnj10−/− mice. The experiment was performed in a cell-attached patch with 140 mM K in the pipette and 5 mM K/140 mM Na in the bath. The holding potential of the pipette (−Vpip) was shown on the top of each trace and the channel closed level is indicated by a dotted line and “C.” B: current (i)/voltage (V) curve yields a slope conductance of 40 pS measured between −60 and 0 mV. C: bar graph summarizes the experiments in which the 40-pS K channel was detected at cell-attached patches in the cTAL of both wild-type (WT; Kcnj10+/+) or knockout (KO) mice (Kcnj10−/−).
Kidney slices were blocked with 2% horse serum for 30 min at room temperature and then incubated with primary antibodies (Kir.4.1, Kir5.1, THP, and NKCC2) for 12 h at 4°C. Slides were thoroughly washed with 1× PBS and followed by addition of second antibodies mixture in 0.4% Triton dissolved in 1× PBS for 2 h at room temperature.

Preparation of protein samples and Western blot. The tissue of renal cortex was homogenized in an ice-cold solution containing 250 mM sucrose, 50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1% protease and phosphatase inhibitor cocktails (Sigma) titrated to pH 7.6. After homogenization, the sample was subjected to centrifugation at 13,000 rpm (10,000 g) for 8 min at 4°C and the protein concentration was measured using the DC Protein Assay Kit (BioRad, Hercules, CA). The proteins were separated by electrophoresis on 4–15% SDS-polyacrylamide gels and transferred to nitrocellulose membrane. The membranes were blocked with LI-COR blocking buffer (PBS). An Odyssey infrared imaging system (LI-COR, Lincoln, NE) was used to scan the membrane at a wavelength of 680 or 800 nm.

Experimental materials and statistics. Kir5.1 and Tammhorsfall protein (THP) antibodies were purchased from Santa Cruz (Santa Cruz, CA). We obtained Kir4.1 antibody from Alomone Lab (Jerusalem, Israel). An antibody for NKCC2 was purchased from Stress Marq (Victoria, Canada). The data are presented as means ± SE. We used χ-squared test, the paired Student’s t-test, or one-way ANOVA to determine the statistical significance.

RESULTS

We first used the patch-clamp technique to examine the expression of K channels in the basolateral membrane of the cTAL. With 140 mM Na and 5 mM K in the bath and 140 mM K in the pipette, we identified two types of K channels in the basolateral membrane of the cTAL in C57bl/6 mice. Figure 1 is a representative recording made in a cell-attached patch at holding potentials from 0 to −60 mV. From the inspection of Fig. 1A, it is apparent that the Po was slightly voltage dependent and it was 0.46 ± 0.05 at 0 mV (n = 5). Moreover, the slope conductance calculated from I–V curve was 40 ± 1 pS (n = 5; Fig. 1B). We observed this 40-pS K channel in 22 patches from total 80 patches and this K channel was similar to the 40-pS K channel identified in the basolateral membrane of the DCT (12, 28), presumably, it is a Kir.4.1/Kir5.1 heterotramer. The notion that Kcnj10 is essential for forming the 40-pS K channel was confirmed by the experiments carried in Kcnj10−/− mice in which the 40-pS K channel was completely absent from all 151 patches (Fig. 1C).

Fig. 2. An 80-pS K channel is expressed in the basolateral membrane of the cTAL. A: channel recording showing the 80-pS K channel activity in the basolateral membrane of the cTAL of Kcnj10−/− mice. The experiment was performed in a cell-attached patch with 140 mM K in the pipette and 5 mM K/140 mM Na in the bath. The channel closed level is indicated by a dotted line and “C” and −Vpip is indicated on the top of each trace. B: I/V curve yields a slope conductance of 80 pS measured between −40 and 0 mV. C: bar graph summarizes the experiments in which the 80-pS K channel was detected at cell-attached patches in the cTAL of both WT and KO mice.
In addition to the 40-pS K channel, we also identified an 80-pS K channel in the basolateral membrane of the cTAL as reported previously (16). Figure 2A is a typical channel recording made in a cell-attached patch showing the channel activity from 40 to −20 mV. Figure 2B is an I-V curve showing that the channel slope conductance was 80 ± 5 pS (n = 4) measured between −40 to 0 mV. Moreover, it is apparent that the $P_o$ was voltage-dependent such that a depolarization increased the $P_o$ (0.68 ± 0.15 at 20 mV, n = 4). This K channel is most likely the Na and Cl-activated large-conductance (154 pS at −80- to −100-mV range) K channel described previously (16). We detected the 80-pS K channel in the cTAL of WT mice in 7 patches from total 80 patches but the probability of finding the 80-pS K channel in Kcnj10−/− mice increased (27 patches with K channel from all 151 patches; Fig. 2C). Because the $P_o$ of the 80-pS K channel was not changed in Kcnj10−/− mice compared with those of WT mice, it is possible that disruption of Kcnj10 in the cTAL increased the channel number in the plasma membrane thereby compensating the loss-of-function of Kcnj10. In the present study, we did not observe the coexistence of 40 and 80 pS in the same patch. However, in a separate experiment performed in the cTAL of the rat kidney, we observed the copresence of two types of K channels in the same patch (Wang’s unpublished observation). This suggests the possibility that two types of the K channels may be present in the same cell of the cTAL.

Because our initial experiments showed that the 40-pS K channel was not uniformly distributed along the cTAL, we then performed the experiments in arbitrarily defined upper part (close to glomerulus) and lower part of cTAL to further characterize this finding (Fig. 3A). We identified the 40-pS K channel in 12 patches from total 25 patches in the upper part (Fig. 3B) while it was only ~7% patches in the lower part (2 patches with the 40-pS K channel from all 28 patches; Figs. 3B and 4A). This suggests that Kcnj10 is not evenly distributed along the cTAL and its expression is higher in the late part of the cTAL close to the DCT than those in the early part of the cTAL. In contrast, the probability of finding this 80 pS was lower in the upper part (4%, 1 patch with 80-pS K channel from all 25 patches) than those in the lower part of the cTAL (14%, 4 patches with the 80-pS K channel from all 28 patches; Figs. 3C and 4B). As mentioned above, the disruption of Kcnj10 completely eliminated the 40-pS K channel in the cTAL but it increased the probability of finding the 80-pS K channel in the top half of the cTAL (17%, 4 patches with the 80-pS K channel in total 23 patches). In contrast, the probability of finding the 80-pS K channel in the low part of the cTAL was not significantly altered (5 patches with K channel in total 27 patches; Fig. 4B).
To further examine whether the expression of Kcnj10 was not uniformly distributed along the cTAL, we also carried out immunostaining to examine Kcnj10 expression in the cTAL of WT and Kcnj10/H11002/H11002 mice. Figure 5, A–C, is double immunostaining showing the expression of Kcnj10 in THP (as a marker of the TAL)-positive tubules in the cortex region. As observed in the patch-clamp experiments, we found that Kcnj10 immunostaining was only detected in few THP-positive cTAL (which was usually next to the glomerulus) while Kcnj10 staining was completely absent in Kcnj10/H11002/H11002 mice (data not shown). Similar to Kcnj10 staining, immunostaining of Kcnj16 was mainly detected also in the cTAL close to the glomerulus (Fig. 5, D and E). Thus, both electrophysiological and immunocytochemical experiments demonstrated that Kcnj10 protein was present in the cTAL but its expression was predominant in the late part of the cTAL close to the DCT.

The basolateral K channels participate in generating the cell membrane potential. We previously demonstrated that the disruption of Kcnj10 caused the depolarization of the DCT because Kcnj10 plays a predominant role in determining the basolateral K conductance in the DCT (29). Since two types of K channels are present and the 80-pS K channel is upregulated in the basolateral membrane of the cTAL in Kcnj10/H11002 mice,

Fig. 4. Disruption of Kcnj10 increases the numbers of the 80-pS K channel in the cTAL. A: group of bar graphs showing the probability of finding the 40-pS K channel in the whole cTAL, at the upper part or lower part of the cTAL in both WT and KO mice, respectively. The experiments were performed in cell-attached patches from total 80 patches in WT mice and 151 patches in KO with 140 mM K in the pipette and 5 mM K in the bath. B: probability of finding 80-pS K channels in the TAL, upper part or lower part of the cTAL, respectively. *Significant difference that was determined using χ-squared test.

Fig. 5. Kcnj10 and 16 are expressed in the basolateral membrane of the cTAL. Immunostaining image showing the expression of Kir4.1 (Kcnj10) and Tammhorsfall protein (THP) in the renal cortex of postnatal day 10 (p10) C57bl/6 mouse with low magnification (A) or with high magnification (B and C). Glomerulus (G) was indicated by *. Immunostaining showing the expression of Kir5.1 (Kcnj16) and THP in the renal cortex of p10 C57bl/6 mouse (D and E). The short bar in each panel represents 10 μm.
we speculate that the disruption of Kcnj10 may not affect the cell membrane potential in the cTAL. Thus, we used the perforated whole cell recording to measure the K reversal potential, an index of the cell membrane potential. First, we examined whether TAL cell was permeable to their neighboring cell using fluorescent dye as described previously (3). As shown in Fig. 6A, the cTAL cells lack the cell coupling as evidenced by the fact that carboxyfluorescein dye injected into the clamped cells did not spread into neighboring cells. We then used the perforated whole cell recording to measure the K reversal potential of the TAL bathed in 140 mM Na/5 mM K containing solution. Figure 6B is a representative recording made in the top half cTAL and it demonstrates that the K reversal potential is around −62 ± 1 mV in WT and −61 ± 2 mV (n = 5) in Kcnj10−/− mice. Also, the K reversal potentials measured with the perforated whole cell recording in the lower half of the cTAL were the same between WT and knockout (KO) mice. This suggests that the disruption of Kcnj10 did not depolarize the cell membrane potential in the cTAL. Because K reversal potential in the cTAL was determined not only by the basolateral K channels but also basolateral Cl channels and apical ROMK channels, we next measured the Ba2+-sensitive K currents in the TAL using the whole cell recording. Since the K currents were measured immediately after Ba2+ was added to the bath, Ba2+ that was diffused to the lumen to block ROMK channels in such time frame should be limited. Thus, the K currents should mainly be a reflex of the basolateral K conductance. Figure 6C is a recording showing the Ba2+-sensitive K currents in the late part of the cTAL (close to the DCT1) of the WT and KO mice. From the inspection of Fig. 6C, it is apparent that the Ba2+-sensitive K currents in the cTAL were similar between WT and KO mice (KO mice, 1,700 ± 180 pA at −60 mV; WT mice 1,820 ± 190 pA at −60 mV, n = 5). This further suggests that the 80-pS K channel could effectively compensate the loss-of-function of Kcnj10 in the cTAL.

A previous study demonstrated that the disruption of Kcnj10 decreased the expression of NCC in the DCT (29). Thus, we next examined whether the disruption of Kcnj10 also affected the expression of NKCC2 in the cTAL. We prepared the cortex tissue from the WT, Kcnj10+/−, and Kcnj10−/− mice and used the Western blot to examine the expression of NKCC2. Figure 7A is a Western blot showing that the disruption of Kcnj10 did not affect the expression of NKCC2 in the cortex (n = 5 mice). This conclusion was also confirmed by immunocytochemical experiments. Figure 7B is an immunostaining image of NKCC2 in the renal cortex and outer medulla of WT and Kcnj10−/− mice. It is apparent the expression of NKCC2 was decreased the expression of NCC in the DCT (29). Thus, we next examined whether the disruption of Kcnj10 also affected the expression of NKCC2 in the cTAL. We prepared the cortex tissue from the WT, Kcnj10+/−, and Kcnj10−/− mice (KO mice). From the inspection of Fig. 6C, it is apparent that the Ba2+-sensitive K currents in the late part of the cTAL (close to the DCT1) of the WT and KO mice. From the inspection of Fig. 6C, it is apparent that the Ba2+-sensitive K currents in the cTAL were similar between WT and KO mice (KO mice, 1,700 ± 180 pA at −60 mV; WT mice 1,820 ± 190 pA at −60 mV, n = 5). This further suggests that the 80-pS K channel could effectively compensate the loss-of-function of Kcnj10 in the cTAL.

![Image A](http://ajprenal.physiology.org/)

**Fig. 6.** Whole cell K currents and K reversal potential of the cTAL in Kcnj10+/− (WT) or Kcnj10−/− (KO) mice. A: fluorescence microscope image showing a carboxyfluorescein-loaded cell in the cTAL of a p9 mouse. The light microscope image shows the image of the same cTAL. The carboxyfluorescein (1.5 mM) was dissolved in the pipette solution containing 140 mM KCl (pH 7.4). After a high-resistance seal was formed, the membrane of the DCT1 cell was ruptured and the cell was automatically filled with dye-containing pipette solution. B: whole cell recording showing K reversal potential in cTAL of p9 WT and KO mice (n = 5). For measurement of K reversal potential, the TAL was bathed in a solution containing 140 mM NaCl + 5 mM KCl while the pipette solution has 140 mM KCl. C: whole cell recording showing Ba2+-sensitive K currents of the cTAL of p9 WT and KO mice (n = 5). The K currents were measured with RAMP protocol from −100 to 100 mV.
DISCUSSION

The previous electrophysiological experiments have identified two types of K channels, a 40-pS inwardly rectifying K channel and a Na and Cl-activated 80- to 150-pS K channel, and a nonselective 20-pS cation channel in the basolateral membrane of the cTAL (12, 16, 26). The 40- to 50-pS K channel is composed of Kir.4.1 (Kcnj10) and Kir5.1 (Kcnj16) while the 80- to 150-pS K channel is possible a homotetramer of KCa4.1 (12, 16). Furthermore, immunostaining has identified the expression of Kir.4.1 in the basolateral membrane of the cTAL in the human and CD1 mouse kidney (11, 20). However, it was inconclusive in previous studies whether Kir.4.1 was expressed in the cTAL of C57bl/6 mice (2, 20). Now we used two methods to confirm that Kcnj10 is expressed in the cTAL of C57bl/6 mice. First, immunostaining has clearly identified the positive staining of Kcnj10 in the THP-positive cTAL. Moreover, the patch-clamp experiments unambiguously detected the 40-pS K channel in the late part of the cTAL. This 40-pS K channel was related to Kcnj10 because the K channel was completely absent in the cTAL from Kcnj10^-/- mice. We speculate that Kir.4.1 expression in C57bl/6 mice started at the late part of the cTAL close to the glomerulus. This speculation is supported by the observation that the probability of finding the 40-pS K channel was higher in the top half of the cTAL close to the glomerulus than those in the lower half.

The TAL is responsible for the absorption of 20–25% filtered Na load and plays an important role for the transcellular calcium and magnesium absorption (4, 7). The absorption of Na is a two-step process: Na enters the cells through the apical NKCC2 cotransporter and leaves the cells through the Na-K-ATPase (9). The basolateral K channels in the TAL participate in generating the cell membrane potential that provides the driving force for the Cl exit through the basolateral Cl channels (8). Thus, the inhibition of the basolateral K channels could lead to the depolarization of the cell membrane potential thereby decreasing Cl exit. A decrease in Cl exit is expected to increase the intracellular Cl concentrations that in turn inhibit the activity of with-no-lysine kinase (WNK) because it is a Cl-sensitive kinase (18). It is well-established that WNK plays an important role in activating Ste20-related proline and alanine-rich kinase (SPAK) and oxidation stress response kinase (OSR) that stimulate NKCC2 function through the phosphorylation (19, 21). Thus, it is conceivable that the inhibition of the basolateral K channel activity could affect the WNK-SPAK/OSR interaction thereby suppressing the function of NKCC2 in the TAL. Relevant to this possibility is our previous finding that the disruption or downregulation of Kcnj10 decreased the SPAK expression and NCC expression in the DCT (27, 29). However, the loss-of-function mutations of Kcnj10 in the human kidney mainly impair the membrane transport in the DCT but not in the TAL, although Kcnj10 is also expressed in the TAL (20). Our present study strongly suggests the possibility that the lack of the effect on the membrane transport in the TAL of patients with loss-of-function mutations of KCNJ10 is
due to the compensation mechanism induced by K channels other than Kcnj10.

This possibility is supported by two lines of evidence as follows: 1) the probability of finding the 80-pS K channel is significantly increased in the cTAL of Kcnj10−/− mice; 2) the disruption of Kcnj10 did not significantly affect the cell membrane potential in the TAL of Kcnj10−/− mice. The lack of changes in the cell membrane potential of the cTAL in Kcnj10−/− mice indicates that Kcnj10 in the TAL is not indispensable. In contrast, the function of Kcnj10 in the DCT is essential for the basolateral K conductance in the DCT because Kcnj10 is a predominant K channel expressed in the basolateral membrane of the DCT (29). Thus, the disruption of Kcnj10 completely eliminated the basolateral K conductance and caused a strong depolarization.

The mechanism by which the disruption of Kcnj10 upregulates the 80-pS K channel in the cTAL is not known. Since the disruption of Kcnj10 inhibits Na transport in the DCT and causes salt wasting and volume depletion, it should increase plasma aldosterone and vasopressin levels in Kcnj10−/− mice. An increase in aldosterone should stimulate epithelial Na+ absorption in the collecting duct. On the other hand, an increase in vasopressin level should stimulate NaCl transport in the Henle’s loop and it may also activate the 80-pS K channel thereby offsetting the function of Kcnj10. Accordingly, the disruption of Kcnj10 eliminates Na-activated 80-pS K channel expression in the collecting duct thereby enhancing Na absorption in the collecting duct.

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