Differential regulation of ROMK expression in kidney cortex and medulla by aldosterone and potassium

H. WALD,¹ H. GARTY,² L. G. PALMER,³ AND M. M. POPOVTZER³

¹Nephrology and Hypertension Services, Hadassah University Hospital, Jerusalem 91120; ²Department of Biological Chemistry, Weizman Institute of Science, Rehovot 76100, Israel; and ³Department of Physiology and Biophysics, Cornell University Medical College, New York, New York 10021

Wald, H., H. Garty, L. G. Palmer, and M. M. Popovtzer. Differential regulation of ROMK expression in kidney cortex and medulla by aldosterone and potassium. Am. J. Physiol. 275 (Renal Physiol. 44): F239–F245, 1998.—This study explores the role of K⁺ and aldosterone in the regulation of mRNA of the ATP-sensitive, inwardly rectifying K⁺ channel, ROMK, in the rat kidney. K⁺ deficiency downregulated ROMK mRNA in cortex to 47.1 ± 5.1% of control (P < 0.001) and in medulla to 56.1 ± 3.4% (P < 0.001). High-K⁺ diet slightly increased ROMK mRNA in medulla to 122 ± 9% (P < 0.05 vs. control). Adrenalectomy (Adx) downregulated cortical ROMK mRNA to 30.7 ± 6.8% (P < 0.001 vs. control), and increased it in medulla to 138 ± 12.9% (P < 0.02 vs. control). In Adx rats, K⁺ deficiency decreased ROMK mRNA in cortex and medulla similar to intact rats. The α₁- and β₁-Na-K-ATPase subunits were regulated in parallel to that of ROMK. In medulla, ROMK mRNA correlated with serum K⁺ concentration at R = 0.9406 (n = 6, P < 0.001) and α₁-Na-K-ATPase mRNA at R = 0.9756 (n = 6, P < 0.01). ROMK2 also correlated with serum K⁺ concentration (R = 0.895; n = 6, P < 0.01). These results show that cortical ROMK expression is regulated by aldosterone and K⁺, whereas the medullary ROMK mRNA is regulated by serum K⁺.

medullary thick ascending limb; cortical collecting duct; adrenalectomy

ROMK IS A RECENTLY CLONED gene encoding inwardly rectifying, ATP-regulated K⁺ channels (11, 31). The mRNA encoding these channels is widely expressed in distal cortical and outer medullary nephron segments (2, 13).

Alternative splicing of ROMK exons yields several different transcripts ROMK1–3 that are differentially expressed along the nephron (2, 10, 13). ROMK2 is the most widely distributed isofrom. ROMK1 is specifically expressed in collecting ducts. ROMK3 is expressed in the earlier nephron segments [medullary thick ascending limb (MTAL), cortical TAL (CTAL), and distal convoluted tubule (DCT)]. ROMK2 is expressed in all the above-mentioned segments except the outer medullary collecting duct (OMCD). The distribution of ROMK channel isoforms is consistent with the possibility that ROMK may represent a major subunit of the low-conductance ATP-regulated secretory K⁺ channel in TAL and principal cells of the collecting duct (10).

Recent studies have suggested a predominant apical location using an ROMK-specific polyclonal antibody, consistent with a role for this channel in K⁺ secretion (30). In the kidney, the apical ATP-regulated secretory channels serve several important roles (10). In the thick ascending limb of Henle (both MTAL and CTAL) K⁺ channels provide a K⁺ efflux pathway for K⁺ entering via the basolateral Na⁺ pump or the apical Na-K-2Cl cotransporter (10), enabling apical K⁺ recycling. In the principal cells of the cortical collecting duct (CCD), K⁺ channels mediate K⁺ secretion into the urine and serve as the major mechanism for maintaining K⁺ balance.

K⁺ homeostasis is controlled mainly by aldosterone and K⁺ load (17, 19, 25, 26). Part of the response to K⁺ load may be mediated by changes in plasma aldosterone (21, 23). However, several studies suggest mineralocorticoid independent effects of K⁺ on K⁺ secretion (5, 12, 15, 22, 23). The present study was designated to explore the role of dietary K⁺ and aldosterone in the regulation of ROMK mRNA in the rat kidney.

MATERIALS AND METHODS

Animal treatment and RNA isolation. Experiments were carried out using male 8- to 10-wk-old Wistar rats. The following six groups of rats were studied: 1) NK, control rats fed a normal chow (10 g KCl/kg, 2.7 g NaCl/kg); 2) LK, rats fed a K⁺-deficient diet (<0.01 g KCl/kg; 1.0 g NaCl/kg; ICN, Cleveland, OH); 3) HK, rats fed a high-K⁺ diet prepared by adding KCl to normal chow (80 g KCl/kg, 2.3 g NaCl/kg); 4) A + NK, adrenalectomized (Adx) rats fed a normal chow like in group 1; 5) A + LK, Adx rats fed a K⁺-deficient diet like in group 2; 6) A + HK, Adx rats fed a high-K⁺ diet like in group 3.

Rats were kept on the different diets 12 days with free access to water.

Animals were killed by cervical dislocation, and kidneys were excised and dissected into cortex and medulla.

Total RNA was prepared from kidney slices using a Tri-Reagent kit (Molecular Research Center, Cincinnati, OH). Plasma electrolytes and aldosterone levels were determined in arterial blood samples drawn immediately after killing the animals. Serum concentrations of Na⁺ and K⁺ were determined by flame photometry, and aldosterone levels were measured using a radioimmunoassay kit (Coat-a-Count aldosterone; DPC, Los Angeles, CA).

Northern hybridization. Aliquots of 10–20 μg total RNA were resolved electrophoretically on 1% agarose gels under denaturing conditions (formamide/formalin backbone). Nucleic acids were transferred to nylon membrane (GeneScreen; New England Nuclear Research Products, Boston, MA). cDNA probes were hybridized for 16–20 h with [32P] labeled cDNA fragments corresponding to ROMK, ROMK2, and α₁-Na-K-ATPase and β₁-Na-K-ATPase under stringent conditions. The radioactive probe was prepared with a Rediprime DNA labeling kit (Amersham). Two ROMK probes were prepared by digesting a full-length clone of ROMK2 (accession number S69385) in pSport 1 (BRL Life Technologies). The first, a ~1.5-kb fragment obtained by digestion with BamH I/Pst I (nucleotide 1–1496), should hybridize with all ROMK isoforms. The
second, a ~300-bp fragment obtained by digestion with Hind III (nucleotide 1577 to end), is specific to ROMK2 and ROMK2b. In addition, hybridizations were performed with the Pst I/Eco R I fragment of the α3-subunit of Na-K-ATPase (nucleotide 3060–3636) and Eco R I fragment of β1-subunit of Na-K-ATPase (nucleotide 343–1600). Membranes were washed and autoradiographed by standard procedures. Bound cDNA probes were removed by 1 to 2 min of boiling in 1× standard sodium citrate + 0.1% sodium dodecyl sulfate, and the same membranes were hybridized with a control probe synthesized from a cloned fragment of 18S ribosomal RNA. The abundance of this RNA species was independent of any of the treatments described in this study. Bindings were quantified by phosphorimaging (Fujix, BHS 1000) and expressed as the ratio of intensities obtained by hybridizing the same stripe with the cDNA studied and 18S cDNA, respectively. Each result was confirmed by repeating the Northern hybridization, and at least two different RNA preparations and more than four animals. Data are expressed as means ± SE, and statistical significance was calculated using a two-tailed t-test. Results

Effect of Adx and modulation of K+ intake on ROMK expression in kidney cortex. The effect of Adx and modulation of K+ intake on the abundance of ROMK mRNA in kidney cortex is depicted in Fig. 1 and Table 1. Each result was confirmed by repeating the Northern hybridization, and Fig. 1B shows the mean results pooled from four to eight rats. LK diet reduced ROMK mRNA expression to 47.1 ± 5.1% of normal (P < 0.001), whereas HK diet did not change it compared with normal. Adx reduced the message to 30.7 ± 6.8% of control (P < 0.001). LK diet fed to Adx rats further reduced ROMK expression to 16.0 ± 9.2%, whereas HK diet given to Adx rats increased it to 55.5 ± 2.1%, above the value observed in Adx rats on NK intake (P < 0.02).

The decreased level of ROMK in the cortex of rats on LK diet may result from combination of hypokalemia and low aldosterone (Table 2), since Adx per se markedly reduced the expression despite high plasma K+ (Table 2) and feeding Adx rats with LK diet further decreased ROMK mRNA expression.

Effect of Adx and modulation of K+ intake on ROMK expression in kidney medulla. The effect of Adx and modulation of K+ intake on the abundance of ROMK mRNA in kidney medulla is depicted in Fig. 1 and Table 1. Each result was confirmed by repeating the Northern hybridization, and Fig. 1B shows the mean results pooled from four to eight rats.

LK diet reduced ROMK expression to 56.1 ± 3.4% of normal (P < 0.001), whereas HK diet slightly increased it (122 ± 9%, P < 0.05 vs. normal). Adx increased the message to 138 ± 12.9% (P < 0.02 vs. normal). LK diet fed to Adx rats reduced ROMK mRNA expression to 73.7 ± 7.7% (P < 0.01 compared with HK and to Adx on NK), whereas HK diet fed to Adx rats increased it to 173.8 ± 6.4% above the value observed in Adx rats on NK intake (P < 0.05 compared with Adx on NK).

Medullary ROMK expression correlated highly with serum K+ concentration ([K+]R)(R = 0.9406; n = 6, P < 0.001) (see Fig. 3A) and was totally independent of aldosterone levels. In contrast, cortical ROMK showed no correlation with serum [K+]R (R = –0.028; n = 6, P = not significant).

Effect of Adx and modulation of K+ intake on α3- and β1-subunits of Na-K-ATPase in kidney cortex and medulla. Since intracellular potassium ([K+]i) is extruded via the ROMK channel and [K+]i is determined primarily by the Na+ pump allowing transcellular K+ extrusion, we also studied the effect of Adx and modulation of K+ intake on the expression of the α3- and β1-subunits of Na-K-ATPase. Figure 2 and Table 1 illustrate the effect of Adx and modulation of K+ intake on the abundance of α3- and β1-Na-K-ATPase mRNA. Figure 2A shows a representative Northern hybridization with α3-Na-K-ATPase, and Fig. 2B shows the measurements pooled from four rats in cortex and medulla for both α3- and β1-Na-K-ATPase.

Similarity is observed in results obtained for Na-K-ATPase mRNA expression and ROMK. Adx markedly reduced the levels of α3-Na-K-ATPase in the cortex (P < 0.001 vs. NK), whereas a tendency to increase was observed in the medulla, but it did not reach statistical significance. β1-Na-K-ATPase was also markedly reduced by Adx in the cortex (P < 0.001 vs. NK) and significantly increased in the medulla (P < 0.025 vs. NK). Modulation of K+ intake affected the level of α3-Na-K-ATPase both in cortex and medulla. LK diet decreased the expression (P < 0.01 vs. NK for both cortex and medulla), whereas HK diet increased it in the cortex (P < 0.01 vs. NK) and also tended to increase it in the medulla, but the value did not reach statistical significance. In the medulla a similar correlation between serum K+ and α3- and β1-Na-K-ATPase expression could be established as for ROMK. For α3-Na-K-ATPase, R = 0.9387 (n = 6, P < 0.001); for β1-Na-K-ATPase, R = 0.9214 (n = 6, P < 0.001). In addition, excellent correlation could be shown between α3-Na-K-ATPase and ROMK expression in the medulla (R = 0.9756; n = 6, P < 0.001) (Fig. 3B). A similar correlation could be shown with regard to β1-Na-K-ATPase (R = 0.9609; n = 6, P < 0.001). In the cortex, as already mentioned, ROMK and Na-K-ATPase levels did not correlate with plasma [K+]R. However, highly significant correlation existed between ROMK expression and α3- and β1-Na-K-ATPase expression (R = 0.880; n = 6, P < 0.01) for α3-Na-K-ATPase and (R = 0.9296; n = 6, P < 0.001) for β1-Na-K-ATPase.

Effect of Adx and modulation of K+ intake on ROMK2 expression in kidney cortex and medulla. For the Northern hybridizations mentioned above, ROMK2 was detected using a cDNA fragment common to the different ROMK isoforms. Thus the regulation of the specific isoforms by Adx and K+ intake modulation could not be defined. Therefore, we used also an ROMK2-specific cDNA fragment.

Figure 4 illustrates the effect of Adx and modulation of K+ intake on the abundance of ROMK2 mRNA. Figure 4A shows a representative Northern hybridiza-
tion in the medulla only, because the signal in the cortex was weak and detectable only on the phosphorimager screen. Figure 4B shows the measurements pooled from four rats in cortex and medulla.

It is evident that in the cortex ROMK2 was decreased by both Adx and LK intake (P < 0.001 vs. NK for both). In the medulla ROMK2 was decreased by LK intake in intact and Adx rats (P < 0.05 for LK vs. NK, and P < 0.02 for A + LK vs. A + NK), increased by HK intake (P < 0.01 vs. NK), and was markedly increased by Adx (P < 0.01 vs. NK) that is associated with hyperkalemia (Table 2). It should be emphasized that in this case the kidney medulla ROMK2 represents exclusively the MTAL ROMK2 (see figure 7 in Ref. 2).

Table 1. Relative mRNA expression of ROMK, ROMK2, and α1- and β1-Na-K-ATPase in cortex and medulla in the groups studied

<table>
<thead>
<tr>
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<th>NK</th>
<th>LK</th>
<th>HK</th>
<th>A + NK</th>
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<td></td>
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<td>ROMK</td>
<td>100 ± 0</td>
<td>47.1 ± 5.1</td>
<td>88.9 ± 10.9</td>
<td>30.7 ± 6.8</td>
<td>16.0 ± 9.2</td>
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<tr>
<td>ROMK2</td>
<td>100 ± 0</td>
<td>44.4 ± 4.9</td>
<td>105.4 ± 11.3</td>
<td>58.3 ± 2.7</td>
<td>52.8 ± 7.9</td>
<td>100.4 ± 12.1</td>
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<td>α1-Na-K-ATPase</td>
<td>100 ± 0</td>
<td>83.7 ± 1.5</td>
<td>130.6 ± 1.6</td>
<td>48.1 ± 1.1</td>
<td>23.7 ± 10.8</td>
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<td>β1-Na-K-ATPase</td>
<td>100 ± 0</td>
<td>82.9 ± 6.7</td>
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<td>54.7 ± 1.0</td>
<td>42 ± 3.6</td>
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<tr>
<td>ROMK</td>
<td>100 ± 0</td>
<td>56.1 ± 3.4</td>
<td>122 ± 9.0</td>
<td>138 ± 12.9</td>
<td>73.7 ± 7.7</td>
<td>173 ± 6.4</td>
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<td>ROMK2</td>
<td>100 ± 0</td>
<td>78.8 ± 7.8</td>
<td>155.4 ± 12.4</td>
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<td>97.9 ± 10.1</td>
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<td>α1-Na-K-ATPase</td>
<td>100 ± 0</td>
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<td>β1-Na-K-ATPase</td>
<td>100 ± 0</td>
<td>86.5 ± 10.1</td>
<td>136.1 ± 12.4</td>
<td>129.3 ± 9.4</td>
<td>86.5 ± 0.5</td>
<td>157.2 ± 14</td>
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Values are means ± SE. See MATERIALS AND METHODS for complete description for diets and adrenalectomy groups. NK, normal potassium diet; LK, potassium-deficient diet; HK, potassium-loaded diet; A, adrenalectomy.
DISCUSSION

The results of this study show that in the cortex ROMK expression is regulated in concert by aldosterone and potassium, whereas in the medulla ROMK expression is regulated by serum K\(^+\) levels irrespective of aldosterone. In the cortex, in intact rats LK decreased ROMK expression, whereas HK returned it to normal. Adx decreased ROMK expression to less than one-third of normal. LK in Adx rats further reduced ROMK expression, whereas HK in Adx rats increased it. In the cortex, ROMK is expressed in the distal segments CTAL, DCT, connecting tubule (CNT), and CCD but not in the proximal nephron. Among these, DCT, CNT, and mainly CCD are aldosterone-responsive segments (3, 8). The dramatic decrease in ROMK expression in Adx rats may therefore be attributed to these segments and mainly to the CCD. The residual expression of ROMK in the cortex of Adx rats may represent the CTAL ROMK, and presumably this portion is decreased by LK intake in Adx rats. Since the downregulation in cortical ROMK expression due to Adx occurred in face of severe hyperkalemia, it may be suggested that the decrease in cortical ROMK expression evident in adrenal-intact rats on LK diet stemmed mainly from decreased aldosterone.

In the medulla, both in intact and Adx rats, LK diet decreased ROMK expression, whereas HK increased it above normal. Adx induced a significant increase in ROMK mRNA expression. A major finding is the existence of a close correlation between the abundance of ROMK mRNA and plasma [K\(^+\)] independent of adrenal function. It is suggested that the increase in ROMK in

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<th>HK</th>
<th>A + NK</th>
<th>A + LK</th>
<th>A + HK</th>
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<tr>
<td>Na(^+), mM</td>
<td>146.1 ± 0.6</td>
<td>143 ± 1.2</td>
<td>148.8 ± 2.8</td>
<td>126.8 ± 2.1</td>
<td>125.3 ± 0.9</td>
<td>138.8 ± 2.7</td>
</tr>
<tr>
<td>K(^+), mM</td>
<td>3.5 ± 0.08</td>
<td>1.9 ± 0.04</td>
<td>5.8 ± 0.35</td>
<td>7.1 ± 0.8</td>
<td>3.6 ± 0.3</td>
<td>7.3 ± 0.5</td>
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<tr>
<td>Aldosterone, nM</td>
<td>1.11 ± 0.20</td>
<td>0.45 ± 0.08</td>
<td>19.6 ± 2.8</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.04</td>
<td>0.08 ± 0.07</td>
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Values are means ± SE. See MATERIALS AND METHODS for complete description of diet and adrenalectomy (A) groups.

Fig. 2. Regulation of \(\alpha_1\)- and \(\beta_1\)-Na-K-ATPase mRNA by K\(^+\) intake and aldosterone. A: Northern hybridization of \(\alpha\)-Na-K-ATPase (top) and 18S control probe (bottom) with RNA from kidney cortex and medulla in intact rats and Adx rats fed NK, LK, and HK rations for 12 days. B: diet- and ADX-induced changes and \(\alpha_1\)-Na-K-ATPase mRNA (solid bars) and \(\beta_1\)-Na-K-ATPase mRNA (open bars) in kidney cortex and medulla. Data are normalized to the amount of 18S cDNA and expressed as percentage of the abundance in intact rats fed normal chow (100%); n = 4.
Adx rats accrued directly from the severe hyperkalemia. In the medulla, ROMK is expressed in the MTAL and in the OMCD. MTAL is relatively an aldosterone-nonresponsive segment (3, 8), whereas OMCD is responsive to aldosterone. Therefore it is assumed that the changes observed in ROMK expression with K intake modulation independently of adrenal function may be attributed mainly to the MTAL. This is further supported by our results with ROMK2. In the medulla, the ROMK2 isoform is located exclusively in the MTAL, and its expression changed similarly to the common core ROMK cDNA fragment in response to Adx and modulation of K intake. These observations emphasize the central role of serum [K+] in the regulation of ROMK expression in the MTAL. With regard to the responsiveness of the MTAL to aldosterone, it should be noted that conflicting results are reported in the literature. Studies by Stanton (20) and by Work and Jamison (29) show inhibition of MTAL NaCl transport in Adx rats and correction to normal by aldosterone. A study by Grossman and Hebert (9) shows increase of MTAL Na-K-ATPase by deoxycorticosterone acetate (DOCA) treatment; however, in the same study, Na+ deprivation that is associated with increased endogenous aldosterone failed to increase MTAL Na-K-ATPase, whereas it increased by 60% CCD Na-K-ATPase. In studies by Garg et al. (8) and Mujais et al. (14), mineralocorticoids administration did not induce any change in MTAL Na-K-ATPase and markedly increased CCD Na-K-ATPase. In the present study, Adx did not reduce medullary Na-K-ATPase expression but rather tended to increase it. Comparison of our expression results with experiments measuring Na-K-ATPase activity in medulla or MTAL of Adx animals does not support our results. However, these apparent discrepancies can be reconciled. Two studies show reduction in MTAL or medullary Na-K-ATPase in Adx animals, but this reduction could not be reversed by mineralocorticoids or glucocorticoid administration (6, 24) as in the cortex or CCD, but rather by NaCl (24). Taken together, these studies suggest that the CCD is the main target of aldosterone, whereas the MTAL Na-K-ATPase is much less dependent on mineralocorticoids. The effect of mineralocorticoid in the MTAL may be direct or indirect in nature, through its effect on sodium balance.

The ROMK channels mediate the downhill movement of K+ from the intracellular compartment. [K+]i is determined mainly by the Na+ pump. Therefore we studied the effect of aldosterone and K+ intake modulation on the expression of the α1- and β1-subunits of Na-K-ATPase. The changes observed in the expression of α1- and β1-Na-K-ATPase were similar to those of ROMK. The expression of cortical α1- and β1-Na-K-ATPase was controlled synergistically by aldosterone and K+, whereas the expression of medullary α1- and β1-Na-K-ATPase correlated highly with serum potassium concentrations. In addition, α1- and β1-Na-K-ATPase expression correlated highly with that of ROMK expression both in cortex and medulla. Parallel observations on the protein level were recently reported by Anzai et al. (1) for Kir 6.1 in rat CCD. It was shown by Western blotting that CCD Kir 6.1 and α1-Na-K-ATPase were 50% decreased by Adx and three- to fourfold increased by DOCA treatment. Taken together, these results show that the modulation of ROMK mRNA and protein by aldosterone and K+ reflects their effect on the Na+ pump. These observations suggest that the regulation of ROMK by aldosterone and K+ may be mediated by Na-K-ATPase and probably depends on [K+]i.

As mentioned in the introduction, the apical ATP-regulated secretory channels serve different roles in the CCD and MTAL. In the CCD, apical K+ channels mediate K+ secretion into the urine, whereas in the
MTAL K⁺ channels provide a K⁺ efflux pathway for K⁺ recycling via the apical Na-K-2Cl cotransporter (10). Our results support the idea that K⁺ secretion in CCD is regulated mutually by aldosterone and K⁺, whereas K⁺ recycling via the apical K⁺ channel in the MTAL depends on plasma K⁺ irrespective of aldosterone. Mineralocorticoids are well known to increase the apical K⁺ conductance of the CCD (18, 19, 27). These observations support our finding that Adx markedly decreased the expression of ROMK in the cortex. In addition, low-K⁺ diet that is associated with decreased aldosterone levels also decreased by 50% ROMK expression. Several studies demonstrated stimulation of CCD apical K⁺ conductance or potassium secretion by high plasma K⁺ independently of mineralocorticoids (15, 16, 22, 28). In some cases, the effect was not complete (22), or the presence of an intact adrenal gland exerted a permissive effect (16). These observations are in line with our finding that low K⁺ intake decreased ROMK expression even in Adx rats. However, high-K⁺ diet did not increase ROMK expression above normal in adrenal-intact rats despite increased aldosterone levels. These observations are in variance with those mentioned above (15, 16, 22, 28). One possibility to reconcile this discrepancy is that the increases observed in K⁺ conductance or K⁺ secretion due to high K⁺ intake stemmed from mobilization of channels from intracellular compartments to the cell membrane rather than increased transcription of new channels.

Inhibition of the cortical or CCD Na-K-ATPase activity in Adx animals is a well-documented phenomenon (3, 6, 24). Treatment of Adx animals with mineralocorticoids restored this activity (6, 24). Administration of DOCA to adrenal-intact rabbits increased Na-K-ATPase activity mainly in the CCD (8). Farman et al. (7), using in situ hybridization, demonstrated reduction in the α₁-subunit of Na-K-ATPase in the CCD and not in the proximal convoluted tubule in Adx rats. These results are in line with our present observations demonstrating more than 50% reduction in the expression of cortical α₁-Na-K-ATPase mRNA in Adx rats. The results of Farman et al. (7) also support our claim that the reduction in cortical α₁-Na-K-ATPase expression represents mainly the CCD α₁-Na-K-ATPase. The same observations on the protein level were reported by Anzai et al. (1) both for α₁-Na-K-ATPase and Kir 6.1 (1).

The Adx rats in this study were not supplemented with glucocorticoids or given NaCl in their drinking water. The reason for this is that previously we have shown (24) that the extreme changes in serum elec-
lytes observed in Adx rats, the effect of which we intended to study, were greatly corrected toward normal by these treatments (see Table 2 in Ref. 24). Normalization in serum K⁺ in Adx rats was obtained by the LK diet (Table 2). This treatment decreased ROMK and Na-K-ATPase expression in most cases studied.

In summary, our present findings show that in the cortex ROMK expression is regulated mutually by aldosterone and K⁺, whereas in the medulla ROMK expression is regulated by serum K⁺ levels irrespective of aldosterone. It may be suggested that different mechanisms regulate K⁺ secretion in the collecting duct and K⁺ recycling in the MTAL. Further studies are required to substantiate this speculation, mainly by looking at the expression of these channels on the protein level by immunoblotting and measuring their activity under the different conditions studied here.

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Address for reprint requests: H. Wald, Nephrology and Hypertension Services, Hadassah Univ. Hospital, POB 12000, Jerusalem 91120, Israel.

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