Potassium restriction downregulates ROMK expression in rat kidney

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Mennitt, Patricia A., Gustavo Frindt, Randi B. Silver, and Lawrence G. Palmer. Potassium restriction downregulates ROMK expression in rat kidney. Am J Physiol Renal Physiol 278: F916–F924, 2000.—The ROMK family of potassium channels has biophysical properties and distribution within the kidney similar to those of secretory potassium channels of the distal nephron. To study the regulation of ROMK during variations in dietary potassium, we measured the abundance of ROMK protein in rat kidney by immunoblotting. Neither 2 nor 5 days of a high-potassium diet had an effect on protein abundance in the cortex or medulla. Potassium deprivation (2 or 5 days) decreased ROMK protein content in both cortical and medullary fractions, to 51 and 40% of controls, respectively. To see whether the Na-K-2Cl cotransporter is similarly affected by potassium restriction, we analyzed immunoblots by using an antibody for the rat type 1 bumetanide-sensitive cotransporter (BSC-1). Like ROMK, BSC-1 protein content was found to decrease significantly in the renal medulla of potassium-deprived rats. In the thick ascending limb of Henle’s loop, a decrease in ROMK and BSC-1 could result in decreased reabsorption of NaCl, a finding associated with hypokalemia.

ascending limb of henle’s loop; cortical collecting tubule; secretory potassium channel; potassium excretion; sodium-potassium-chlorine cotransporter; type 1 bumetanide-sensitive cotransporter

REGULATION OF SERUM POTASSIUM levels takes place in the kidney in part through an inwardly rectifying potassium channel. This channel, termed SK for secretory potassium channel, has been well characterized by using the patch-clamp technique in the cortical collecting tubule (CCT) and in the thick ascending limbs of Henle’s loop (TALH) (7, 31). In the CCT the SK channel is upregulated to increase potassium secretion in response to increased potassium intake (22, 24, 30). The SK channel in the TALH is believed to primarily act to recycle potassium, which is essential for the reabsorption of NaCl through the apical Na-K-2Cl cotransporter (9). The TALH may also play a role in potassium adaptation as part of the pathway of potassium recycling (14) from the medullary collecting duct (MCD), into the medullary interstitium, and then into the loop of Henle. Altered dietary potassium intake could therefore affect SK channel activity in both the CCT and the TALH.

The cloning of the ROMK family of potassium channels (3, 12, 34) has enabled further study of potassium channel regulation at the molecular level. These channels are believed to correspond to SK, because they have similar biophysical properties (23) and distribution (3, 15, 20, 33) within the kidney. So far, four rat ROMK isoforms have been cloned, ROMK1–3 (3, 12, 34) and ROMK6 (16). These isoforms are derived from different splicing of the ROMK gene, generating proteins that differ in the amino acid sequence and length of their NH2 termini but are identical to one another in the rest of the peptides. Previous patch-clamp studies have shown a three- to fourfold increase in SK channel density from CCT of animals fed a high-potassium diet (22, 24, 30). Wald et al. (27) studied the effects of changes in potassium intake and adrenalectomy on the abundance of ROMK mRNA in rat kidney. Increased intake did not alter the cortical mRNA level, and it augmented only slightly the medullary abundance of message. Low potassium intake markedly reduced cortical and medullary ROMK mRNA levels both in intact and adrenalectomized animals. ROMK mRNA levels in isolated rat CCTs were not altered by feeding animals a high-potassium diet (8). No information is available yet on the regulation of ROMK protein expression under these conditions.

The purpose of the present study was to examine the effects of potassium intake on the regulation of ROMK protein in the renal cortex and medulla. We found ROMK protein levels do not change with increased dietary potassium, but there was a marked decrease with potassium depletion. This decrease in ROMK protein with potassium depletion was paralleled by a decrease in the apical membrane Na-K-2Cl cotransporter protein.

METHODS

Antibodies. Two affinity-purified rabbit polyclonal antibodies to the COOH-terminal of the rat ROMK1 channel were used in this study. The APC-001 (Alomone Laboratories, Jerusalem, Israel) antibody is directed against amino acids 342–391, and LL309 anti-ROMK is directed against amino acids 369–391. Both of these peptides are identical in all four ROMK isoforms. The LL309 antibody has been previously characterized in our laboratory (20). In addition, an affinity-purified rabbit polyclonal antibody to the Na-K-2Cl cotransporter BSC-1, which is specific to the cortical and medullary TALH in rat (5) was provided by Dr. Mark Knepper (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD).
Institutes of Health, Bethesda, Md.). A mouse monoclonal anti-actin antibody (AC-40) was obtained from Sigma Chemical (St. Louis, MO).

Preparation of membrane fractions. Adult Sprague-Dawley rats (100–140 gm) were fed matched diets containing either high K (HK, 10% KCl), low K (LK, no KCl added), or control K (CK, 1.2% KCl) for 2 or 5 days. All diets were obtained from Harlan-Teklad (Madison, WI). Rats were killed by cervical dislocation, and both kidneys were removed and placed in chilled PBS. The kidneys were dissected to obtain cortical, outer medullary and inner medullary sections. These sections were homogenized with a polytron in ice-cold lysis buffer (250 mM sucrose, 1 mM ethylenediaminetetraacetic acid and 10 mM trithanolamine, pH 7.5 with HCl) containing 0.1 mg/ml phenylmethylsulfonyl fluoride and 1 µg/ml leupeptin. The homogenized tissue was centrifuged at 1,000 g for 20 min at 4°C, and the supernatant was removed and saved. The pellet was rehomogenized and centrifuged again at 1,000 g for 20 min. This high-speed supernatant is cytosol enriched. In this supernatant the pellet was removed and centrifuged at 2,500 rpm for 5 min at 4°C. This final supernatant resulting from the high-speed pellet is membrane enriched. The 1,000 g supernatant was accomplished by using a refrigerated Eppendorf microcentrifuge. The 200,000 g spin was carried out with a Beckman TI-100 Ultracentrifuge fitted with a TLA100.2, S.N.1033 rotor. The high-speed supernatant and pellet fractions were analyzed for protein concentration (Micro BCA Protein Assay Reagent Kit, Pierce, Rockford, IL). The membrane fractions were solubilized at 60°C for 15 min in Laemlll sample buffer containing 30 mg/ml of dithiothreitol (DTT).

Oocytes were prepared and injected as previously described (34). After incubation for 3 days in Barth’s solution at 19°C, seven control oocytes and seven ROMK2 cRNA-injected oocytes were incubated in 2 ml of PBS with 5 mM EGTA for 10 min at 4°C. The oocytes were broken by trituration with a Pasteur pipette and homogenized by using a 2-ml Dounce homogenizer with a Teflon pestle, in ice-cold lysis buffer as above. Homogenates were spun in an Eppendorf microcentrifuge 2,500 rpm for 5 min at 4°C to remove nuclei and incompletely homogenized cells. Then the supernatant was resuspended in an Eppendorf microcentrifuge at 14,000 rpm for 2 h at 4°C. The membrane fractions were solubilized at 60°C for 15 min in Laemmli sample buffer containing 30 mg/ml of DTT.

Electrophoresis and immunoblotting of membranes. Samples of 4–25 µg of total protein were separated by SDS-PAGE on minigels of 8% polyacrylamide for assessment of ROMK and actin expression and on minigels of 6% polyacrylamide for BSC-1 protein expression. The gels were then electrophoretically transferred to polyvinylideine difluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, MA). The membranes were blocked with 5% milk blocker (150 mM NaCl, 50 mM sodium phosphate, 50 µl/100 ml Tween-20, and 5% nonfat dry milk) for 30 min, and then probed with one of the antibodies (LL309, APC-001, or BSC-1 at 1:200 dilution or anti-actin at 1:500 dilution) overnight. The antibodies were prepared in an antibody diluent containing 150 NaCl, 50 mM sodium phosphate, 1.5 mM sodium azide, 50 µl/100ml Tween-20, and 0.1% bovine serum albumin (BSA). Membranes were then washed six times with the antibody dilution buffer (without BSA or sodium azide) before incubation with horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:30,000) or anti-mouse IgG (1:8,000) (Pierce). After washing, sites of antibody-antigen reaction were visualized by using luminol-based enhanced chemiluminescence (SuperSignal Substrate; Pierce) and exposure to X-ray film (Biomax MR; Eastman Kodak, Rochester, NY) for 5–30 min. The control for the APC-001 antibody consisted of APC-001 serum preadsorbed with a fourfold excess of APC-001 fusion protein. Specificity of ROMK LL309 and BSC-1 antibodies was previously demonstrated (5, 20).

Densitometric analysis of immunoblots. The bands in the films were scanned using an AGFA ARCUS II scanner. Relative quantitation of the immunoblott band densities was then carried out using National Institutes of Health Image 1.60. For each film the densitometry values were averaged for samples from rats on a CK diet and on either a HK or LK diet. Rat HK/CK or HK/KC ratio was then computed for each blot. Ratios from different blots were averaged and data are presented as mean ± SE, where the number of observations corresponds to the number of different blots. Both the number of animals used and the number of blots analyzed are given in the text. Comparisons were always made between signals from equal amounts of total protein on the same film. To verify equality of loading, a few representative gels were not transferred to membranes but instead stained with Brilliant Blue G-Colloidal (Sigma). The gels were then scanned and regions spanning a similar size range in each lane were analyzed by densitometry. With the cell membrane fractions no difference was found between the density of the bands in lanes containing HK and CK (HK/KC ratio, cortex = 1.05, outer medulla = 1.03, 6 rats, 2 measurements/rat, n = 2 gels) or HK and CK (HK/KC ratio for rats on diet for 2 days, cortex = 1.04, medulla = 1.22, 6 rats, 2 measurements/rat, n = 2 gels) and for rats on diet for 5 days, cortex = 0.94, medulla = 1.01, 2 measurements/rat, n = 2 gels).

Tissue preparation for immunocytochemistry. Adult Sprague-Dawley rats were fed either a HK or a CK diet for 7–14 days. Rats were anesthetized with methoxyflurane (Mallinckrodt Veterinary, Mundelein, IL), and their kidneys were perfused via the aorta, first with heparinized PBS for 1–2 min, then with 2% paraformaldehyde in PBS for 3–5 min at a pressure of ~100 mmHg. Once fixed, kidneys were sliced (~3 mm thickness), immersed in PBS containing 30% sucrose at 4°C for 4 h, and immersed for an additional 30 min in Tissue-Tek OCT (Sakura Finetech USA, Torrance, CA). Subsequently the kidney tissue was frozen over dry ice in Tissue-Tek OCT, cut into 5- to 8-µm thick sections with a cryostat, and sections were picked up on Superfrost Plus microslides (VWR, West Chester, PA).

Immunocytochemistry. Sections were incubated with fetal calf serum for 30 min at 37°C to block nonspecific antibody binding and then permeabilized with 0.2% Triton X-100 in PBS for an additional 30 min at 37°C. Sections were incubated overnight at 4°C with either anti-ROMK LL309 (1:100 dilution) or APC-001 (1:25 dilution). After five washes in PBS the sections were incubated for 30 min at 37°C with FITC-conjugated donkey (1:160) anti-rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA). Controls for the APC-001 anti-ROMK consisted of either omitting the primary or the secondary antibodies or staining with APC-001 antibody preincubated with a fourfold excess of APC-001 fusion protein. Controls for LL309 anti-ROMK were previously demonstrated (20).

Stained sections were viewed and photographed on Kodak film by using a Zeiss Axioskop fluorescence microscope equipped with a fluorescein filter, differential interference contrast (DIC) optics, and an automatic camera.
RESULTS

ROMK immunocytochemistry. We performed indirect immunofluorescence labeling of cryosections of paraformaldehyde-fixed rat kidneys with LL309 and APC-001 anti-ROMK sera to compare the localization of ROMK by the two antibodies. The LL309 anti-ROMK was previously characterized in our laboratory (20).

The antibodies labeled similar tubules of both the cortical and outer medullary sections of the kidney. Figure 1A shows the distribution of immunoreactivity of the APC-001 anti-ROMK in a cortical section. No specific staining was observed in glomeruli or proximal tubules, although the proximal tubules show punctate yellow autofluorescence. The two CCTs (marked by C) show that some of the cells are apically stained. This is consistent with our previous study (20), using the LL309 anti-ROMK, which demonstrated CCT cells that were positive for LL309 did not stain with the H-ATPase antibody (an intercalated cell marker), suggesting that they were principal cells. Connecting tubules also express the APC-001 anti-ROMK on their apical membrane (not shown).

There are also numerous apically stained TALH tubules, two indicated by arrows, in this cortical section. In one tubule with an open lumen, only a fraction of the cells are positive for ROMK. Many more open and closed, as well as longitudinally cut, TALH tubules labeled with the APC-001 anti-ROMK can be seen in the medullary section shown in Fig. 1B (two closed TALH identified by arrows). One clearly identifiable apically stained outer medullary collecting duct (OMCD) is shown in this panel (marked by C). There was no staining of the inner medulla (Fig. 1C). No labeling with the APC-001 anti-ROMK was detected in sections preincubated with a fourfold excess of APC-001 fusion protein (Fig. 1D, two TALH indicated by arrows) nor in sections incubated with only the primary or secondary antibody (not shown).

Fig. 1. Immunolocalization of ROMK protein stained with APC-001 antibody (FITC) in rat kidney sections. A: cortical section. Apically stained cortical collecting tubule (CCT) and thick ascending limbs of Henle’s loop (TALH) (2 of them, 1 open and 1 closed, are marked with arrows) are shown. No specific staining of proximal convoluted tubules is observed, although they show yellow autofluorescence. Magnification ×554. B: medullary section. One apically stained outer medullary collecting duct (OMCD) and numerous apically stained TALH tubules are shown. Most of TALH are collapsed (2 marked with arrows), but a few are open and 2 of them are longitudinally cut. Magnification ×360. C: inner medullary section. No specific staining is seen. Magnification ×90. D: cortical section stained with APC-001 anti-ROMK preincubated with a 4-fold excess of APC-001 fusion protein. No specific staining is visible, although autofluorescence of proximal tubules persists. Two TALH are marked with arrows. Magnification ×360.
Immunoblot analysis. Figure 2 compares the selectivity of the APC-001 and LL309 ROMK antibodies as evaluated by immunoblot of rat kidney membrane preparations. Twenty-five micrograms of either high-speed supernatant (S) or pellet (P) preparations from outer medulla were loaded in each lane.

The regional localization of APC-001 labeling in the rat kidney was studied by loading 25 µg of protein per lane of high-speed pellet of cortical, outer, and inner medullary preparations (Figure 3). As a positive control, a ROMK2 cRNA-injected oocyte preparation was loaded and is shown in the lane labeled OI. The single band in the oocyte lane is at the same molecular weight as those seen in the kidney preparation lanes. It was not detected in the membranes of control oocytes (not shown). The strongest intensity is seen in the outer medullary preparation, with less intensity in the cortex and the least in the inner medulla. Figure 4 shows the distribution of LL309 anti-ROMK labeling in homogenates of outer and inner medullary and cortical preparations (20 µg of protein/lane). The 60-kDa band is most intense in the inner medulla, with less intensity in the outer medulla and the least in the cortex. A faint lower band at ~45 kDa is only visible in the inner medulla.

Figure 5 shows an immunoblot, of which one-half is stained with APC-001 anti-ROMK and the other with the APC-001 anti-ROMK preincubated with a fourfold excess of APC-001 fusion protein. Twenty micrograms of protein per lane of high-speed pellet from cortical or outer medullary preparations were loaded. The APC-001 anti-ROMK stained half showed one band of ~45 kDa in both the cortex and outer medulla.
medulla, with the medullary band dominating. These signals were both ablated in the presence of the peptide. On the basis of the above results, we concluded that the \( \sim 45\)-kDa band recognized by the APC-001 antibody is ROMK.

Regulation of ROMK by potassium intake. We used immunoblots of the APC-001 antibody to compare the effects of changes in potassium intake on ROMK protein expression. First, a calibration curve was made to ensure the method could detect differences in protein concentration per lane. Increasing concentrations (from 4 to 32 \( \mu \)g of protein) of a high-speed pellet preparation were used. Figure 6 shows the measured 45-kDa band densities plotted against the protein concentration. Within this range the signal is a monotonic function of protein concentration. We therefore used 10 \( \mu \)g of protein per lane for all of the comparisons between membranes prepared from animals with various potassium intakes.

Next, to confirm that the animals were potassium replete or depleted, serum samples were taken from three animals on each of the diets. Table 1 lists the measured serum sodium and potassium values. There is no difference between the serum sodium values for any of the rats. Predictably, the serum potassium values increased with a 2-day HK diet (from 3.62 \( \pm \) 0.11 to 4.44 \( \pm \) 0.18 meq/l), and decreased with a 5-day LK diet (2.33 \( \pm \) 0.10 meq/l).

Figure 7 shows results comparing the effects of three diets on the cortex (Fig. 7A) and medulla (Fig. 7B) of six representative animals. Figure 8 summarizes the relative densities of the ROMK bands from the cortex (Fig. 8A) and medulla (Fig. 8B) of all of the rats studied.

Five days on a HK diet had no effect on ROMK protein levels in the cortex or the medulla. The HK/CK densitometry ratio was 1.04 \( \pm \) 0.08 (6 rats, 3 blots) in the cortex and 0.85 \( \pm \) 0.14 (6 rats, 3 blots) in the outer medulla. We also looked at the effects of 2 days on the HK diet on ROMK protein levels. Again, there were only minor differences in the cortex (HK/CK ratio = 1.18, 6 rats, 2 blots gave the same value) and a small decrease in the outer medulla (HK/CK ratio = 0.73 and 0.79, 6 rats, 2 blots). We did not carry out further studies to evaluate the statistical significance of these small effects.

We then examined the effect of an LK diet compared with a matched CK diet. Five days on an LK diet led to a marked decrease in ROMK protein levels in both the cortex (LK/CK = 0.51 \( \pm \) 0.07, 12 rats, 7 blots) and the outer medulla (LK/CK ratio = 0.40 \( \pm \) 0.06, 12 rats, 7 blots). One measurement was made by using a single pair of rats maintained on the LK and CK diets for 2 days. The shorter period of potassium depletion led to a similar decrease in ROMK protein levels (LK/CK ratio

### Table 1. Serum electrolytes

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<th>Control K</th>
<th>Low K (5 Days)</th>
<th>High K (2 Days)</th>
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<tr>
<td>Na, mM</td>
<td>137 ( \pm ) 1</td>
<td>136 ( \pm ) 1</td>
<td>139 ( \pm ) 2</td>
</tr>
<tr>
<td>K, mM</td>
<td>3.6 ( \pm ) 0.1</td>
<td>2.3 ( \pm ) 0.1</td>
<td>4.4 ( \pm ) 0.2</td>
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Values are means \( \pm \) SE for 3 rats. Rats were fed control diet for 5 days, low-K diet for 5 days, or high-K diet for 2 days.
of the densitometry values, cortex = 0.17, medulla = 0.29).

As an additional test for the effects of LK diet on the kidney, we examined the regulation of the Na-K-2Cl cotransporter, BSC-1, after 5 days of LK diet. Figure 9A shows results from four representative animals, and Fig. 9B summarizes the densities from all the rats studied. Similar to the ROMK results, we found a marked decrease in BSC-1 levels in the outer medulla of potassium-depleted rats (densities determined from the 140-kDa band, LK/CK = 0.62 ± 0.01, 12 rats, 5 blots). A similar decrease was also found in the outer medulla from the pair of rats maintained on the diets for 2 days (LK/CK ratio of the densitometry value = 0.62). 4–10 µg of protein per lane were run for these studies.

As a negative control we examined the relative abundance of actin. For these measurements the homogenates of the same samples analyzed for ROMK and BSC-1 were used. There was no measurable difference in the relative abundance of actin (LK/CK = 1.20 and 1.03, 8 rats, 2 blots). The mean change in ROMK abundance in the same samples was LK/CK = 0.44, similar to what was found in membrane fractions from the same kidneys.

**DISCUSSION**

Our studies characterize the APC-001 anti-ROMK serum and compare it with the previously characterized LL309 anti-ROMK. We found that the antibody recognizes epitopes at the apical surface of both medullary and cortical TALH, connecting tubules, CCT, and outer MCD. This distribution of ROMK is similar to that seen previously by us (20) and others (15, 33), all using anti-ROMK COOH-terminal antibodies. The pep-
tides used to make these antibodies are common to all four ROMK isoforms found in the kidney.

Results of immunoblot studies from these laboratories, using the same antibodies as for the localization studies, were somewhat more variable. Xu et al. (33) found a ~45-kDa band, as well as several species between 85 and 90 kDa, in blots of renal cortex and outer medulla. Faint bands at ~45 and 85 kDa were also detected in the inner medulla. They found similar labeling with an antibody to an NH2-terminal peptide common to all four ROMK isoforms. They suggested the 85- to 90-kDa proteins could represent homodimeric and/or heterodimeric complexes formed by ROMK isoforms. The antibody in the study by Kohda et al. (15) recognized ~42, 44-, and 77-kDa bands in whole kidney homogenate. They also made an antibody to the outer medulla. Faint bands at 85-90 kDa, in blots of renal cortex and found a 45-kDa band, as well as several species between 42–45 kDa, close to the predicted molecular size of ROMK2 cRNA-injected oocytes (Fig. 2).

In this study, we find the LL309 antibody recognizes proteins at ~45 and 60 kDa. These presumably correspond to the 42- and 75-kDa species we identified previously (20). A few adjustments to our immunoblot technique were made in this study which may be responsible for the differences. In particular, we have utilized a differential centrifugation technique to separate cytosolic from membrane fractions and switched to transferring our gels to a PVDF membrane instead of nitrocellulose and hence switched our solutions accordingly. The APC-001 antibody recognizes one band at ~45 kDa, close to the predicted molecular size of ROMK3 (39–43 kDa without glycosylation) and consistent with the band seen in ROMK2 cRNA-injected oocytes (Fig. 2).

Our further characterization of the APC-001 antibody supports the idea that it specifically recognizes ROMK in immunoblots. The 45-kDa species is found predominately in the high-speed pellet fraction, which is membrane enriched, consistent with the immunocytochemical localization of ROMK to the apical surface. The appearance of the band, albeit at lower intensity, in the high-speed supernatant is unexpected. Although no cytoplasmic staining of ROMK was found by immunocytochemistry, it is possible ROMK exists in a subapical area that is visible indiscernible from the membrane or in a form or amount undetectable to immunocytochemistry. The band is more dominant in the outer medulla than the cortex, as predicted by the abundance of ROMK in the TALH. The faint species in the inner medulla was not expected given the lack of staining of sections of this part of the kidney, although Xu et al. (33) also found ROMK bands in the inner medulla, and Lee and Hebert (18) reported a low abundance of ROMK. The ubiquitous distribution of ROMK6 mRNA in various rat tissues (16) makes this isoform a possible candidate for immunoblot detection in the inner medulla. No labeling was seen when the APC-001 antibody was preadsorbed with the APC-001 fusion protein.

All of these studies (15, 20, 33) have in common their recognition of a protein at ~42–45 kDa, which is most likely ROMK. We therefore believe this lower-molecular-weight species can be used to study regulation of ROMK protein in the kidney. In particular, APC-001 anti-ROMK's predominant detection of the lower band makes it a good choice for further studies.

The identities of the higher molecular weight proteins and what, if any, relationship they have to ROMK remains to be understood. In the case of the predominant 60-kDa band recognized by the LL309 antiserum, the facts that most of the protein was found in the cytosolic fraction and that it was not recognized by APC-001 suggest that it may be a nonmembrane protein unrelated to ROMK. However, we cannot rule out the possibility that it represents a potassium channel protein within a very light vesicular compartment.

Patch-clamp studies previously identified a three- to fourfold increase in active potassium channel density from the CCT of rats fed a HK diet (22, 24, 30). Yet we identified no increase in the expression of ROMK protein in the cortex or medulla of rats fed a HK diet. Our results, consistent with studies finding a potassium-enriched diet, did not change ROMK mRNA abundance in the rat CCT (8) or in total rat cortex (27). In the medulla, Wald et al. (27) saw a slight increase in ROMK mRNA (122% of control). The CCT and MCD are minor fractions of the ROMK-expressing tubules in the whole cortex and whole medulla, thus this method may not be sensitive enough to identify changes in ROMK expression specific to those segments. Our results imply there is no effect of a potassium-enriched diet on expression of ROMK protein, at least in the TALH. Electrophysiological measurements indicated that the density of conducting, ROMK-like channels in the TAL was not changed under these conditions (29).

In rats fed a LK diet for 5 days we found a large decrease in ROMK protein content in both whole cortex and medulla. The results of our studies on potassium deprivation correlate well with the mRNA expression studies of Wald et al. (27). They found a potassium-deficient diet downregulated ROMK mRNA expression in both the cortex (47% of control) and the medulla (56% of control). The cortex of adrenalectomized rats also showed a decrease in ROMK mRNA expression which could be further reduced by a LK diet, suggesting separate aldosterone-dependent and aldosterone-independent regulatory processes (27). In the medulla, they found ROMK mRNA depended on serum potassium levels irrespective of aldosterone. The preponderance of ROMK in the TALH suggests that one effect of potassium deprivation is a decrease in ROMK mRNA and protein in TALH. Single-tubule studies will be required to determine whether expression in the CCT is similarly affected.
Impaired NaCl reabsorption in the TALH has been shown to occur in potassium depletion (10, 19) although the mechanisms by which this occurs are controversial. Potassium-depleted rats have a reduction in luminal potassium concentration (10, 28), but it is minimal and therefore potassium may not be rate-limiting for NaCl transport. Potassium deprivation was found to decrease expression of mRNA coding for $\alpha$- and $\beta$-subunits of Na-K-ATPase (27) and the apical Na-K-2Cl cotransporter (1). These studies support our finding that BSC-1 protein expression is downregulated in the outer medulla of potassium depleted rats. A decrease in SK and Na-K-2Cl cotransporter function in the TALH would result in decreased absorption of NaCl and may explain the reduced concentrating ability found with chronic hypokalemia (21).

Is decreased ROMK expression in potassium depletion an adaptive response? If the primary purpose for ROMK expression in CCT is to secrete potassium, then downregulation of ROMK in the CCT might be adaptive by suppressing secretion and promoting potassium retention. In addition, functional studies (17, 32) suggest one of the primary effects of an LK diet on CCT and MCD is enhancement of H-K-ATPase activity for absorption of potassium. A consequence of upregulating H-K-ATPase and downregulating ROMK, should be a decreased urine potassium. However, we cannot conclude from our data that the CCT expresses less ROMK protein. In fact, patch-clamp studies showed no detectable change in the density of conducting channels in the CCT (24).

In the TALH, it is believed that the primary role of SK is to recycle potassium across the apical membrane, to support the function of the Na-K-2Cl cotransporter (9). According to the mouse mTALH model of Hebert and Andreoli (11) there is also a net secretion of potassium into the lumen through SK. Thus a reduction in ROMK with potassium deprivation could reduce secretion in this segment and potentially give rise to an increase in the amount of potassium reabsorbed through a transcellular pathway. However, the amount of Na-K-2Cl cotransporter was similarly reduced, arguing against a role for these effects in conserving potassium. The interpretation is further complicated by the finding that mutations in ROMK, as well as Na-K-2Cl, can cause Bartter’s syndrome, a disease associated with sodium and potassium wasting, hypokalemic acidosis, and major defects in urinary concentrating and diluting capacity (25, 26). It seems unlikely that downregulation of ROMK and BSC-1 proteins is an adaptive response to low potassium intake, because the malfunctioning of either protein causes a disease characterized by hypokalemia.

The combined decrease of ROMK, BSC-1, and Na-K-ATPase, may implicate an overall suppression of transporters in the TALH during potassium depletion. In addition, Imbert-Teboul et al. (13) found a reduced adenylate cyclase response to arginine vasopressin and glucagon in mTAL of potassium-depleted rats. Arginine vasopressin and glucagon have been shown to stimulate reabsorption of electrolytes in the TALH (2, 4). Morphotically, Elger et al. (6) showed that although overall hypertrophy of the kidney occurs with potassium depletion, there is decreased growth of all segments of both short and long loops of Henle except that corresponding to the outer stripe of the medulla.

In summary we studied the regulation of ROMK expression at the protein level by immunoblotting. Our findings show ROMK expression is downregulated by potassium depletion in both the cortex and the medulla. Under the same conditions, in the medulla, we find a decrease in BSC-1 protein expression. No effect was identified in rats fed a HK diet. Further work is required to identify the mechanisms responsible for the modulation of ROMK and BSC-1 expression by potassium depletion.

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