Regulation of Potassium Channel Kir 1.1 (ROMK) Abundance in the Thick Ascending Limb of Henle’s Loop

CAROLYN A. ECELBARGER,* GHEUN-HO KIM,† MARK A. KNEPPER,‡ JIE LIU,‡ MARGARET TATE,‡ PAUL A. WELLING,‡ and JAMES B. WADE‡

*Division of Endocrinology, Georgetown University, Washington, DC, †Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, and ‡Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland.

Abstract. The renal outer medullary potassium channel (ROMK) of the thick ascending limb (TAL) is a critical component of the counter-current multiplication mechanism. In this study, two new antibodies raised to ROMK were used to investigate changes in the renal abundance of ROMK with treatments known to strongly promote TAL function. These antibodies specifically recognized protein of the predicted size of 45 kD in immunoblots of rat kidney or COS cells transfected with ROMK cDNA. Infusion of 1-deamino-(8-D-arginine)-vasopressin (dDAVP), a vasopressin V2 receptor-selective agonist, for 7 d into Brattleboro rats resulted in dramatic increases in apical membrane labeling of ROMK in the TAL of dDAVP-treated rats, as assessed by immunocytochemical analyses. Using immunoblotting, a more than threefold increase in immunoreactive ROMK levels was observed in the outer medulla after dDAVP infusion. Restriction of water intake to increase vasopressin levels also significantly increased TAL ROMK immunolabeling and abundance in immunoblots. In addition, dietary Na⁺ levels were varied to determine whether ROMK abundance was also affected under other conditions known to alter TAL transport. Rats fed higher levels of sodium, as either NaCl or NaHCO₃ (8 mEq/250 g body wt per d), exhibited significantly increased density of the 45-kD band, compared with the respective control animals. Moreover, in rats fed a low-NaCl diet (0.25 mEq/250 g body wt per d), a 50% decrease in band density for the 45-kD band was observed (relative to control rats fed 2.75 mEq/250 g body wt per d of NaCl). These results demonstrate that long-term adaptive changes in ROMK abundance occur in the TAL with stimuli that enhance transport by this segment.

NaCl transport by the thick ascending limb (TAL) of the loop of Henle plays a central role in renal water and electrolyte homeostasis. Uptake of NaCl across the luminal membrane of this segment occurs via a bumetanide-sensitive Na⁺/K⁺/2Cl⁻ cotransporter (NKCC2). Potassium brought into the cell by this cotransporter, which is also known as BSC1 (1,2), is largely recycled back into the lumen via apical K⁺ channels (3,4). Indeed, K⁺ channels represent the principal conductive path - way in this membrane (5), and their blockade nearly abolishes NaCl transport by this segment (3). Many observations indicate that the Kir 1.1 renal outer medullary potassium channel (ROMK) gene product, which was originally identified by Ho et al. (6) in 1993, encodes the major functional subunit of the secretory K⁺ channel (7,8). The critically important role of ROMK in TAL function has been illustrated by the finding that loss-of-function mutations in this gene cause the severe concentrating defect and salt-wasting disorder known as Bartter’s syndrome (9,10).

Chronic elevation of vasopressin (AVP) levels is known to cause profound structural (11,12) and functional (13) adaptive changes in the TAL that promote renal concentrating ability. Studies using semiquantitative immunoblotting recently demonstrated that chronic infusion of the AVP analog 1-deamino-(8-D-arginine)-vasopressin (dDAVP) or restriction of water intake causes a consistent and marked increase in NKCC2 expression in the renal outer medulla (14). These treatments seem to selectively affect pathways central to the concentrating mechanism, because consistent increases in expression of the TAL proteins NHE3 and Tamm-Horsfall protein were not observed. NKCC2 expression has also been observed to be increased by chronic NaCl loading (15,16). In view of the central role of apical K⁺ recycling in TAL function, these studies were undertaken to test the hypothesis that ROMK abundance might also be altered in these adapted states.

Materials and Methods

Antibodies

To produce polyclonal antibodies against ROMK, a short peptide sequence was synthesized as follows, on the basis of the predicted amino acid sequence of rat ROMK1 (amino acids 370 to 391, NH₂-CRKYDNPNVVLSEVEDEETTDQTMCOOH). The sequence was chosen for specificity, antigenicity, and an absence of likely posttranslational modifications predicted by computer analysis, as described previously (14,15). The peptides were purified by HPLC, conjugated to keyhole limpet hemocyanin, and used for immunization of rabbits (L567) and chickens (LC35), using standard immunization...
protocols. The resulting antisera were affinity-purified against the immunizing peptide, as described previously (14).

**ROMK Expression in COS Cells and Immunoblotting**

ROMK1 and ROMK2 cDNA were subcloned into pCDNA 3.0 (Invitrogen, Carlsbad, CA) for expression in COS cells. COS cells were grown to approximately 70 to 80% confluence [in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10 mM Hepes] for transfection. After cells were washed once with serum-free DMEM, they were incubated for 5 h with lipofectamine and 1 µg of DNA in OptiMEM (Life Technologies-BRL, Grand Island, NY). After the initial incubation with the transfection mixture, cells were supplemented (at a 1:1 ratio) with DMEM containing 20% fetal bovine serum and 4 mM L-glutamine. Twenty-four h after transfection, transfection medium was replaced with maintenance medium supplemented with 2 mM sodium butyrate, to enhance protein expression. Forty-eight h after transfection, cells were washed with 1250 U of peptide N-glycosidase (PNGaseF; New England Biolabs, Beverly, MA) for expression in COS cells. COS cells were used at 1:10,000 for detection with the enhanced chemiluminescence system (Amersham, Arlington Heights, IL). For Western blotting, L567 and LC35 antibodies were used at 1 to 5 µg/ml. Horseradish peroxidase-conjugated goat anti-rabbit antibody and rabbit anti-anti-chicken antibody (Jackson Immunoresearch, West Grove, PA) were used at 1:10,000 for detection with the enhanced chemiluminescence system (Amersham), according to the recommendations of the manufacturer.

**PNase Digestion**

Triton-solubilized COS or kidney cell lysates (20 to 30 µg) were denatured for 10 min at 60°C in 0.5% sodium dodecyl sulfate/1% β-mercaptoethanol, cooled, and then incubated for 60 min at 37°C with 1250 U of peptide N-glycosidase (PNaseF; New England Biolabs, Beverly, MA) in 50 mM sodium phosphate containing 1% Nonidet P-40.

**Animals and Study Design**

For these experiments, three different groups of animals were studied. In the first study, 12 male Brattleboro rats (210 to 260 g; Harlan Sprague Dawley, Indianapolis, IN), under light methoxyflu- rane (Metofane; Schering-Plough Animal Health Corp., Union, NJ) anesthesia, were implanted with osmotic mini-pumps (Alzet model 2001; Alza Corp., Palo Alto, CA) for administration of 20 ng/h of a V2 receptor-selective agonist (n = 6) or saline vehicle (n = 6) for 7 d. In the second study, male Sprague-Dawley rats (195 to 225 g; Taconic Farms, Germantown, NY) were water-restricted for 7 d by provision of a limited amount of water as part of a slurry diet (14). ”Restricted” rats (n = 6) were given an amount of water chosen to match respiratory losses, i.e., 19 ml water/250 g body wt per d, mixed with 15 g of powdered food. Control rats (n = 6) were given 46 ml water/250 g body wt per d, mixed with 15 g of powdered food. Rats in both groups consumed all of their food daily and maintained their weight throughout the 7-d period. This treatment resulted in urinary osmolalities of 549 ± 22 mosmol/kg H2O for control rats and 2913 ± 175 mosmol/kg H2O for water-restricted rats, as previously reported (14). In the third study, 24 male Sprague-Dawley rats (205 to 255 g) were fed either a control diet (2.75 mEq Na+/250 g body wt per d) (n = 6), a low-sodium diet (0.25 mEq Na+/250 g body wt per d) (n = 6), a neutral high-sodium diet (8 mEq Na+/250 g body wt per d) (n = 6), or an alkaline high-sodium diet (8 mEq Na+/250 g body wt per d, added as NaHCO3) (n = 6) for 7 d. The effects of a NaCl diet on NKCC2 in these animals were previously reported (16). In all studies, rats were euthanized by decapitation, and both kidneys were rapidly removed and either frozen on dry ice for later processing or immediately dissected and homogenized in buffered isolation solution, as described below.

**Immunolocalization**

Kidneys from ketamine/pentobarbital-anesthetized rats were fixed for immunolocalization by retrograde perfusion through the abdominal aorta, and antibodies were immunolocalized on frozen sections as described previously (17). Sections were incubated overnight at 4°C with primary antibodies diluted to 10 µg/ml. Secondary antibodies were species-specific donkey anti-chicken and donkey anti-rabbit antibodies (Jackson Immunoresearch) coupled to Alexa 488 and Alexa 568, respectively (Molecular Probes, Eugene, OR).

**Immunoblotting of Kidney Samples**

**Preparation of Samples.** Immediately after euthanasia (or after thawing), kidneys were placed in chilled buffered isolation solution containing: 250 mM sucrose, 10 mM triethanolamine (Calbiochem, La Jolla, CA), 1 µg/ml leupeptin (Bachem, Torrance, CA), and 0.1 mg/ml phenylmethylsulfonl fluoride (United States Biochemicals, Toledo, OH), adjusted to pH 7.6. Whole right kidneys were homogenized using a tissue homogenizer (Omni 2000; Omni International Inc., Warrenton, VA), fitted with a 10-mm micro-sawtooth generator, in 10 ml of isolation solution on ice. The left kidneys were dissected into the cortex and inner stripe of the outer medulla. Each region was separately homogenized using three bursts of 10 s at approximately 15,000 rpm, in either 10 ml (cortex) or 1 ml (outer medulla) of isolation buffer, on ice.

Protein concentrations of the homogenates were measured using the Pierce BCA protein assay reagent kit (Pierce, Rockford, IL). All samples were then diluted with isolation solution to a protein concentration of 1 to 3 µg/µl and were solubilized at 60°C for 15 min in Laemmli sample buffer. Samples were stored at −80°C until gel analysis.

**Immunoblotting.** To ensure equal protein loading of samples from different rats and treatment groups, each sample set was initially tested by electrophoresis. Five micrograms of protein from each sample were loaded into individual lanes, subjected to electrophoresis on 12% polyacrylamide gels (precast; Bio-Rad, Hercules, CA), and then stained with Coomassie blue dye [G-250 (Bio-Rad); 0.04% solution in 3.5% perchloric acid]. Gels were destained with water, and selected bands were scanned (Scan Jet 6100C; Hewlett Packard, Palo Alto, CA) to determine densities (NIH Image software) and the relative amounts of protein loaded in each lane. When necessary, protein concentrations were adjusted on the basis of these measurements.

For immunoblotting, 10 to 30 µg of protein from each sample were loaded into individual lanes of precast minigels of 7, 10, or 12% polyacrylamide (Bio-Rad). The proteins were electrophoretically resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a nitrocellulose membrane (Amersham, Arlington Heights, IL). For Western blotting, L567 and LC35 antibodies were used at 1 to 5 µg/ml. Horseradish peroxidase-conjugated goat anti-rabbit antibody and rabbit anti-anti-chicken antibody (Jackson Immunoresearch, West Grove, PA) were used at 1:10,000 for detection with the enhanced chemiluminescence system (Amersham), according to the recommendations of the manufacturer.
transferred from the gels to pure nitrocellulose membranes (Bio-Rad). After 30 min of blocking with 5% milk, membranes were probed overnight at 4°C with the desired affinity-purified polyclonal antibody. For probing blots, all antibodies were diluted into a solution containing 150 mM NaCl, 50 mM sodium phosphate, 10 mg/ml sodium azide, 50 mg/ml Tween-20, and 0.1 g/dl bovine serum albumin (pH 7.5). The secondary antibodies were goat anti-rabbit IgG conjugated to horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) and rabbit anti-chicken IgG conjugated to horseradish peroxidase (Jackson Immunoresearch), used at a concentration of 0.1 μg/ml. Sites of antibody-antigen reaction were observed using luminol-based enhanced chemiluminescence (LumiGLO; Kirkegaard and Perry Laboratories) before exposure to x-ray film (Fujifilm; Fuji Medical Supplies, Stamford, CT).

Statistical Analyses

The relative intensities of the resulting immunoblot bands were determined by laser scanning densitometry (Scan Jet 6100C), followed by analysis using NIH Image software. The statistical significance of the effects of the various treatments on expression was determined using an unpaired t test of densitometry values when SD values were equivalent or using Welch’s t test when SD values were significantly different (GraphPad Prism software; GraphPad, San Diego, CA). P < 0.05 was considered statistically significant.

Results

Characterization of Antibodies to ROMK

Antibodies raised to a ROMK-specific peptide in rabbits (L567) and chickens (LC35) were characterized by immunoblotting, as presented in Figure 1. Because COS cells do not normally express detectable ROMK, we evaluated control cells (Figure 1A, lane pCDNA) and cells transfected with ROMK2, to determine whether the antibodies recognized authentic ROMK. The L567 antibody (Figure 1A) detected a broad band (possibly a doublet) at approximately 45 kD, as did the chicken antibody LC35 (Figure 1B). A band of the same size was also detected in COS cells transfected with ROMK1 (data not shown). Labeling with both antibodies was ablated by peptide absorption. The rabbit antibody (L567) also detected a 75- to 78-kD protein in kidney but not in transfected cells. Recombinant ROMK aggregated to run at >80 kD (observed at the top of the blots in Figure 1, A and B). These aggregates were clearly larger than the distinct 75- to 78-kD band observed in kidney. The LC35 antibody raised in chickens strongly recognized a protein of 45 kD but did not significantly recognize the 75- to 78-kD band. Collectively, these observations demonstrate that ROMK is a 45-kD protein and that the 75- to 78-kD band in kidney that was recognized by rabbit antibody L567 and was observed in other studies may be a cross-reacting protein unrelated to ROMK, as argued by others (18,19).

It should be noted that there was a small difference in molecular mass between recombinant ROMK and native ROMK in the kidney. This size difference was eliminated by PNGase treatment (Figure 1C), suggesting that it arose from differences in glycosylation. The remaining higher-molecular mass bands observed in the kidney sample after PNGase treat-

![Figure 1](image-url). Western immunoblots of lysates of COS cells transfected with renal outer medullary potassium channel 2 (ROMK2) and kidney outer medulla. Control COS cells (pCDNA), COS cells transfected with ROMK2, and homogenates of rat outer medulla (Kidney) were probed with antibodies. (A) L567, without (−) or with (+) an excess of immunizing peptide. (B) LC35, without (−) or with (+) an excess of immunizing peptide. (C) Lysates, with (+) or without (−) peptide N-glycosidase treatment.
ment (Figure 1C) probably resulted from limited effectiveness of the PNGase treatment. Figure 1C also demonstrates that the broad ROMK band observed in the kidney sometimes appeared as a doublet, depending on the gel development conditions; this was also observed with recombinant ROMK2 (Figure 1, A and B).

**Immunolocalization of ROMK**

The antibody LC35 specifically localizes to the TAL and collecting ducts. Figure 2 demonstrates co-labeling with this antibody (Figure 2, A and B) and the antibody to NKCC2 (Figure 2, C and D). There was dramatic enhancement of TAL labeling in dDAVP-treated animals (Figure 2B), compared with control animals (Figure 2A). Collecting ducts in the outer medulla were also weakly labeled (Figure 2). Note that, as described previously for other antibodies to ROMK (20,21), there was distinct heterogeneity of labeling, such that some of the NKCC2-positive TAL cells were not labeled by the ROMK-specific antibody (Figure 2, arrows).

**Increased Abundance of ROMK with Chronic dDAVP Infusion**

To independently assess whether dDAVP increases ROMK levels, we performed semiquantitative immunoblotting, comparing the abundance of ROMK in homogenates of the outer medulla from vehicle- and dDAVP-treated Brattleboro rats. Figure 3A presents an immunoblot containing samples from rats given a 7-d infusion of either vehicle (n = 6) or dDAVP (20 ng/h) (n = 6), probed with the LC35 antibody. Each lane was loaded with a sample from a different rat. As observed for the transfected COS cells (Figure 1), this antibody exclusively detected a 45-kD band in both groups of animals. Importantly, immunoreactive ROMK levels consistently increased with dDAVP treatment. After dDAVP infusion, the average density of the ROMK band increased by 343 ± 34% (P < 0.005), compared with vehicle-infused control animals (Figure 3B). Samples from the same animals were also probed with the L567 antibody (Figure 4). Two bands (45 and 75 to 78 kD) were observed in these outer medullary samples. The average
density of the 45-kD band was also strongly increased in dDAVP-infused rats (to $593 \pm 73\%$ of the value for vehicle-infused rats, $P < 0.001$). However, the density of the 75- to 78-kD band did not change with dDAVP infusion (the average band density for dDAVP-infused rats was equal to $119 \pm 11\%$ of the vehicle-treated control mean value). Therefore, increased labeling of the 45-kD band in immunoblots was correlated with the striking increase in immunolabeling of the TAL we observed in dDAVP-treated animals (Figure 2). When animals were exposed to dDAVP for only 1 h, no significant change in abundance of the 45-kD band or TAL labeling could be detected (data not shown).

Effect of Water Restriction on ROMK Abundance

To further assess whether ROMK abundance is increased in antidiuretic states, we also evaluated ROMK levels in normal Sprague-Dawley rats with restricted water intake. Water restriction causes an increase in endogenous levels of circulating AVP and a homeostatic decrease in water excretion by the kidney. Figures 5 and 6 illustrate the effect of water restriction (19 ml water/250 g body wt per d) for 7 d, relative to the water-replete control animals (46 ml water/250 g body wt per d). Immunolocalization with the LC35 antibody in cross-sections through the outer medulla demonstrated increased labeling with water restriction, similar to that observed with dDAVP treatment (Figure 2). Figure 5 illustrates the difference in labeling observed in low-magnification views of longitudinal sections of the outer medulla. Compared with control animals (Figure 5A), water restriction in Sprague-Dawley rats resulted in a strong increase in TAL labeling by the antibody to ROMK (Figure 5B).

An immunoblot of outer medullary samples from six control rats (water-replete) and six water-restricted rats is presented in Figure 6A. Water restriction significantly increased the average density of the 45-kD band for the water-restricted rats, to

Figure 3. Effect of dDAVP infusion on ROMK expression in the renal outer medulla of Brattleboro rats as assessed by antibody LC35. (A) Immunoblots of outer medullary homogenates from Brattleboro rats that received subcutaneous infusions of dDAVP (20 ng/h) or vehicle, via osmotic minipumps, are shown. Each lane was loaded with a protein sample from a different rat. (Parallel 12% Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gels confirmed equal loading among lanes.) Blots were probed with the chicken polyclonal ROMK-specific antibody LC35. (B) Densitometric analysis of the 45-kD band demonstrated a significant increase in labeling of this band. $*P < 0.005$.

Figure 4. Effect of dDAVP infusion on ROMK expression in the renal outer medulla of Brattleboro rats as assessed by antibody L567. Immunoblots of outer medullary homogenates from Brattleboro rats that received subcutaneous infusions of dDAVP (20 ng/h) or vehicle, via osmotic minipumps, are shown. Blots were probed with the rabbit polyclonal ROMK-specific antibody L567. Note that levels of the 45-kD band were increased in dDAVP-treated animals but levels of the 75- to 78-kD band were unaltered by dDAVP treatment.

Figure 5. Immunolocalization of ROMK in low-magnification longitudinal sections of the inner stripe of the renal outer medulla (OM). Labeling of the apical membrane of the outer medullary TAL was performed using the ROMK-specific antibody LC35. Vascular bundles (VB) and the adjacent inner medulla (IM) were not labeled. (A) Control rats. (B) Water-restricted rats. Labeling of the apical membrane of the outer medullary TAL by the ROMK-specific antibody was increased by water restriction. Scale bar, 12.5 μm.
173% of the value for control rats ($P < 0.05$) (Figure 6B). A qualitatively similar result was observed when a duplicate blot was probed with the L567 antibody; the density of the 75- to 78-kD band was not affected (data not shown), as in the case of dDAVP infusion.

**Changes in Dietary Sodium Alter Abundance of ROMK**

In previous studies (15,16), we demonstrated that the abundance of NKCC2 is increased by dietary sodium load. Because of the close relationship of NKCC2 and ROMK with NaCl entry across the apical plasma membrane of TAL cells, we hypothesized that ROMK abundance might be altered in response to NaCl intake, in parallel with NKCC2 levels. Figure 7 presents immunoblots of the whole outer medulla from rats with different levels of sodium intake as either NaCl or NaHCO$_3$ (Figure 7, A, C, E, and G), as well as a bar graph summary of the average densitometric findings for the 45-kD band in each blot (Figure 7, B, D, F, and H). Relatively increased levels of dietary sodium, as either sodium chloride or sodium bicarbonate, resulted in significantly increased densities of the ROMK band. No difference in band density was observed for the 45-kD band in rats receiving 8 mEq/250 g body wt per d of NaCl versus 8 mEq/250 g body wt per d of NaHCO$_3$ (Figure 7H).

**Discussion**

There is strong evidence that the Kir 1.1 channel, which is widely known as ROMK (6), has a role in apical K$^+$ secretion performed by the TAL and collecting duct segments. Our results demonstrate that treatments known to strongly alter TAL function dramatically enhance the abundance of this channel, as observed in immunolocalization assays and immunoblots of the renal outer medulla. Although the central role of apical K$^+$ conductance in NaCl reabsorption by the TAL is well established (3,5), our findings indicate that increases in the amount of ROMK occur in concert with upregulation of NKCC2. This response provides further evidence of the importance of ROMK in TAL function.

Many groups have described antibodies to ROMK (18,20,21). Although three splice isoforms of ROMK, differing at the amino terminus, have been identified (22,23), currently available antibodies are all directed at conserved sites. Only some of the antibodies developed to ROMK, especially antibodies to the carboxyl terminus, have proven suitable for both immunoblotting and immunolocalization. Although those antibodies localized to the TAL, as expected, they recognized multiple bands (18,20,21). The work presented here takes advantage of newly developed antibodies against ROMK that unambiguously detect ROMK in immunoblots and immunolocalization studies in kidney. In immunoblots of cell lines transfected with ROMK, as well as in the renal outer medulla, ROMK consistently migrates at approximately 45 kD, with variation attributable to glycosylation.

**Immunolocalization of ROMK**

Immunolocalization assays using the new antibody, LC35, demonstrated strong localization in the TAL and much weaker localization in collecting duct principal cells. This confirms previous studies that demonstrated localization of ROMK at these sites (18,20,21). In particular, the strong localization of ROMK in the apical membrane of a subfraction of TAL cells reflects the cellular heterogeneity of this segment noted in previous studies (24–27). The functional significance of this cell-type variation remains to be determined.

**Regulation of ROMK Abundance by AVP**

The medullary TAL is an important site of action of AVP. Acutely, AVP increases NaCl transport by this region (28–30). The K$^+$ conductance of the apical membrane has been demonstrated to be strongly increased by AVP (5). Patch-clamp studies indicate that AVP exposure increases the density of K$^+$ channels, via a protein kinase A (PKA)-mediated pathway, in both the cortical collecting ducts and TAL (31,32). PKA has been demonstrated to be a regulator of ROMK channels expressed in oocytes (17), and site-directed mutagenesis of the three putative PKA sites demonstrated that at least two of the three sites are required for channel activation (33). Long-term exposure to AVP has been demonstrated to have much more dramatic effects in enhancing the lumen-positive transepithelial potential and ion transport rates, compared with short-term exposure to AVP (13). This study indicates that long-term AVP exposure also has a striking effect on ROMK abundance, as demonstrated by immunolocalization findings and semi-quantitative immunoblotting results. These observations reveal

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**Figure 6.** Effect of restriction of water intake on ROMK expression in the rat renal outer medulla. (A) The blot was probed with antibody LC35. (B) Densitometry showed that the band density for the 45-kD ROMK protein was significantly increased by chronic restriction of water intake. *$P < 0.05$. 

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**Figure 7.** Immunoblots of the whole outer medulla from rats with different levels of sodium intake as either NaCl or NaHCO$_3$. (A) Bar graph summary of the average densitometric findings for the 45-kD band in each blot. (B) Relatively increased levels of dietary sodium, as either sodium chloride or sodium bicarbonate, resulted in significantly increased densities of the ROMK band. No difference in band density was observed for the 45-kD band in rats receiving 8 mEq/250 g body wt per d of NaCl versus 8 mEq/250 g body wt per d of NaHCO$_3$.
a physiologically relevant mechanism of ROMK regulation that had previously escaped detection.

Outer medullary collecting ducts also express ROMK, and changes in ROMK abundance may occur in this region. However, because of the very high expression of ROMK in the TAL, the changes in ROMK abundance detected in immunoblots undoubtedly reflect changes in TAL rather than collecting duct expression. Further work will be required to determine whether the abundance of ROMK in the collecting duct and other distal regions is also altered by AVP. Previous studies also demonstrated strong effects of long-term AVP treatments on the abundance of the TAL cotransporter NKCC2 (14) and aquaporin 2 in the collecting duct (34). Therefore, AVP contributes to the renal concentrating ability by both short-term and long-term actions. This seems to produce a coordinated increase in expression of the transporters needed to maximally concentrate the urine.

Regulation of ROMK Abundance by Na\(^+\) Intake

Because previous investigations noted that NKCC2 abundance could be affected by Na\(^+\) intake (15,16), we also assessed the effect of Na\(^+\) intake on the abundance of ROMK. There were strong effects of the high-Na\(^+\) diet to increase and the low-Na\(^+\) diet to decrease the abundance of ROMK. Volume expansion results in a marked increase in NaCl delivery to the TAL (35) and increased reabsorption by this segment (36). Chronic elevation of Na\(^+\) intake has been demonstrated to increase the bicarbonate absorptive capacity of the medullary TAL (37). The physiologic role of enhanced reabsorption by the TAL when overall renal NaCl excretion is increased is very likely related to the need to maintain water balance even when urinary osmolality is increased with NaCl. Increased NaCl reabsorption with recycling of K\(^+\) via ROMK tends to elevate interstitial NaCl levels in the medulla and allow excretion of Na\(^+\) loads without impairment of water balance. Further work

Figure 7. Effect of dietary Na\(^+\) on ROMK expression in the rat renal outer medulla. (A, C, E, and G) Blots probed with antibody LC35. (B, D, F, and H) Corresponding densitometric analyses of the 45-kDa band. *P < 0.05. (A and B) Control Na\(^+\) diet (2.7 mEq/250 g body wt per d) compared with the high-NaCl diet (8 mEq/250 g body wt per d), demonstrating that ROMK abundance is increased by high Na\(^+\) intake. (C and D) Control diet (2.75 mEq/250 g body wt per d) compared with the low-Na\(^+\) diet (0.25 mEq/250 g body wt per d), demonstrating that ROMK abundance is reduced by low Na\(^+\) intake. (E and F) Control diet (2.75 mEq/250 g body wt per d) compared with the high-NaHCO\(_3\) diet (8 mEq/250 g body wt per d), demonstrating that high Na\(^+\) intake increases ROMK abundance when the high dietary Na\(^+\) intake is from sodium bicarbonate. (G and H) High-NaCl diet (8 mEq/250 g body wt per d) compared with the high-NaHCO\(_3\) diet (8 mEq/250 g body wt per d), demonstrating that there is no significant difference in ROMK abundance with the alkaline diet.
is needed to confirm these findings, but these results suggest that ROMK and NKCC2 may be coordinately regulated by an unknown mechanism, as well as by AVP.

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