Differential regulation of ROMK (Kir1.1) in distal nephron segments by dietary potassium

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Wade JB, Fang L, Coleman RA, Liu J, Grimm PR, Wang T, Welling PA. Differential regulation of ROMK (Kir1.1) in distal nephron segments by dietary potassium. Am J Physiol Renal Physiol 300: F1385–F1393, 2011.—ROMK channels are well-known to play a central role in renal K secretion, but the absence of highly specific and avid-ROMK antibodies has presented significant roadblocks toward mapping the extent of expression along the entire distal nephron and determining whether surface density of these channels is regulated in response to physiological stimuli. Here, we prepared new ROMK antibodies verified to be highly specific, using ROMK knockout mice as a control. Characterization with segmental markers revealed a more extensive pattern of ROMK expression along the entire distal nephron than previously thought, localizing to distal convoluted tubule regions, DCT1 and DCT2; the connecting tubule (CNT); and cortical collecting duct (CD). ROMK was diffusely distributed in intracellular compartments and at the apical membrane of each tubular region. Apical labeling was significantly increased by high-K diet in DCT2, CNT1, CNT2, and CD (P < 0.05) but not in DCT1. Consistent with the large increase in apical ROMK, dramatically increased mature glycosylation was observed following dietary potassium augmentation. We conclude 1) our new antibody provides a unique tool to characterize ROMK channel localization and expression and 2) high-K diet causes a large increase in apical expression of ROMK in DCT2, CNT, and CD but not in DCT1, indicating that different regulatory mechanisms are involved in K diet-regulated ROMK channel functions in the distal nephron.

ROMK antibody; ROMK localization

THE CRITICAL ROLE of ROMK (Kir1.1) and BK (Maxi-K) as the major potassium secreting channels in the kidney is supported by strong evidence (24, 33). Knockout studies in mice established a definitive link. Indeed, ablation of the ROMK gene eliminates the most predominant and active potassium channel in the mouse collecting duct (CD) (15). Removal of BK α or other BK subunit genes causes an attenuation of the kaliuretic response that is evoked by increased urinary flow (22, 23, 39). Interestingly, knockout of either channel gene is compensated by upregulation of the other (1, 22). It would seem that the kidney is equipped with at least two separate potassium secretory pathways to ensure high-capacity potassium excretion and protect against hyperkalemia.

The two channel types are differently regulated to meet different physiologic demands. The unique properties of the BK channels allow the potassium secretion apparatus to be especially sensitive to the urinary flow rate (39). By contrast, ROMK channels are constitutively active and are thus generally considered to mediate basal potassium secretion (8, 20). ROMK channels are also regulated by dietary potassium, increasing with dietary loading and decreasing with restriction (6, 7). How this occurs has been the subject of great interest.

Because ROMK channels exhibit an open probability near unity (8), regulated changes in ROMK function are thought to be brought about by alterations in the density of functional channels at the apical surface. In principle, this could occur by switching channel activity on and off, or by regulated channel trafficking processes that change channel expression at the apical membrane. Numerous studies pointed toward the involvement of different trafficking mechanisms, but the extent to which the surface density of ROMK channels changes in response to physiological stimuli along the distal nephron remained an important unresolved question.

It also is unknown whether ROMK is similarly regulated in all of the potassium-secreting segments. Micropuncture studies established that potassium secretion is principally regulated in the late distal nephron (17, 25) involving different cell types in the distal convoluted tubule (DCT), the connecting tubule (CNT), and the initial portion of CD. Yet, exploration of ROMK regulation has largely focused on the CD because it is most tractable to patch-clamp analysis. Careful examination of ROMK function in the other potassium-secreting segments has been limited. One recent study revealed that ROMK in the CNT exhibits a quantitatively more robust response to dietary potassium than in the CD (7), but the mechanism has not been elucidated. The extent to which ROMK is regulated in the DCT is completely unknown.

The absence of highly specific and avid-ROMK antibodies has presented a significant roadblock toward addressing these important and unresolved issues. Widely used commercial antibodies often detect spurious bands that have been misidentified as ROMK, leading to considerable confusion. We (4) and others in the field (10, 18, 40) previously developed anti-ROMK antibodies, adequate for localizing the abundant ROMK in the thick ascending limb of the loop of Henle. But their low avidity and uncertain specificity precluded rigorous immunolocalization studies in the late distal nephron segments where ROMK abundance at the apical membrane is thought to be low (21). Here, we exploit the availability and extensive characterization of ROMK knockout (KO) mice (14, 15, 30), together with new sample preparation methods to validate the specificity of newly prepared ROMK antibodies. These antibodies allowed for the first time a careful characterization of ROMK localization along the entire distal nephron and a quantitative evaluation of ROMK segmental responses to variation in K diet in the mouse kidney.
METHODS

Animals. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine. Male C57 BL/6 mice (9–11 wk of age) were fed compositionally matched diets consisting of either a control K diet (1% K; Harlan Teklad TD.88238), a K-deficient diet (15–30 ppm K; Harlan Teklad TD.88239), or a 10% KCl diet (Harlan Teklad TD.09075) for 4 days.

Antibodies. New antipeptide antibodies were raised in rabbits to COOH-terminal sequences of ROMK. One group of three rabbits was immunized with the sequence CKRGYDNPNFVLSEVDETDDTQM and a second group of three rabbits was immunized with the same sequence but lacking the terminal 3 amino acids of PDZ-binding domain (CKRGYDNPNFVLSEVDET). A cysteine residue was added at the NH2-terminal end for coupling to KLH and to sulfa-link columns (Thermo Sci) for affinity purifications. Antibodies to aquaporin-2 (AQP2) raised in chicken (29) and NCC raised in guinea pig (3) were used in colabeling studies with the ROMK antibodies. Mouse anti-ezrin and mouse anti-calbindin D28 were obtained from Sigma.

Immunoblotting. Mice were anesthetized with isoflurane. Kidneys were flash-frozen in liquid nitrogen. The most effective solubilization of ROMK for immunoblotting was achieved as follows: samples were placed in ice-cold HEENG buffer [20 mM HEPES, pH 7.6, 25 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, pH 7.6, containing protease inhibitor cocktail (P8340; Sigma), 1% Triton X-100, and 0.5% SDS], homogenized on ice using a Polytron tissue homogenizer, and then rotated at 4°C for 1 h. The samples were centrifuged at −15,000 g for 10 min at 4°C to pellet-insoluble material. Protein concentration was measured using a bicinchoninic acid protein assay reagent kit (Pierce). Equal amounts of kidney protein were suspended in Laemmli buffer (room temperature for 45 min) and loaded on 10% SDS-PAGE gels for Western blot analysis with rabbit antibodies raised against ROMK as described above.

Immunolocalization of ROMK. Anesthetized mice were fixed by perfusion with 2% paraformaldehyde in PBS via the left ventricle for 5 min at room temperature. The kidneys were then removed and fixed (24 h at 4°C), rinsed in PBS, and embedded in paraffin. Cross-sections 3-µm-thick, cut at the level of the papilla, were picked up on chrome-alum gelatin-coated glass coverslips and dried on a warming plate. The sections were then deparaffinized in two xylene baths and two absolute ethanol baths, 5 min each, and rehydrated in a graded ethanol series to distilled water.

For epitope retrieval, the coverslips were placed in a pH 8 solution (1 mM Tris, 0.5 mM EDTA, and 0.02% SDS). The retrieval solution and sections were heated to boiling in a microwave oven, transferred to a conventional boiling water bath (15 min), and then cooled to room temperature before the sections were thoroughly washed in distilled water to remove the SDS.

Sections were preincubated for 30 min with 2% BSA, 0.2% fish gelatin, and 0.2% sodium azide in PBS. Incubations with specific antibodies to ROMK were performed overnight at 4°C. After washing, sections were incubated for 1 h with the appropriate secondary antibody, washed again, and then mounted with a drop of n-propyl gallate to prevent fading.

Fig. 1. Western blot screen of ROMK antisera. Kidney homogenates from ROMK null (KO) and wild-type (WT) mice were used to distinguish antisera R78 which cross-reacts with antigens present in KO mice (A) from antisera R79 which shows minimal cross-reactivity with KO homogenates and a strong response to ROMK bands in WT mice (B).

Fig. 2. Characterization of anti-ROMK antibodies. Antisera from 2 rabbits, R79 (immunized with the full-length COOH-terminal peptide) and R80 (immunized with COOH-terminal peptide lacking the final 3 amino acids at the end of the COOH terminal representing the PDZ-binding domain), and a second group of three rabbits was immunized with the same peptide but lacking the terminal 3 amino acids of PDZ-binding domain (CKRGYDNPNFVLSEVDETDDT). A cysteine residue was added at the NH2-terminal end for coupling to KLH and to sulfa-link columns (Thermo Sc) for affinity purifications. Antibodies to aquaporin-2 (AQP2) raised in chicken (29) and NCC raised in guinea pig (3) were used in colabeling studies with the ROMK antibodies. Mouse anti-ezrin and mouse anti-calbindin D28 were obtained from Sigma.
antibodies (listed above), diluted in PBS containing 1% BSA, 0.2% fish gelatin, 0.1% Tween 20, and 0.2% sodium azide, took place overnight in a humid chamber at 4°C. After thorough washing in high-salt wash (incubation medium plus added sodium chloride at 0.5 M), the anti-ROMK was detected with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Rockland) and enhanced with Alexa Fluor 488-conjugated donkey anti-goat IgG (Jackson Laboratories). Anti-guinea pig sodium chloride cotransporter was detected with Alexa Fluor 568-conjugated donkey anti-guinea pig IgG (Jackson Laboratories), while mouse anti-calbindin D28 was detected with Alexa Fluor 633-conjugated donkey anti-mouse IgG (Invitrogen). Unconjugated secondary antibodies from Jackson Laboratories and Rockland were coupled to the respective fluorophores using kits from Invitrogen.

Quantitative analysis of images. Segmental ROMK localization images were acquired with a Zeiss LSM 410 confocal microscope. For quantification of cytoplasmic ROMK, system gain was adjusted so that no pixels in the tubules of interest would be saturated. A fluorescence standard (FocalCheck, Invitrogen) was used to adjust system sensitivity to allow comparisons between sessions.

For quantification of apical label, a conventional Zeiss fluorescent microscope was used because it gave more uniform and sensitive labeling likely due to the higher resolution of its CCD camera. A flat-field correction was applied to these images to compensate for uneven illumination. With this correction, measured fluorescence of a test object placed at different positions in the image field deviated from the average fluorescence for all positions by no more than 2%.

Total ROMK per tubule, expressed as the average pixel intensity for all cytoplasmic pixels, was determined using Photoshop (Adobe). Background label was subtracted based on the level of labeling in nearby intercalated cells.

Tubule boundaries were defined and total pixel number (i.e., the area) and the average pixel intensity for each segment region were measured using Photoshop. Intercalated cells were excluded from analysis. Nuclear area was subtracted from the total tubule area for each nephron segment. On a per tubule basis, the average cytoplasmic pixel intensity was calculated by dividing the total cytoplasmic pixel intensity by the number of cytoplasmic pixels.

Apical ROMK labeling intensity was determined using Scion Image (www.scioncorp.com). A plot profile line with a width of three pixels was drawn exactly perpendicular to the cell apical membrane at the point to be measured, and the density profile was plotted. The peak intensity value was taken along with the pixel intensity three pixels from the peak in the direction of the cytoplasm. This later value provided a measure of background label and ROMK label not associated with the apical membrane and was subtracted from the peak intensity. Three cells per tubule and at least 10 tubules per tubule type were measured for each animal.

Statistics. ANOVA and a Newman-Keuls multiple comparison test were used to test Western blot differences between animals on low-K, control, and high-K diets. An unpaired Student’s t-test was used to compare ROMK labeling results of high- and low-K diets on a control or low-K diet (*P < 0.05; n = 5). When changes in the ROMK density (B) in high-K diet mice was significantly higher than that of mice on a control or low-K diet (*P < 0.05; n = 5). When changes in the complex glycosylated ROMK band were separately quantified (C), its abundance was found to be significantly greater in high-K than control diet (*P < 0.05; n = 5) while low-K diet strongly reduced the complex glycosylated band (#P < 0.001) and K diet did not significantly affect abundance of the core + unglycosylated ROMK.
on the 50- to 55-kDa band but eliminated the band at 40 kDa and left the band at 37 kDa. These observations are consistent with previous reports with recombinant ROMK, indicating that unglycosylated ROMK runs at ~37 kDa, its core glycosylated form runs at ~40 kDa and the post-Golgi complex glycosylated form of ROMK runs at 50–55 kDa (4, 41).

To determine whether variation in K diet alters the abundance of ROMK bands, Western blots were used to examine homogenized kidney cortex from animals placed on low-K and high-K diets for 4 days. Compared with animals on a control K diet with plasma K (P_K) values of 3.9 ± 0.1 mmol/l (n = 5), the mice on the K-deficient diet had P_K values of 3.4 ± 0.1 mmol/l (n = 5; P < 0.05) while mice on the high-K diet had P_K values of 4.9 ± 0.3 (n = 4; P < 0.05). Thus, while varying K intake measurably shifted plasma K values were within the physiological range. Plasma Na values were not significantly different between the groups. As shown in Fig. 3, variation in K diet is associated with significant changes in ROMK expression. Total ROMK was increased by high-K diet but unchanged by low-K diet (Fig. 3B). Quantitative evaluation of the different glycosylated species of ROMK showed that the abundance of the complex glycosylated form of ROMK was significantly increased by high-K diet and decreased by low-K diet compared with control diet (Fig. 3C). While the abundance of the unglycosylated form of ROMK was not affected by K diet, the abundance of core glycosylated form was increased by either low-K diet or high-K diet compared with control diet (Fig. 3C).

**Immunolocalization of ROMK antibodies.** To further test the specificity of the ROMK antibodies, immunolocalization studies were carried out on fixed tissue from WT and ROMK KO mice. While ROMK-specific antibodies strongly labeled the thick ascending limb of the loop of Henle and cortical CD in WT mice, as expected, labeling was absent in ROMK KO mice (Fig. 4A). ROMK labeling was also detectable in DCT identified by thiazide-sensitive sodium-chloride cotransporter (NCC) labeling but this labeling by ROMK antibody was completely absent in ROMK KO mice (Fig. 4B).

**Effect of K diet on segmental localization.** Using antibody to AQP2 to identify CD, ROMK shows a strong cytoplasmic labeling with a perinuclear localization irrespective of K diet (Fig. 5). ROMK labeling is absent from intercalated cells (*; Fig. 5). To determine whether K diet affects the abundance of ROMK in the principal cell apical membrane, colabeling was

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**Fig. 4.** A: localization of ROMK antibodies in WT and KO mice. Antibody R80 strongly and specifically labeled well-established sites of ROMK expression in collecting duct (CD) and thick ascending limb of the loop of Henle (TAL) in WT mice but not in ROMK KO mice. The heterogeneous labeling of TAL cells has been previously described (40). Bar = 10 μm. B: localization of ROMK in distal convoluted tubule (DCT). In WT mice, DCT segments identified by labeling with antibody to thiazide-sensitive sodium-chloride cotransporter (NCC; arrows) are strongly labeled by antibodies to ROMK (B). In ROMK KO mice, DCT segments labeled by NCC (arrows) show no labeling by ROMK antibody (arrows). Note that some DCTs in ROMK-null mice show strong hypertrophy, as previously reported (30). Bar = 40 μm.
Fig. 5. Localization of ROMK in CD. Colabeling with antibody raised in chicken to aquaporin-2 (AQP2) was used to ensure identification of CD in mice adapted to low-K and high-K diets. With either diet, intercalated cells (*) identified by the absence of AQP2 labeling failed to show significant ROMK labeling. Principal cells in the CD show strong perinuclear cytoplasmic labeling in both animals on low-K and high-K diet. Bar = 10 µm.

Fig. 6. Apical localization of ROMK in CD. To assess possible changes in apical ROMK with K diet, colabeling was carried out with an apical membrane marker, ezrin (red), and ROMK (green). Bottom: as shown in the colored combined panels, there is little overlap and yellow color at the apical surface in low-K-diet animals but strong overlap resulting in yellow color for animals on a high-K diet. This shows there is elevated apical ROMK in CD principal cells associated with high-K diet. Intercalated cells (*). Bar = 10 µm.
carried out with ROMK and ezrin, an apical membrane marker. To maximize our ability to identify those segments responsive to variation in K diet, we compared animals on low-K diet to those on high-K diet. Dietary treatment was limited to 4 days avoiding the renal changes found to occur in animals K-depleted for 2 wk or more (19). Previous studies characterized the effect of modest periods on these K diets on plasma K concentration and other functional parameters (2, 21, 27). In animals on a low-K diet, we found little or no colabeling with ezrin but animals on a high-K diet showed strong colabeling of

Fig. 7. Localization of ROMK in DCT. To evaluate ROMK expression along the DCT and its response to variation in K diet, antibody raised in guinea pig to NCC was used along with antibody raised in mice to calbindin D28 to distinguish DCT1 from DCT2. This identification was further confirmed by identification of intercalated cells (*) lacking NCC labeling in DCT2 regions but not in DCT1 regions. For DCT1, ROMK labeling was of similar intensity whether mice were on a low-K or a high-K diet. In DCT2, apical labeling was distinctly higher in animals on a high-K diet than for those on a low-K diet. Bar = 10 μm.

Fig. 8. Localization of ROMK in connecting tubule (CNT). To identify regions of CNT, the guinea pig anti-NCC antibody used above was employed along with mouse anti-calbindin D28 (simultaneous labeling with 3 different secondary fluorophores) to identify early CNT regions (CNT1) adjacent to DCT2 segments. Dashed lines represent boundaries between DCT2 and CNT1. Late CNT (CNT2) regions were identified based on an absence of NCC label and characteristically stronger calbindin D28 labeling than found in CD. Bar = 10 μm.
ROMK with ezrin (Fig. 6), indicating that variation in K diet results in large changes in apical expression of ROMK. By contrast, ROMK labeling of the thick ascending limb was strongly apical and not detectably affected by variation in K diet.

Antibody to the NCC was used to identify DCT regions. The early region of the DCT, known as DCT1, was identified based on its weak expression of calbindin D28 (13). This region was found to express ROMK but the level of expression was not affected by K diet (DCT1; Fig. 7). The late DCT, known as DCT2, is marked by the presence of calbindin and intercalated cells (*, DCT2; Fig. 7). ROMK expression in this region of the DCT is distinctly more apical in animals on a high-K diet. Note also (Fig. 7) that NCC expression levels are high in animals on a low-K diet compared with NCC levels on a high-K diet, consistent with the recent report that K diet influences NCC expression (27). This effect on NCC is seen in both DCT1 and DCT2 regions of the DCT.

In examining ROMK localization, we observed some variation along the CNT. At the beginning of the CNT adjacent to the DCT2, ROMK labeling is similar to that in the DCT2. There is weak cytoplasmic labeling in animals on a low-K diet and a very strong apical labeling pattern in some CNT cells with high-K diet (CNT1; Fig. 8). In the later regions of the CNT (CNT2; Fig. 8), cytoplasmic labeling is increased by high-K diet but apical labeling is not as striking as in the DCT2 and CNT1 regions.

Quantification of changes in ROMK due to variation in K diet. Quantitative assessment of cytoplasmic ROMK labeling in nephron segments indicates that the DCT regions do not significantly change ROMK abundance in response to changes in K diet. However, total cytoplasmic ROMK labeling increased by 25% in CNT and 18% in CD (Fig. 9). Quantitative evaluation of apical ROMK labeling indicates that apical labeling is not significantly increased in the DCT1 segment but showed a 2-fold increase in DCT2, a 5-fold increase in CNT1, a 1.5-fold increase in CNT2, and a 5-fold increase in apical labeling in the CD (Fig. 9). Strikingly, while the apical labeling intensity of DCT2 and CNT1 is similar, the intensity of apical labeling declines in more distal regions with progressively weaker apical labeling in CNT2 and CD (Fig. 9).

DISCUSSION

While the central role of ROMK channels in renal K secretion is well-established (20, 33, 37), the specific nephron segments where ROMK is regulated in response to changes in dietary K have remained uncertain, largely because of limitations of the available anti-ROMK antibodies. Here, we took advantage of the availability of ROMK KO mice to screen antibodies produced in six rabbits immunized with COOH-terminal peptides of ROMK. The specificity of the antibodies was confirmed in three ways: 1) its use in Western blot experiments identified the three major forms of ROMK at the correct molecular weight (unglycosylated, 37 kDa; core glycosylated, 40 kDa; maturely glycosylated, 50–55 kDa), 2) its use in immunocolocalization studies identified ROMK not just in the thick ascending limb of the loop of Henle but also in distal nephron and CD, 3) its use with protein and tissue from ROMK KO mice produced negative results. Using these criteria, only two of the six rabbits immunized with the ROMK peptides produced antibodies specific to ROMK. Each of the other antibodies reacted with unknown proteins in ROMK KO mice. This indicates that antibodies previously produced to COOH-terminal peptides of ROMK that have not been tested against ROMK KO tissue may well cross-react with non-ROMK proteins.

Our ROMK-specific antibodies and nephron-segment-specific antibodies (NCC, calbindin D28, and AQP2) raised in other species made possible a definitive assessment of ROMK expression along the distal nephron. These studies demonstrate specific ROMK labeling in both DCT regions, DCT1 and DCT2, in addition to the CNT and CD. While DCT labeling was reported in one early description of ROMK (40), this was not widely accepted because it was not universally observed with other anti-ROMK antibodies.

The extensive expression pattern of ROMK along the entire distal nephron, compared with the more restricted localization of the flow-dependent BK channel in the CNT and CD, may have important physiological implications. As illuminated in mathematical simulations of the kaliuretic response (34, 35), forces for potassium secretion rapidly diminish in the antidiuretic hormone (ADH)-responsive segments (particularly the...
CD and to a lesser extent in the CNT) during antidiuresis because water reabsorption causes the concentration of luminal potassium to rise toward equilibrium. The abundant apical expression of ROMK in the water-impermeable DCT2 and initial portion of the CNT (undetectable or only low levels of AQP2 are found in CNT1 in animals on a high-K diet, data not shown) where potassium secretion is predicted to be driven by optimal forces (35) would ensure that a large component of potassium excretion is not coupled to ADH-mediated water reabsorption.

This emerging view of the importance of ROMK in the DCT2 also casts a fresh perspective on the WNK1/4 signaling pathway. WNK1 and WNK4, kinases mutated in a familial disorder of renal potassium retention and hypertension (38), are thought to be essential components of a signaling pathway that switches the aldosterone response of the kidney to be either kaliuretic or antinatriuretic, depending on whether aldosterone is induced by a change in potassium or by an alteration in the extracellular fluid volume (36). Evidence suggests the signaling pathway differentially regulates the NCC and ROMK. One explanation is that WNK kinases indirectly modulate potassium secretion by directly affecting NCC in the DCT and thereby the amount of sodium available for Na/K exchange in the CD. Numerous data support the idea that the WNK kinases directly affect ROMK (9, 11, 12, 28). Our new observations that dietary potassium loading increases ROMK and decreases NCC simultaneously in DCT2 cells underscore the importance of such a signaling pathway that has the capacity to directly and reciprocally regulate both transporters in the same cell. The specific hormones and factor(s) responsible for mediating the changes in apical ROMK due to variation in K diet remain to be identified. Although changes in K intake are well-known to cause an increase in aldosterone levels, changes in aldosterone alone do not appear to be sufficient to produce the response (21).

Development of the highly specific and avid anti-ROMK antibodies permitted detection of changes in apical membrane expression along the distal nephron. Extending recent whole kidney biotinylaton studies (6), we discovered the response is heterogeneous. Potassium loading produced the largest increase within the DCT2 and the earliest portion of the CNT, reinforcing the chief importance of these segments in the potassium adaptation response. Quantitatively, the change in apical expression within the CNT and CD is strikingly similar to the increase in channel activity that was detected by patch-clamp analysis (21). The observations reinforce the critical role of membrane-trafficking processes in the physiologic regulation of apical K conductance.

The discovery that ROMK is differentially regulated in the distal nephron also provides information to explain the axial variation of potassium secretion that has been observed in these segments. Comparison of K+ secretion in the early and late distal tubules by free-flow microperfusion and micropuncture techniques (5, 16, 25, 26, 32) established that potassium is secreted at relatively low rates in the “early” distal tubule compared with the “late” distal tubule, where basal potassium secretion is much more robust and where the regulatory response to high-K diet is restricted. In modern anatomical terms, micropuncture samples from the early distal tubule likely represent those collected from DCT1, where we found ROMK is present but not regulated. By contrast, late distal tubule punctures undoubtedly evaluated the collective function of DCT2, CNT, and the initial part of the CCD, where we found high-K diet affected a large increase in apical ROMK expression. Because the largest regulatory response was achieved within the DCT2 and the CNT, where the electrochemical driving forces for potassium are maximal (35), it seems likely the bulk of potassium secretion is actually achieved by this intermediate portion of the distal nephron. Future studies will be required to tease out the cellular and molecular basis for the differential response. We speculate that heterogeneous responses to hormones like ANG II, which reduces K+ secretion in late distal tubule but not in the early distal tubule (31), may play an important role.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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