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Potassium and sodium transport along the loop of Henle: Effects of altered dietary potassium intake

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Potassium and sodium transport along the loop of Henle: Effects of altered dietary potassium intake. We assessed the effects of changes in potassium (K^+) balance on the function of the loop of Henle by a combination of renal clearance and microperfusion experiments. Rat superficial cortical nephrons were perfused *in vivo* at 20 nl · min^{-1} from late proximal to early distal tubule with an artificial end-proximal solution containing either 3.8 or 1.8 mM potassium. Rats were fed a control diet, a low-potassium diet for at least three weeks, or a high-potassium diet for 10 to 14 days. When compared with the appropriate end-proximal potassium concentration in the perfusion fluid, potassium absorption along the loop of Henle (J_K) increased in potassium-depletion whereas sodium (J_{Na}) and fluid (J_v) absorption decreased. In rats fed a high-potassium diet, absorption of potassium, sodium and fluid was depressed. We propose that changes of external potassium balance affect the transport of electrolytes and fluid along the loop of Henle *in vivo* by modulating the transport of potassium and sodium primarily in the thick ascending limb. Changes in potassium reabsorption may also be affected by alterations of potassium-recycling.

Several *in vitro* studies have shown that the thick ascending limb (TAL) of the loop of Henle (LOH) is an important site of sodium, potassium, bicarbonate and ammonium transport [1, 2]. Moreover, potassium is recycled through the medullary interstitium by a process involving the exit of potassium from the medullary collecting duct and entry into the descending limb of the LOH [3]. Potassium reabsorption in the TAL occurs via an electroneutral Na^+-$2Cl^-$-K^+ cotransport system [1] that is dependent on the low intracellular sodium and chloride concentrations which are maintained, directly in the case of sodium and indirectly in the case of chloride, by the activity of the basolateral Na^+-K^+-ATPase. The luminal cotransporter is also thought to be regulated and dependent upon a potassium-recycling step across the apical cell membrane [4, 5]. In addition, the lumen-positive transepithelial potential difference is responsible for a significant passive component of transepithelial cation absorption, including that of potassium [1].

Evidence is also available to suggest a contribution of potassium recycling to the overall handling of potassium along the loop of Henle [6]. Accordingly, the mechanisms accounting for altered potassium transport in the loop in states of altered potassium balance may include changes in potassium recycling.

It is also well established that K^+-depletion impairs the urine concentrating mechanism [7, 8], but the relationship between changes in potassium balance and electrolyte and fluid transport, particularly with respect to potassium along the LOH, remains to be fully elucidated. We have chosen an experimental approach which minimized changes of delivery of potassium to the loop which might have occurred by alterations of glomerular filtration rate and potassium reabsorption along the proximal tubule. Accordingly, we undertook an *in vivo* microperfusion study of the LOH to examine the relationship between dietary potassium intake and sodium, potassium and fluid transport. Care was taken to match the concentration of potassium in the perfusate with the concentration expected in the lumen in free-flow in each dietary state.

Our renal clearance data confirm that fractional potassium excretion was decreased following dietary K^+-depletion and increased during K^+-adaptation. Renal sodium excretion was lower in K^+-depleted, but unchanged in high-K^+ rats. Data from microperfusion of the LOH show a significant increase in potassium absorption and a decline in sodium absorption in K^+-depleted animals and a decrease in the transport of sodium, potassium, and fluid in high-K^+ rats. Reduction of potassium concentration in the perfusate fluid in control rats lowered the absorption of sodium and potassium.

Methods

Preparation of animals

Experiments were carried out on a total of 32 male Sprague-Dawley rats grouped in cages at 21°C and maintained in controlled daylight. The animals received food until the time of the experiments and were anesthetized intraperitoneally with Inactin (Promonta, Germany) using a dose of 120 mg · kg^{-1} body wt. Following tracheotomy, they were placed in the right lateral position on a thermoregulated table (37°C) and prepared for micropuncture [9–11]. The right carotid artery was catheterized to record blood pressure and collect blood samples for measurements of hematocrit and plasma total CO_2, sodium and potassium concentrations. The left jugular vein was cannulated with PE-50 tubing for intravenous (i.v.) infusion via a syringe pump (Harvard Apparatus Co., Inc., S. Natick, Massachusetts, USA) of a Ringer-saline solution (125 mM NaCl + 25 mM $NaHCO_3$) at 4 ml · hr^{-1}. The left kidney was exposed through a flank incision, freed of

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perirenal fat and immobilized in a lucite cup with 3% Agar in 0.9% saline. The kidney was bathed with prewarmed (37°C) paraffin oil.

Microperfusion

We perfused LOH of superficial nephrons *in vivo* to measure sodium, potassium and fluid transport under conditions of constant flow rate and electrolyte delivery [9]. A perfusion pipette was inserted in the last surface loop of proximal tubule, a castor oil block placed upstream of the perfusion pipette and microperfusion started at 20 nl · min^{-1} with a thermally shielded microperfusion pump (Hampel, Frankfurt, Germany). Special care was taken to initiate perfusion of the loop from the very latest part of the proximal tubule and to collect fluid from the very earliest loop of the distal convoluted tubule. The perfusion solution contained the following (in mM): NaCl, 128; $NaHCO_3$, 13; $MgCl_2$, 1; NaH_2PO_4, 0.38; Na_2HPO_4, 1.62; FD&C blue dye (0.07 g%) and ^{14}C-inulin (12.5 μCi · ml^{-1}). In each group two different LOH perfusate solutions were used containing either 3.8 or 1.8 mM potassium to match the plasma potassium concentrations of control or low-K^+ diet rats, respectively. These potassium concentrations were chosen because free-flow micropuncture studies have uniformly shown that the end-proximal tubule fluid/plasma potassium concentration ratio remains close to unity under control, low- and high-K^+ conditions [9, 10]. Accordingly, it is reasonable to assume that the concentration of potassium in the tubule fluid entering the LOH is similar to the potassium concentration in the plasma of control and experimental animals. Net fluid flux (J_v) and net sodium (J_{Na}) and potassium (J_K) transport rates were measured in three groups of rats receiving: (a) control diet (NaCl 5 g · kg^{-1} and KCl 12 g · kg^{-1}); (b) potassium-free diet (NaCl 5 g · kg^{-1} and KCl 10 g · kg^{-1}) for at least three weeks; (c) high potassium diet (NaCl 5 g · kg^{-1} and KCl 100 g · kg^{-1}) for ten to fourteen days (Teklad diets, Madison, Wisconsin, USA). Transport data are expressed per individual loop (without normalization for perfused length) because it has been shown that the LOH of superficial tubules of the rat is a segment of essentially constant length (ca. 6 to 7 mm) [12].

Analytical methods

Tubule fluid sodium and potassium concentrations were measured by furnace atomic absorption spectrophotometry (Video 22, Instrumentation Laboratory, Lexington, Massachusetts, USA) using a modified dilution method [11]. Each sample analysis was bracketed by standards of known sodium and potassium concentrations. Plasma total CO_2 was measured by a CO_2 analyzer (Corning model 960). ^{14}C-inulin radioactivity was measured by liquid scintillation counting (Searle model P2, Chicago, Illinois, USA). Plasma sodium and potassium concentrations were measured by flame photometry (Corning model 480).

Renal clearance experiments

In a separate set of experiments we measured glomerular filtration rate (GFR) and renal sodium and potassium excretion rates in rats on the control and experimental diets. Experiments were done on three groups of male Sprague-Dawley rats (263.3 ± 10.1 g body wt). Animals were prepared as described for micropuncture above, but no flank incision was made and the bladder was catheterized suprapubically. All rats were given a priming dose of 20 μCi ^{3}H-inulin in 0.5 ml 0.9% saline, followed by a continuous infusion of ^{3}H-inulin at 20 μCi · hr^{-1} in Ringer-saline solution (125 mM NaCl + 25 mM $NaHCO_3$) solution at a rate of 4 ml · hr^{-1}, as described for microperfusion. After 45 minutes equilibration, the first of four 30-minute urine collections began. There was an interval of 60 minutes between the second and third urine collections. Carotid artery blood samples were taken at the start and end of each collection period.

Calculations and statistical analysis

In the micropuncture experiments, the perfusion rate *in vivo* was obtained from the rate of fluid collected from the early distal tubule, multiplied by the ratio of inulin concentrations in collected/perfused fluids (TF/P inulin). A sample collection was considered acceptable if the calculated perfusion rate was within 15% of the calibrated microperfusion pump rate. The perfusion pump was calibrated by timed collections of perfusion fluid delivered directly into counting vials for measurements of ^{14}C-inulin concentrations. Calculated J_v was the difference between perfusion and collection rates. J_{Na} and J_K were calculated from the amount delivered in the perfusion pipette minus the amount collected in the collection pipette.

Statistical analysis of the three diet groups was first by one-way analysis of variance and covariance (ANOVA and ANCOVA), followed by comparison with the relevant variable in the control diet group and appropriate perfusate potassium concentration by unpaired *t*-test. Because of small variations in potassium and sodium load to the LOH in the microperfusion experiments, the load values of potassium and sodium were used as a covariate in the ANCOVA of loop J_K and J_{Na}, respectively. In the renal clearance experiments, GFR (inulin clearance) and fractional excretions of sodium and potassium were calculated in the usual way. All data are given as mean ± SEM; $P < 0.05$ was considered statistically significant. (The number of observations of J_{Na} are slightly fewer than of J_K because it was not possible to measure sodium concentration in all tubule samples obtained.)

Results

Clearance experiments and renal function

Table 1A summarizes data on weights, plasma electrolytes and packed cell volume in control and experimental conditions. Results are from animals in which microperfusions of the LOH were carried out. Whereas no significant changes in plasma sodium concentration were observed, plasma potassium concentration was sharply reduced in low-K^+ animals. These animals also had elevated plasma bicarbonate concentration values, typical of the metabolic alkalosis that occurs in prolonged chronic K^+-depletion [13, 14]. We did not observe changes in plasma sodium or potassium concentrations in the high-K^+ animals. Moderately elevated plasma potassium levels were observed in another study of chronic hyperkalemia [15]. It should be noted that the animals in the latter study received a diet containing more potassium than ours and were also infused during the experiments with a high-K^+ solution.

Table 1B summarizes data from separate sets of experiments in which kidney function was evaluated in an identically treated group of control, low and high-K^+ animals. Glomerular filtration rate (GFR), plasma potassium concentration as well as absolute and fractional excretion rates of potassium were sharply reduced in hypokalemic animals. It should be noted that despite the

Table 1A. Body weight, plasma electrolytes (P[Na], P[K]), serum bicarbonate concentration ($P[HCO_3]$; total CO_2) and packed cell volume (PCV) in each diet treatment group (control, potassium-depletion–low-K and potassium-adaptation–high-K; 3 to 5 weeks and 10 to 14 days, respectively)

Group (diet)	N	Weight g	P[Na] mM	P[K] mM	$P[HCO_3]$ mM	PCV %
Control	21	251.9 ± 10.4	148.8 ± 1.5	4.09 ± 0.06	28.2 ± 0.7	48.8 ± 0.6
Low K	8	248.4 ± 12.6	146.6 ± 1.4	1.98 ± 0.04[a]	35.0 ± 0.9[a]	47.1 ± 1.3
High K	7	283.3 ± 18.3	151.1 ± 1.2	3.80 ± 0.08	28.1 ± 0.6	48.1 ± 2.0

Comparison with control by unpaired *t*-test; mean ± SEM.
N is the number of animals.
[a] $P < 0.01$

Table 1B. Representative renal clearance data in rats on control, low-potassium (K^+-depletion) and high-potassium (K^+-adaptation) diets

Group (Diet)	N	P[Na] mM	P[K] mM	GFR $ml \cdot min^{-1}$	$\mu l \cdot min^{-1}$	$U_{Na}V$ $\mu mol \cdot min^{-1}$	U_KV $\mu mol \cdot min^{-1}$	FE_{Na} %	FE_K %
Control	5	144.2 ± 1.4	4.17 ± 0.13	0.90 ± 0.07	15.3 ± 2.2	1.02 ± 0.21	0.50 ± 0.08	0.87 ± 0.21	13.1 ± 2.1
Low K	5	151.2 ± 1.8[b]	2.21 ± 0.05[a]	0.40 ± 0.01[a]	2.5 ± 0.6[a]	0.20 ± 0.07[a]	0.02 ± 0.01[b]	0.34 ± 0.13	1.8 ± 0.8[a]
High K	5	148.5 ± 0.8[b]	4.24 ± 0.19	0.82 ± 0.14	13.8 ± 3.6	0.95 ± 0.35	1.79 ± 0.19[a]	0.84 ± 0.28	67.0 ± 11.0[a]

Abbreviations are: P[Na] and P[K], plasma sodium and potassium concentrations, respectively; GFR, glomerular filtration rate; V, urine flow rate; $U_{Na}V$ and U_KV, FE_{Na} and FE_K, absolute and fractional excretion of sodium and potassium, respectively. GFR, V, $U_{Na}V$ and U_KV are given per 100 g body weight. Comparisons with control are by unpaired *t*-test; mean ± SEM. *N* is the number of animals.
[a] and [b] $P < 0.01$ and $P < 0.05$, respectively

Table 2A. Effect of chronic low (low-K^+; 3 to 5 weeks) potassium diet on loop of Henle potassium reabsorption (J_K)

Group (diet)	N	Collection $[K^+]$ mM	Perfusion rate $nl \cdot min^{-1}$	Collection rate $nl \cdot min^{-1}$	TF/P inulin	J_v $nl \cdot min^{-1}$	Potassium load $pmol \cdot min^{-1}$	$^*J_K$ $pmol \cdot min^{-1}$	FR_K %
Control	15	1.6 ± 0.2	19.7 ± 0.3	12.2 ± 0.3	1.62 ± 0.03	7.4 ± 0.2	35.4 ± 0.6	15.7 ± 2.2	44.5 ± 6.2
Low-K^+	23	1.2 ± 0.2	20.3 ± 0.3	11.8 ± 0.3	1.73 ± 0.05	8.4 ± 0.4	36.6 ± 0.6	22.7 ± 1.7[a]	61.8 ± 4.1[a]

Abbreviations are: TF/P inulin, tubule fluid to plasma inulin concentration ratio; J_v, fluid reabsorption; FR_K, fractional potassium reabsorption. Comparison with Control matched for perfusate K^+ concentration of 1.8 mM by one-way ANOVA ($^*J_K$; adjusted for potassium load as a covariate in the ANOVA); mean ± SEM; *N* is the number of tubules.
[a] $P < 0.05$

marked fall of GFR, proximal and distal transit times underwent no major changes. Mean value of proximal transit times were 8.4 ± 0.3 seconds on control and 11.8 ± 0.5 seconds in K-deprived rats. Distal transit times were 43.3 ± 1.1 seconds and 57.7 ± 2.3 seconds, respectively (both $P < 0.01$). Although different from control values, it is clear that tubule flow rates and nephron function was fairly well maintained. Walker, Shore and Shirley also reported only moderate changes in transit time in K-deficient rats [16].

Renal sodium excretion was also significantly reduced in rats on a low-K^+ diet. As expected, rats on a high-K^+ diet had adapted well as reflected by the observation that absolute and fractional potassium excretion rates were sharply elevated, yet plasma potassium concentration was almost identical to comparable values in control animals.

Potassium, sodium and fluid transport along the loop of Henle

Tables 2A, 2B, 3A, 3B, and Figures 1 and 2 indicate that derangements of potassium balance change the pattern of electrolyte transport along the LOH *in vivo.*

J_K, J_{Na} and J_v during perfusion with a solution containing 1.8 mM K^+

Data on electrolyte and fluid transport in perfusion studies with low potassium concentration in the perfusate are summarized in Tables 2A, 2B and Figure 1. The low-K^+ containing solution was chosen because it approximates the concentration of potassium in the tubule fluid that reaches the LOH in our group of K^+-depleted hypokalemic animals. Perfusion with such a low-K^+ solution in control and K^+-adapted rats provides further insights into the behavior of the loop with respect to sodium and potassium transport when it is exposed to different potassium loads in the lumen.

From inspection of Table 2A it is apparent that compared to control animals (J_K = 15.7 ± 2.2 pmol · min^{-1}) *potassium transport* was significantly enhanced in low-K^+ animals (22.7 ± 1.7 pmol · min^{-1}). In contrast, as shown in Figure 1, J_K fell in high-K^+ animals (9.4 ± 2.3 pmol · min^{-1}; $P < 0.05$). The change in potassium transport along the loop is also reflected by a similar directional change in fractional transport rate for potassium in low-K^+ rats (Table 2A).

Table 2B and Figure 1 summarize the results of perfusion experiments in which the effect of a low-K^+ diet on *sodium transport* was investigated. Whereas low-K^+ animals reabsorbed sodium at a slightly, but significantly, reduced rate (control J_{Na} 1.84 nmol · min^{-1}, low-K^+ 1.63 nmol · min^{-1}), no change in sodium transport was observed in high-K^+ animals (Fig. 1). The modest decline in J_{Na} in low-K^+ animals is associated with a similar fall in fractional sodium absorption (control FR_{Na} 70%, low-K^+ 59%).

Table 2B. Effect of chronic low and high (high-K^+) potassium diet on loop of Henle sodium reabsorption (J_{Na})

Group (diet)	N	Collection [Na^+] mM	Perfusion rate	Collection rate	TF/P inulin	J_v	Sodium load	$^*J_{Na}$	FR_{Na}
			$nl \cdot min^{-1}$			$nl \cdot min^{-1}$	$nmol \cdot min^{-1}$		%
Control	15	65.6 ± 3.5	19.7 ± 0.3	12.2 ± 0.3	1.62 ± 0.03	7.4 ± 0.2	2.64 ± 0.04	1.84 ± 0.06	69.7 ± 1.8
Low-K^+	26	88.7 ± 6.1[a]	20.3 ± 0.3	12.0 ± 0.3	1.72 ± 0.04	8.3 ± 0.4	2.72 ± 0.04	1.63 ± 0.10[b]	59.2 ± 3.3[b]

Abbreviations are: TF/P inulin, tubule fluid to plasma inulin concentration ratio; J_v, fluid reabsorption; FR_{Na}, fractional sodium reabsorption. Comparison with Control matched for perfusate K^+ concentration of 1.8 mM by one-way ANOVA ($^*J_{Na}$; adjusted for sodium load as a covariate in the ANOVA); data are mean ± SEM; *N* is the number of tubules.
[a] and [b], $P < 0.01$ and <0.05, respectively

Table 3A. Effect of chronic low and high (high-K^+) potassium diets on loop of Henle potassium reabsorption (J_K)

Group (diet)	N	Collection [K^+] mM	Perfusion rate	Collection rate	TF/P inulin	J_v	Potassium load	$^*J_K$	FR_K
			$nl \cdot min^{-1}$			$nl \cdot min^{-1}$	$pmol \cdot min^{-1}$		%
Control	48	2.4 ± 0.2	19.8 ± 0.3	11.0 ± 0.3	1.84 ± 0.05	8.7 ± 0.3	76.2 ± 3.2	50.0 ± 2.4	65.6 ± 2.9
High-K^+	29	2.4 ± 0.2	19.2 ± 0.3	11.8 ± 0.4	1.66 ± 0.06[b]	7.3 ± 0.4[a]	72.8 ± 1.2	44.0 ± 2.3[b]	60.6 ± 2.8

Abbreviations are: TF/P inulin, tubule fluid to plasma inulin concentration ratio; J_v, fluid reabsorption; FR_K, fractional potassium reabsorption. Comparison with Control matched for perfusate K^+ concentration of 3.8 mM by one-way ANOVA ($^*J_K$; adjusted for potassium load as a covariate in the ANOVA); data are mean ± SEM. *N* is the number of tubules.
[a] and [b] $P < 0.01$ and < or = 0.05, respectively

Table 3B. Effect of chronic low and high (high-K^+) potassium diets on loop of Henle sodium reabsorption (J_{Na})

Group (diet)	N	Collection [Na^+] mM	Perfusion rate	Collection rate	TF/P inulin	J_v	Sodium load	$^*J_{Na}$	FR_{Na}
			$nl \cdot min^{-1}$			$nl \cdot min^{-1}$	$nmol \cdot min^{-1}$		%
Control	45	61.8 ± 2.3	19.8 ± 0.3	11.0 ± 0.3	1.86 ± 0.06	8.8 ± 0.3	2.65 ± 0.04	2.01 ± 0.05	75.2 ± 1.5
High-K^+	29	64.0 ± 3.0	19.2 ± 0.3	11.8 ± 0.4	1.67 ± 0.06[b]	7.3 ± 0.4[a]	2.55 ± 0.05	1.76 ± 0.06[a]	69.7 ± 1.7[b]

Abbreviations are: TF/P inulin, tubule fluid to plasma inulin concentration ratio; J_v, fluid reabsorption; FR_{Na}, fractional sodium reabsorption. Comparison with Control matched for perfusate K^+ concentration of 3.8 mM by one-way ANOVA ($^*J_{Na}$; adjusted for sodium load as a covariate in the ANOVA); data are mean = SEM; *N* is the number of tubules.
[a] and [b] $P < 0.01$ and <0.05, respectively

We observed no significant changes in J_v in low-K animals compared with corresponding control values.

J_K, J_{Na} and J_v during perfusion with a solution containing 3.8 mM K^+

Perfusion of the LOH with solutions containing 3.8 mM potassium approximated the concentration of potassium in end-proximal tubule fluid in control and K^+-adapted animals. Table 3A and Figure 2 show that a small, but significant, fall in J_K occurred in the animals on a high-K^+ diet.

A modest reduction of J_v was observed in both low- and high-K^+ animals (Fig. 2). Compared with a control value of 8.7 ± 0.3 nl · min^{-1}, J_v fell to 6.9 ± 0.4 nl · min^{-1} in low-K^+ (Fig. 2), and to 7.3 ± 0.4 nl · min^{-1} in high-K^+ animals (Table 3A).

Table 3B and Figure 2 show comparable data of sodium absorption in control and high-K^+ animals. Similar to the reduction of J_v in low- and high-K^+ animals, J_{Na} also declined in low- and high-K^+ animals (Fig. 2). These changes in absolute sodium absorption correspond to similar alterations in fractional sodium reabsorption. Whereas 75.2 ± 1.5% of sodium were absorbed under control conditions, only 59.5 ± 3.1% of sodium were transported in low-K^+ animals ($P < 0.01$). The corresponding value in animals treated with a high-K^+ diet was 69.7 ± 1.7% (Table 3B).

Load-dependence of potassium transport

Inspection of Figures 1 and 2 shows that changes in perfusate potassium concentration from 1.8 to 3.8 mM significantly affect both sodium and potassium transport in all experimental groups. Comparing the left panels of Figures 1 and 2 shows that net potassium reabsorption is uniformly stimulated at the perfusate concentration of 3.8 mM. The concentration-dependent stimulation of potassium transport also resulted in elevated fractional reabsorption rates. Comparing potassium transport at low and high potassium concentrations in the perfusate, fractional potassium reabsorption increased from 44% to 66% in control animals, from 62% to 73% in low-K^+ animals and from 35% to 61% in K^+-adapted rats. Stimulation was observed not only in control, but also in low-K^+ and high-K^+ animals. We conclude from these data that potassium transport was not saturated at the lower potassium concentration in the perfusate.

Changing the perfusate potassium concentration in control animals also led to a small but significant enhancement of sodium absorption. At a potassium concentration in the perfusate of 1.8 mM J_{Na} was 1.84 ± 0.06 nmol · min^{-1} and the comparable value at a perfusate potassium concentration of 3.8 mM was 2.01 ± 0.05 nmol · min^{-1} ($P < 0.05$). No significant changes in sodium transport were observed in low-K^+ and high-K^+ animals when the

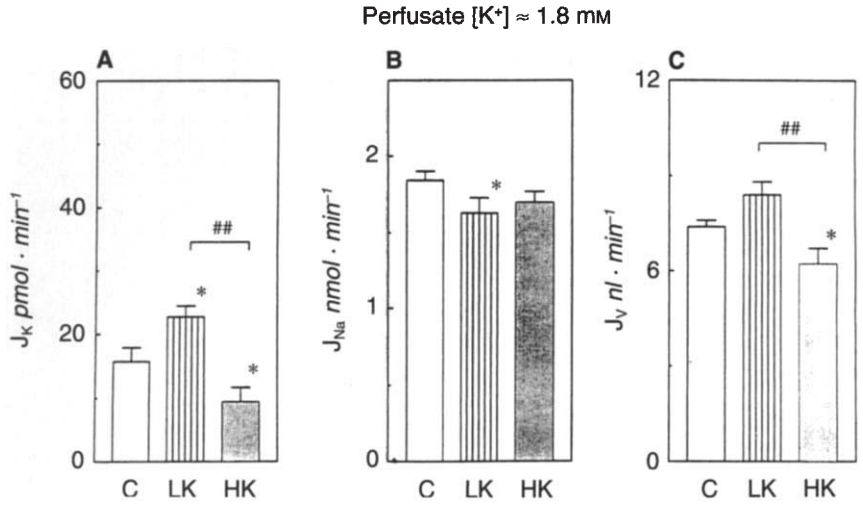

Fig. 1. *Perfusate potassium concentration 1.8 mM.* **A.** Potassium absorption (J_K) along the loop of Henle (LOH) perfused with 1.8 mM potassium in control (C), potassium depleted (LK) and potassium adapted (HK) rats. $^*P <$ 0.05 compared with C; $^{**}P < 0.01$ comparing LK vs. HK; data are mean ± SEM. **B.** Sodium absorption (J_{Na}) along the loop of Henle (LOH) perfused with 1.8 mM potassium in control (C), potassium depleted (LK) and potassium adapted (HK) rats. $^*P < 0.05$ compared with C; data are mean ± SEM. **C.** Fluid reabsorption (J_v) along the loop of Henle (LOH) perfused with 1.8 mM potassium in control (C), potassium depleted (LK) and potassium adapted (HK) rats. $^*P < 0.05$ compared with C; $^{**}P < 0.01$ comparing LK vs. HK; data are mean ± SEM.

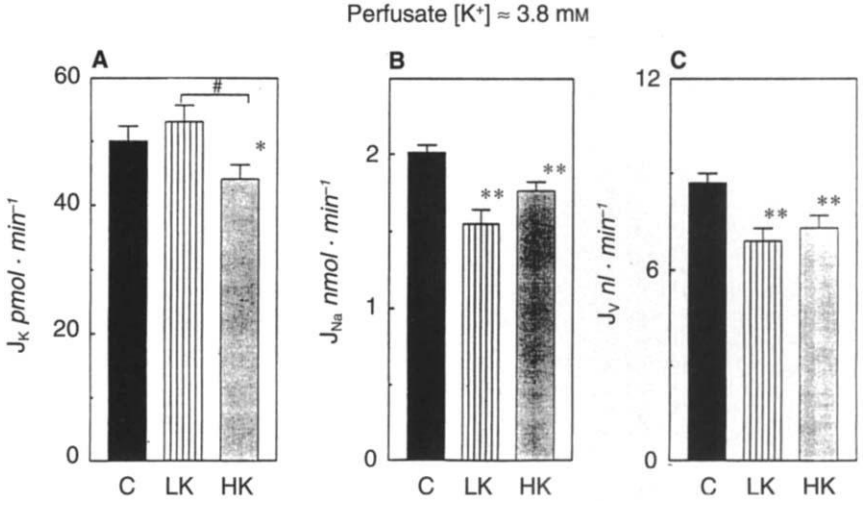

Fig. 2. *Perfusate potassium concentration 3.8 mM.* **A.** Potassium absorption (J_K) along the loop of Henle (LOH) perfused with 3.8 mM potassium in control (C), potassium depleted (LK) and potassium adapted (HK) rats. $^{**}P <$ 0.01 compared with C; $^*P < 0.05$ comparing LK vs. HK; $^*P = 0.05$ compared with C; data are mean ± SEM. **B.** Sodium absorption (J_{Na}) along the loop of Henle (LOH) perfused with 3.8 mM potassium in control (C), potassium depleted (LK) and potassium adapted (HK) rats. $^{**}P < 0.01$ compared with C; data are mean ± SEM. **C.** Fluid reabsorption (J_V) along the loop of Henle (LOH) perfused with 3.8 mM potassium in control (C), potassium depleted (LK) and potassium adapted (HK) rats. $^{**}P <$ 0.01 compared with C; data are mean ± SEM.

transport rates during perfusion with the different potassium concentrations were compared.

Discussion

Assessment of transport in the loop of Henle

The technique of continuous microperfusion of the LOH offers both advantages and disadvantages compared with studies on isolated segments of the loop *in vitro.* Perfusion of the LOH *in vivo* permits control of volume and composition of the fluid entering this nephron segment, and function is assessed during exposure of the loop to the renal interstitium, its physiological environment. While this approach permits the evaluation of the integrated response of the LOH to changes in potassium balance, it does not allow precise assignment of changes of potassium transport to individual tubule segments. The perfused nephron components include parts of the pars recta of the proximal tubule, the thin descending limb, the TAL and early portions of the distal convoluted tubule. While these segments may, to a variable extent, contribute to the overall transport of potassium along the loop, it is likely that a large portion of the net movement of potassium is controlled by two transport activities: reabsorption of potassium in the TAL and recycling of potassium. Thus, significant changes of potassium and sodium transport occur along the loop under conditions that induce similar changes of transport in the TAL. Representative examples include the drastic effects on electrolyte transport along the isolated TAL of several loop diuretics, hormones and cell messengers *in vitro,* effects that are closely mimicked by the integrated response of the LOH *in vivo* [17–19].

Several lines of evidence suggest that K^+-recycling is also modulated by changes in K^+-balance [6]. Potassium-recycling involves the addition of potassium from the interstitium to the tubule fluid in the descending limb of Henle's loop. Since the amount of potassium entering the interstitium is determined by the amount of potassium secreted by the initial and cortical collecting tubule, variations in secretion in high- and low-K^+ animals ultimately affect the rate at which potassium is added to the loop. Thus, it is likely that changes in external K^+-balance exert effects on the transport of potassium along the LOH by modifying the delivery to the loop from the interstitium in

addition to effects on specific tubule cell transport mechanisms, particularly in the TAL. However, it should be realized that the present perfusion studies involved short loops of Henle. Accordingly, it is safe to assume that potassium recycling contributed less to the observed changes in net potassium transport than in long loops of the nephron.

In a recent and careful study of the effects of K^+-depletion on reabsorption of chloride along the LOH, perfusion was carried out with low-sodium and mannitol-containing solutions designed to minimize fluid and ion transport in nephron segments upstream of the TAL [20]. This approach has the significant advantage of minimizing transport in these tubule segments, but may result in modification of the transport function of the TAL by the altered composition of the perfusion fluid. While we realize that using perfusion solutions approximating late proximal tubule fluid imposes limitations with respect to the precise location of the observed transport changes, we believe that the results obtained by this approach permit valid inferences concerning the TAL.

Sodium and potassium transport along the loop of Henle in vivo

The main findings of the present study are significant changes in net potassium and sodium transport along the LOH of cortical nephrons in conditions of altered potassium balance. Whereas changes in fluid, sodium and potassium delivery may vary significantly under free-flow conditions during changes in potassium balance, such factors cannot contribute to the observed alterations in transport because the present perfusion studies prevent such changes by keeping constant the volume and the composition of the fluid reaching the LOH.

Several factors may contribute to the observed changes in potassium transport following the alterations of potassium balance. The present view of the tubule mechanisms controlling potassium reabsorption along the LOH involves both passive and active mechanisms [19]. Concerning the pars recta of the proximal tubule and the thin descending limb of the loop, it is virtually certain that passive movement of potassium, either by diffusion or solvent drag, largely determines the rate of potassium transport [3, 19]. The extent to which changes in the transport pattern of these structures contribute to the observed effects of altered potassium balance in cortical short LOH is not known.

Net absorption of K^+ with Na^+ and two Cl^- ions along the TAL is ultimately driven by active exchange of three Na^+ for two K^+ ions across the basolateral membrane. It is the latter mechanism that generates the low cell sodium concentration, and indirectly, the low chloride concentration, which provide favorable electrochemical potential gradients for the entry of Na^+, K^+ and Cl^- ions across the apical membrane [17, 19, 20]. The results of our perfusion studies show that the cotransport mechanism responds to the increase of potassium concentration in the lumen with enhanced sodium and potassium absorption. We conclude that over the expected physiological range of potassium delivery saturation of transport has not been reached. It is also well established that a significant fraction of sodium in the thick ascending limb of rat kidneys is reabsorbed by exchange with hydrogen [2]. However, no changes in net bicarbonate absorption were observed in a perfusion study under similar conditions of potassium depletion [22].

The apical membrane is also the site of an important potassium channel which allows significant recycling of K^+ ions back into the lumen [5, 21]. It is the balance between cotransport-driven potassium absorption and passive diffusion, either secretory from cell to lumen via potassium channels, or reabsorptive through the paracellular pathway, that determines the rate of net potassium absorption. Changes of the apical potassium permeability are significantly affected by the potassium balance in the amphibian diluting segment which shares most transport functions with the mammalian TAL [23].

Effects of chronic hypokalemia

Several studies have provided strong evidence that K^+-depletion compromises LOH function [16, 20, 23–25]. There is general agreement that a negative potassium balance and low plasma potassium levels inhibit sodium and chloride reabsorption [23–25]. *In vitro* perfusion studies have confirmed that lowering perfusion fluid and bath potassium concentration below 5 mM reduces sodium absorption [26]. It is of interest that the transport defect of sodium reabsorption in hypokalemia can be alleviated by acute infusion of potassium [25]. A recent study in which the V_{max} of the hydrolytic activity of the Na-K, ATPase and ouabain-sensitive uptake of R6 were measured in hypokalemic rats showed no changes in cortical TAL [27]. These findings lend support to the interpretation that the reduction of plasma K is responsible for the reduction of transport of Na and K. Additional evidence showed that restoration of sodium transport was independent of aldosterone changes [20] and that luminal potassium delivery was not rate limiting [20, 25]. Taken together, these data support the view that the low plasma potassium levels in K^+-depletion compromise the activity of basolateral Na^+-K^+-ATPase. As a consequence of this transport impairment during K^+-deprivation, cells of the TAL may gain sodium and lose potassium.

The action of vasopressin on the cortical collecting tubule is similar to that on the TAL where it has been shown to stimulate sodium transport [28]. It is noteworthy that in the cortical collecting tubule stimulation by vasopressin of sodium absorption is significantly diminished in hypokalemia [29]. It is possible that hypokalemia also compromises the action of vasopressin on sodium transport in the TAL and may contribute to the reduction of sodium transport in the present setting of hypokalemia.

It should be noted that in the present studies urinary sodium excretion fell, despite inhibition of sodium transport along the LOH. This decline in sodium excretion may be mediated by the low GFR that was observed in the hypokalemic animals. In addition, overall sodium excretion may have been also reduced in K^+-depletion owing to enhanced reabsorption in more distally located tubule segments. Such stimulation was most likely initiated by volume depletion and high renin release which occurs in K^+-deficiency [30, 31]. Indeed serum aldosterone concentration rose in K^+-depleted animals (974 ± 158 pg · ml^{-1}; $N = 6$) to levels four times greater than those observed in animals on a normal potassium diet (230 ± 39 pg · ml^{-1}; $N = 14$; $P < 0.01$). As expected aldosterone was also sharply elevated in high-K^+ adapted rats (624 ± 91 pg · ml^{-1}; $N = 7$).

The high aldosterone levels in the present setting of K depletion deserve mention in view of the fact that several studies have shown reduced plasma aldosterone concentrations in low-K conditions [20, 32–34]. It is likely that low aldosterone levels occur more typically in mild to moderate states of K-depletion, usually achieved by shorter periods of K-deprivation. In contrast, when plasma K levels drop below 2.0 mEq/liter, severe K-depletion may be associated with volume depletion (low GFR and chloride loss)

and therefore activate aldosterone release. The latter would also be favored by the high renin levels that have been reported in K-deficiency [29, 30]. It is also of interest that in a study of hypokalemic metabolic alkalosis, associated with increased renin levels and reduced GFR levels, aldosterone levels in the plasma fail to decline despite low plasma K levels. Interestingly, plasma volume contraction was observed in these experiments [35].

It is also of interest that high prostaglandin levels have been observed in hypokalemia [36]. Prostaglandins have been shown to inhibit sodium transport in the TAL [36, 37] and they may contribute to the observed reduction of sodium transport.

Transport inhibition in the LOH explains diminished sodium absorption but fails to account for enhanced potassium transport from lumen to peritubular fluid during K^+-depletion. We propose the following sequence of events to account for enhanced potassium reabsorption. It is possible that basolateral inhibition of Na^+-K^+-ATPase activity induces a fall in cell pH because the expected increase in cell sodium concentration compromises apical Na^+-H^+ exchange. Also relevant are the findings that apical potassium channel activity in the TAL of rats is depressed by intracellular acidosis [21]. Accordingly, it is reasonable to postulate that the apical potassium conductance decreases in K^+-depletion so that backflux of potassium from cell to lumen diminishes. Evidence to support this thesis comes from observations in the *Amphiuma* early distal tubule in which the apical potassium permeability was proportional to potassium balance [38]. Potassium reabsorption in the TAL would be expected to fall if the decline of apical potassium channel activity were to exceed the inhibition of apical cotransport-mediated potassium absorption following inhibition of basolateral Na^+-K^+-ATPase activity.

An additional mechanism that is likely to contribute to the stimulation of apparent net potassium absorption along the LOH could be reduced recycling of potassium in hypokalemia [3]. A significant decrease in potassium concentration in vasa recta of hypokalemic rats was demonstrated by micropuncture studies [3, 6] and would be responsible for suppression of potassium entry into the descending limb of the loop and an apparent reduction in net LOH potassium absorption.

It should be noted that the present experiments were carried out under conditions in which the delivery of potassium was kept constant. Potassium absorption, compared at equal loads, was shown to increase in potassium depletion. However, it is important to note that in free-flow conditions, the delivery of potassium to the loop is sharply diminished owing to the decline in filtered load of potassium. Under these conditions, the absolute amount of potassium absorption in potassium depleted animals will decrease when compared to control animals in which a significantly larger load of potassium reaches the loop of Henle.

Effects of chronic potassium-loading

Sodium and fluid absorption along the LOH were reduced in K^+-adaptation and final urine excretion of potassium was increased. Inhibition of sodium absorption in the LOH following K^+-loading would provide excess fluid and sodium to the potassium secretory site in the initial and cortical collecting tubules and as a consequence, potassium secretion would be enhanced. In addition, as shown in the present study as well as in acute loading experiments [34, 38–40] diminished potassium absorption in the LOH provides a larger than normal fraction of potassium for excretion in the final urine.

The present studies show that the administration of a high-K^+ diet led not only to a fall of sodium absorption, but also of potassium absorption along the LOH. It has been proposed that high peritubule potassium concentrations, expected to be present in K-loaded animals [37], could depolarize the negative potential of the cells of the TAL and impede passive chloride exit from cell to peritubule fluid. Accordingly, cell chloride concentration would increase, lower the driving force for apical Na^+-$2Cl^-$-K^+ cotransport and thus ultimately reduce net sodium reabsorption.

Two factors may account for diminished potassium absorption along the LOH. An increase in apical potassium permeability, similar to that seen in the amphibian diluting segment in high-K^+ animals [41], would favor enhanced backflux of potassium from cell to lumen and together with diminished cotransporter activity, decrease net potassium absorption. Although we have not observed an increase in plasma potassium concentration, it is possible that the corticomedullary potassium concentration gradient is steeper in K^+-adapted animals, a condition likely to be associated with a raised interstitial potassium concentration [40, 42].

Similar to the situation in hypokalemic animals changes in potassium recycling could also contribute to the observed modulation of potassium transport in K loaded animals. A significant increase in the concentration of potassium would be expected to enhance potassium entry into the descending limb of the loop and be responsible for apparent reduction of net potassium absorption. It is presently not clear to what extent these changes in recycling contribute to the overall changes in net transport that were observed.

Conclusion

Potassium and sodium transport are significantly altered in states of K^+-deprivation and K^+-loading when care is taken to adjust the composition of perfusion fluid of the LOH to approximate late proximal tubule fluid. Whereas K^+-depletion stimulates potassium absorption and blocks Na^+ transport, chronic K^+-loading lowers potassium and sodium absorption. These changes are interpreted to involve alterations in the TAL in both basolateral Na^+-K^+-ATPase activity and apical potassium channel function. Altered potassium-recycling may also contribute to the changes in apparent net reabsorption of potassium.

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