PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 179, 96–100 (1985)

Urea Reabsorption along the Papillary Collecting Duct in Potassium-Deficient Rats (42069)

SARAH K. WEBSTER,[1] H. JOHN REINECK, AND JAY H. STEIN

Departments of Physiology and Medicine, University of Texas Health Sciences Center, San Antonio, Texas 78284

Abstract. These experiments were designed to evaluate the hypothesis that K^+ deficiency may be associated with decreased delivery of urea to the renal papillary collecting duct and/or decreased reabsorption of urea from the papillary collecting duct. Either of these factors would result in diminished capacity for urea recycling and might explain the mechanism of the urinary concentrating defect that is observed in K^+ depletion. Munich–Wistar rats were fed 25 ml of water and 12 g of normal (CON) or K^+-deficient (KD) diet each day for 21 days. Papillary collecting duct samples were obtained by micropuncture through the intact ureter. Fractional delivery of H_2O to the base and tip of the papillary collecting duct was increased in KD as compared to CON rats (1.50 ± 0.30% in KD vs 0.72 ± 0.09% in CON at the base, $P < 0.01$; and 0.55 ± 0.08% in KD vs 0.30 ± 0.05% in CON at the tip, $P < 0.01$). However, fractional delivery of urea to the base and tip of the papillary collecting duct was not different between KD and CON rats (26.9 ± 5.6% in KD vs 21.4 ± 3.3% in CON at the base, $P > 0.05$; and 12.4 ± 1.5% in KD vs 10.4 ± 1.4% in CON at the tip, $P > 0.05$). Furthermore, reabsorption of water or urea between the base and tip of the papillary collecting duct was not decreased in KD as compared to CON rats (water reabsorption was 57.8 ± 4.4% in KD and 55.9 ± 5.11% in CON and urea reabsorption was 45.0 ± 6.5% in KD and 45.9 ± 5.4% in CON, $P > 0.05$). These results demonstrate that water reabsorption, but not urea reabsorption, is impaired in renal tubules proximal to the accessible papillary collecting duct in hydropenic rats.

Potassium depletion induced by dietary potassium deprivation is associated with an inability to maximally concentrate urine in rats (1–3), dogs (4–7), and humans (8, 9). This concentrating defect occurs in association with decreases in the concentration of solutes in the medullary interstitium (3), which results in a decreased osmotic gradient for the reabsorption of water from the collecting duct to the interstitium during antidiuresis. One of the factors which may be responsible for the decreased inner medullary osmolality in K^+ depletion is the decreased delivery of urea to the papilla via juxtamedullary nephrons. In previous experiments, Whinnery and Kunau (10) found that papillary plasma flow is decreased in K^+-depleted rat kidneys. Assuming that this observation reflects a decrease in single nephron glomerular filtration rate and hence in delivery of solute to the medulla, this observation could at least partially explain the decrease in inner medullary tonicity.

In addition to the possible impairment of the delivery of urea to the renal papilla, reabsorption of urea along the papillary collecting duct and thus recirculation of urea in the renal medulla may be impaired in K^+ depletion. The current studies were designed to test these possibilities by measuring urea and water delivery to the base papillary collecting duct and reabsorption along the papillary collecting duct using micropuncture techniques.

Materials and Methods. Male Munich–Wistar rats weighing 130 to 170 g were fed 12 g of normal (CON) or K^+-deficient (KD) diet per day for 21 days. The diet contained 18% casein (ICN Nutritional Biochemicals, Cleveland, Ohio), 0.1% $CaHPO_4$, 0.06% NaCl, and 0.068% Na_2HPO_4 (in K^+-depleted group) or 0.083% K_2HPO_4 (in control group). The rats were restricted to 25 ml drinking water per day to prevent the effects of polydipsia. This dietary regimen resulted in an inability to maximally concentrate urine after 24 hr of water deprivation in K^+-depleted

[1] To whom reprint requests should be addressed: Department of Physiology and Biophysics, Mayo Clinic and Foundation, Rochester, Minn. 55905.

0037-9727/85 $1.50

rats (urine osmolality 1898 ± 63 mOsm/kg) as compared to control rats (2421 ± 83 mOsm/kg, $P < 0.02$), and a decrease in kidney potassium content (518 ± 16 mg/kg protein vs 764 ± 36 in CON) and skeletal muscle potassium content (447 ± 22 meq/kg protein vs 796 ± 24 in CON).

Rats were anesthetized with Inactin (100 mg/kg: Promonta, Hamburg, W. Germany) and placed on a thermoregulated table to maintain body temperature at 38°C. Polyethylene catheters were placed in the trachea for maintenance of airway, carotid artery for monitoring blood pressure and collecting blood samples, jugular vein for infusing solutions, and bladder for collecting right kidney urine samples. A left subcostal incision was made and the left kidney was gently separated from the adrenal gland and contiguous perirenal fat and placed in a Plexiglas, kidney-shaped cup. The surface was illuminated with a fiberoptic light source and the kidney was bathed with 38°C mineral oil equilibrated with isotonic saline to prevent dehydration and cooling. A catheter was placed in the left ureter for collection of left kidney urine samples.

Following a bolus injection of 30 μCi, [*Methoxy*-^{3}H]inulin (2.2 mCi/mmole, New England Nuclear, Boston, Mass.) in 0.1 ml isotonic saline, the rats were infused at the rate of 2 ml/hr with 4% albumin in 0.9% saline containing 20 μCi/ml [^{3}H]inulin. Animals with mean arterial pressures less than 100 mm Hg were discarded. After the infusion had been maintained for 1.5 to 2 hr, superficial fascia and perirenal fat were dissected away from the surface of the ureter. Samples were obtained through the ureter from collecting ducts at the base of the papilla, closest to the papillary cortical junction, and at the tip of the papilla, using sharpened micropipets with external tip diameters of 12–15 μm and containing mineral oil stained with Sudan black dye. After inserting the pipet into a base collecting duct, a small oil droplet was injected into the duct to verify the position of the pipet. After the oil droplet passed into the urine, the samples were collected at free flow rates, slow enough to prevent changes of fluid flow rates through the collecting duct. Collecting time ranged from 30 sec to 2 min for each sample of 10 to 50 nl. The distance between the collection sites was measured with an eyepiece micrometer and ranged from 2 to 3 mm. Urine from the right kidney was collected from the bladder catheter, and a blood sample was collected from the carotid catheter at the end of the collection period.

All tubular fluid samples were kept under oil and analyzed within 24 hr. All microsamples were analyzed for [^{3}H]inulin by transferring known volumes of 2–5 nl with calibrated constriction pipet into 10 μl distilled H_2O, transferred to 10 ml Biofluor (New England Nuclear) containing 0.5 ml H_2O and counted in a Beckman liquid scintillation counter (Model LS-7000). An additional aliquot, 2–5 nl, was diluted into 5 μl and assayed for urea in duplicate as described by Marsh *et al.* (11). Right kidney urine and plasma samples were analyzed for [^{3}H]inulin by counting 5 μl of sample in 10 ml Biofluor containing 0.5 ml H_2O in the Beckman liquid scintillation spectrometer; for urea by diluting 1:5000 and 1:50, respectively, and assaying in duplicate as described by Marsh *et al.* (11); for osmolality with a Wescor Vapor Pressure Osmometer (Wescor Model 5130B, Logan, Utah).

Urea was assayed in urine, plasma, and tubule samples as described by Marsh *et al.* (11) as modified from Beale and Croft (12). Plasma samples were diluted 1:50 and 5 μl was assayed. Urine and tubule samples were diluted by pipetting 2–5 nl with special calibrated pulled quartz pipets into 5 μl of H_2O. Seventy-five microliters of reagent containing 0.05% diacetylmonoxime (Eastman Co., Rochester, N.Y.), 0.001% glacial acetic acid, 0.005% *N*-phenylanthranilic acid (Eastman Co.), 0.05% $NaHCO_3$, 1% ethanol, 12.6% NaH_2PO_4, 1.6% $MnCl_2$, 1.6% HCl, and 0.32 m*M* Na_2NO_3 was added. The samples were incubated at 100°C for 11 min and read in a Beckman Model DU-8 spectrophotometer (Beckman Instruments, Palo Alto, Calif.) at 535 nm in a 2-mm wide cuvette. Concentration of urea in the samples was calculated from a linear standard curve with a range of 0.05 to 0.5 m*M* urea. Dilution of plasma samples from 1:25 to 1:100 and urine samples from 1:2000 to 1:5000 gave linear results.

Glomerular filtration rate (GFR) was determined by inulin clearance (C_{In}) according to the formula $(U/P)_{In} \times V$, where $(U/P)_{In}$ is

the ratio of urine to plasma inulin concentration and V is urinary flow rate. Fractional excretion of urea (FE_{urea}) was determined by clearance of urea (C_{urea})/clearance of inulin (C_{inulin}). Fractional delivery of urea (FD_{urea}) to the base or tip of the papilla was determined by tubule fluid to plasma urea concentration ($(TF/P)_{urea}$)/tubule fluid to plasma inulin concentration ($(TF/P)_{inulin}$). Percentage reabsorbed from base to tip was calculated as (FD to base) − (FD to tip)/(FD to base) × 100. Comparisons between CON and KD were evaluated using Student's t test for group comparisons. Changes from base to tip were evaluated using Student's t test for paired analysis.

Results. In order to evaluate the effect of K^+ depletion on the delivery and reabsorption of H_2O and urea from the papillary collecting duct, micropuncture samples were obtained from the renal papilla with the ureter intact.

As shown in Fig. 1, fractional delivery of water (FD_{H_2O}) which decreases from base to tip in CON and KD rats ($P < 0.05$) is significantly greater in KD than in CON rats ($P < 0.01$) at both the base and tip. These results demonstrate that delivery of H_2O to the base is greater, and hence reabsorption of H_2O from the tubule proximal to the papillary collecting duct is impaired in K^+ depletion.

Urea concentration at the base is significantly less in KD than CON rats ($P < 0.05$) and slightly less (but not statistically significant) at the tip ($P > 0.05$) (Fig. 2). This difference between control and K^+-depleted rats must be due almost entirely to the difference in H_2O reabsorption since fractional delivery of urea to the base and to the tip is not significantly different between CON and KD rats (Fig. 3). Furthermore, the percentage reabsorption of water and urea from base to tip is not different in KD as compared to CON rats ($P > 0.05$) (Fig. 4). These results demonstrate that water or urea reabsorption along the accessible papillary collecting duct is not impaired in K^+ depletion.

As shown in Table I, mean arterial pressure (MAP) was slightly less in the KD rats as compared to that in CON. Plasma K^+ concentration was significantly less, while plasma concentration of urea and plasma osmolality were not different in KD as compared to that in CON. GFR as measured by inulin clearance and urinary osmolality were less in

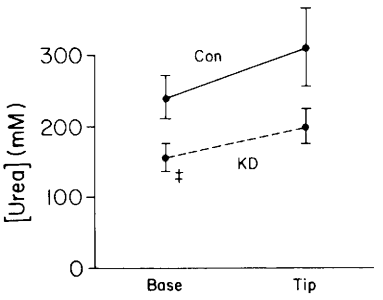

FIG. 2. Urea concentration of tubular fluid obtained from the base and tip of papillary collecting ducts of CON (solid line) and KD (dotted line) rats with the ureter intact. †$P < 0.05$ KD vs CON. $n = 15$ in CON; $n = 9$ in KD. Values are means ± standard errors.

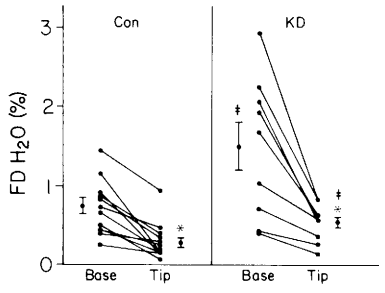

FIG. 1. Fractional delivery of water to the base and tip of papillary collecting ducts in CON and KD rats with the ureter intact. *$P < 0.05$ tip vs base, †$P < 0.01$ KD vs CON. $n = 15$ in CON; $n = 9$ in KD. Vertical lines depict means ± standard errors.

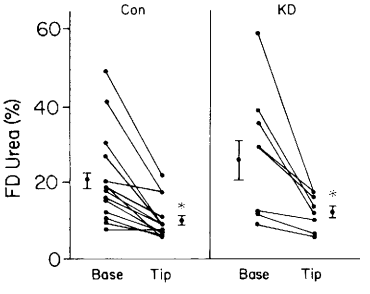

FIG. 3. Fractional delivery of urea to the base and tip of papillary collecting duct obtained with the ureter intact from CON and KD rats. *$P < 0.005$ base vs tip. $n = 15$ in CON; $n = 9$ in KD. Vertical bars depict means ± standard errors.

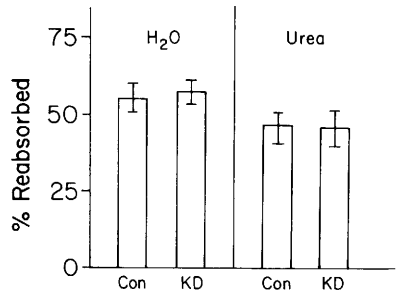

FIG. 4. Percentage of the water and urea delivered to the base of the papillary collecting duct that is reabsorbed from base to tip. N = 15 in CON; n = 9 in KD. Bars are means ± standard errors.

KD than CON. These results are consistent with those of other investigators (3, 13). Urinary flow rate (V) was not different between the two groups.

Discussion. A dietary regimen deficient in potassium, with restricted water intake to prevent the effects of polydipsia, resulted in the characteristic deficiency in maximal urinary concentrating ability in association with depression of muscle, kidney and serum potassium concentration in these Munich–Wistar rats.

According to the models of Stephenson (14) and Kokko and Rector (15), a series of processes are involved in the renal concentrating mechanisms. Therefore, the concentrating defect which occurs in the presence of potassium depletion could arise from any of several causes related to each of these processes. In the present series of studies, the possible impairment of delivery of urea to the papillary collecting duct and the ability to reabsorb urea along the collecting duct were directly evaluated.

Papillary plasma flow is decreased in K^+ depletion (10). Assuming that this observation reflects a relative decrease in single nephron glomerular filtration rate of juxtamedullary nephrons, it is probable that delivery of solute to the renal medulla may be compromised in K^+ depletion. Urea delivery to the renal papilla by way of the descending limbs of Henle's loop cannot be directly measured without removing the ureter, which impairs the concentrating mechanism (16).

The results obtained in these experiments demonstrated an impairment in reabsorption of water in renal tubules of potassium-depleted rats proximal to but not along the accessible papillary collecting duct. On the other hand, urea reabsorption was not significantly impaired. These results do not support the hypothesis that the delivery of urea to or the reabsorption of urea from the papillary collecting duct is impaired in potassium depletion. Rather, these results are consistent with the hypothesis that a decreased reabsorption of water, independent of urea, either from the descending limb of the loop of Henle or from the collecting duct proximal to the papillary collecting duct, or both, is impaired in K^+ depletion. These two possibilities are not mutually exclusive, and both may contribute to the concentrating defect in K^+ depletion.

Several factors, such as levels of vasopressin (17), catecholamines, and volume status, may contribute to the differences between the control and K^+-depleted rats. Thus, the mechanism of the impairment of H_2O reabsorption may be multifactorial. The present experiments do demonstrate that the concentration of urea at the base of the papillary collecting duct was decreased, possibly resulting in a decreased gradient for the reabsorption of urea from the collecting duct. Interstitial concentrations of urea were not evaluated in these experiments. Therefore, quantitation of these driving forces for urea cannot be determined. However, according to the models of Stephenson (14), and Kokko

TABLE I. CHARACTERISTICS OF CONTROL AND K-DEPLETED RATS DURING MICROPUNCTURE EXPERIMENTS

n:	Control 15	K depleted 9
MAP (mm Hg)	124 ± 2	115 ± 3**
P_K (meq/L)	4.04 ± 0.08	1.74 ± 0.09*
P_{osm} (mOsm)/kg)	306 ± 2	301 ± 6
P_{urea} (mM)	7.97 ± 0.63	8.33 ± 0.78
V (μl/min)	1.89 ± 0.26	2.38 ± 0.56
C_{Inulin} (ml/min)	0.78 ± 0.10	0.46 ± 0.07**
U_{osm} (mOsm/kg)	1161 ± 102	831 ± 84**

Note. Values are means ± SE. MAP, mean arterial pressure; P_K, plasma potassium; P_{osm}, plasma osmolality; P_{urea}, plasma urea; V, urinary excretion rate; C_{Inulin}, inulin clearance; U_{osm}, urinary osmolality.

* $P < 0.005$.

** $P < 0.05$.

and Rector (15), a decreased concentration of urea in the interstitium could result in a decreased gradient for NaCl reabsorption along the thin ascending limb, and hence a decreased gradient for water reabsorption in the descending limb of the loop of Henle.

The authors gratefully acknowledge the secretarial assistance of June M. Hanke. This study was supported by National Institutes of Health Grant AM 17387.

1. Beck N, Webster SK. Impaired urinary concentrating ability and cyclic AMP in K^+-depleted rat kidney. Amer J Physiol **231**:1204–1208, 1976.
2. Hollander W, Winters RW, Williams TF, Bradley J, Oliver J, Welt LG. Defect in the renal tubular reabsorption of water associated with potassium depletion in rats. Amer J Physiol **189**:557–563, 1957.
3. Manitius A, Levitin H, Beck D, Epstein FH. On the mechanism of impairment of renal concentrating ability in potassium deficiency. J Clin Invest **39**: 684–692, 1960.
4. Abbrecht PH. Effects of potassium deficiency on renal function in the dog. J Clin Invest **48**:432–442, 1969.
5. Bennett CM. Urine concentration and dilution in hypokalemic and hypercalcemic dogs. J Clin Invest **49**:1447–1457, 1970.
6. Giebisch G, Lozano R. The effects of adrenal steroids and potassium depletion on the elaboration of an osmotically concentrated urine. J Clin Invest **38**: 843–853, 1959.
7. Kunau, RT Jr. Function of ascending loop of Henle in the potassium deficient dog. Amer J Physiol **219**: 1071–1079, 1970.
8. Relman AJ, Schwartz WB. The kidney in potassium depletion. Amer J Med **24**:764–773, 1958.
9. Rubini ME. Water excretion in potassium deficient man. J Clin Invest **40**:2215–2224, 1960.
10. Whinnery MA, Kunau RT. Effect of potassium deficiency on papillary plasma flow in the rat. Amer J Physiol **237**:F226–F231, 1979.
11. Marsh DJ, Frasier C, Decter J. Measurement of urea concentration in nanoliter specimens of renal tubular fluid and capillary blood. Anal Biochem **11**:73–80, 1965.
12. Beale RN, Croft D. A sensitive method for the colorimetric determination of urea. J Clin Pathol **14**:48–424, 1961.
13. Siegel RR, Lotspeich WD. Potassium and sodium excretion and potassium homeostasis during acute hypokalemia. Amer J Physiol **208**:1143–1152, 1965.
14. Stephenson JL. Concentration of urine in a central core model of the renal counterflow system. Kidney Int **2**:85–94, 1972.
15. Kokko JP, Rector, FC Jr. Countercurrent multiplication system without active transport in inner medulla. Kidney Int **2**:214–223, 1972.
16. Chuang EL, Reineck HJ, Osgood RW, Kunau RT Jr, Stein JH. Studies on the mechanism of reduced urinary osmolality after exposure of the renal papilla. J Clin Invest **61**:633–639, 1978.
17. Rutecki GW, Cox JW, Robertson GW, Francisco LL, Ferris TF. Urinary concentrating ability and antidiuretic hormone responsiveness in the potassium-depleted dog. J Lab Clin Med **100**:53–60, 1982.

Received November 12, 1984. P.S.E.B.M. 1985, Vol. 179.
Accepted January 23, 1985.