

The Role of the Medullary Collecting Ducts in Postobstructive Diuresis

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ABSTRACT Medullary collecting duct function was studied by direct microcatheterization techniques in rats undergoing postobstructive diuresis. Significant net addition of water and sodium to the duct was demonstrated during postobstructive diuresis after relief of 24-h bilateral ureteral ligation. This striking abnormality in function was associated with reduced delivery of sodium and water to the collecting duct compared to sham-operated controls. To examine the role of circulating factors in this phenomenon, another group of rats was studied that underwent 24 h of total urine reinfusion into the femoral vein. Natriuresis and diuresis were similar to the postobstructive group, but absolute collecting duct reabsorption of sodium and water was normal. The natriuresis and diuresis in rats with urine reinfusion resulted from increased delivery of fluid and sodium to the medullary collecting duct. A third group of rats was studied with 24-h unilateral ureteral ligation as well as urine reinfusion from the contralateral normal kidney. Without urine reinfusion there was no diuresis-natriuresis but with urine reinfusion the diuresis and natriuresis after relief of unilateral obstruction was similar to that after relief of bilateral obstruction. Moreover, net addition of sodium and no significant water reabsorption were demonstrated in the medullary collecting duct of such animals.

The results indicate that (a) the medullary collecting duct is the critical nephron segment affected by ureteral obstruction, since postobstructive diuresis occurred despite reduced delivery of fluid from the more proximal

nephron; (b) the net addition of sodium to the medullary collecting duct observed during postobstructive diuresis is probably a direct effect of obstruction, since it was found during postobstructive diuresis after relief of bilateral or unilateral ureteral ligation, but not with urine reinfusion alone; and (c) blood-borne factors are important in the development of postobstructive natriuresis and diuresis, and probably act by increasing the fraction of filtered sodium and water delivered from the proximal and distal tubule to the collecting duct.

INTRODUCTION

Decreased salt and water reabsorption in the proximal, and particularly in the distal nephron, has been demonstrated during postobstructive diuresis by micropuncture methods (1-3). Impaired reabsorption in the collecting ducts and disproportionate impairment of function in the deep as compared to the superficial nephrons of the post-obstructive kidney have also been suggested indirectly by these studies. Blood-borne factors, including accumulation of urea, probably have a major role in producing postobstructive diuresis, as indicated by recent urine reinfusion (4) and cross-circulation experiments (5).

The importance of the collecting ducts in determining urinary excretion of salt and water during saline-induced natriuresis has recently been demonstrated by Sonnenberg (6, 7), using a method of direct cannulation of the medullary collecting ducts (8) in the rat. This technique measures the contribution of both superficial and deep nephrons to the final urine, thus eliminating any changes due to abnormalities in distribution of nephron function. Accordingly, it was decided to directly study collecting duct function in the postobstructive kidney after relief of bilateral or unilateral ureteral ligation, and to further examine the role of circulating natriuretic factors in postobstructive diuresis.

The results indicate that the marked diuresis-natriuresis after relief of bilateral ureteral ligation is associated

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with net addition of sodium and water to the medullary collecting duct, presumably due to decreased reabsorption and increased back diffusion. An increase in the fraction of filtered sodium remaining along the collecting duct of the postobstructive kidney was found despite reduced absolute delivery of fluid to the duct. Unilateral ureteral ligation with urine reinfusion from the contralateral kidney produced a similar diuresis-natriuresis and net addition of sodium to the medullary collecting duct. Total urine reinfusion for 24 h also resulted in a marked diuresis-natriuresis but was associated with increased delivery of fluid to the collecting duct and no intrinsic abnormality in duct function.

We conclude (a) that the medullary collecting duct is the critical nephron segment affected by ureteral obstruction; (b) that the net addition of sodium to the collecting duct during postobstructive diuresis is probably a direct effect of obstruction, and (c) that circulating factors are important in the development of post-obstructive diuresis and probably act by decreasing fractional reabsorption in the nephron proximal to the collecting duct.

METHODS

Male Sprague-Dawley rats (weight range 202–278 g) were maintained on Purina lab chow (Ralston Purina Co., St. Louis, Mo.) and water ad lib. 24 h before an experiment, animals were lightly anesthetized with pentobarbital and subjected to one of four surgical procedures as follows: sham operation (group I), both ureters were exposed but not ligated through a midline suprapubic incision; bilateral ureteral ligation (group II), both ureters were ligated through a midline suprapubic incision; urine reinfusion (group III), the bladder was cannulated through a midline incision and the catheter was passed through the body wall in the groin area and inserted into a femoral vein; unilateral ureteral ligation with urine reinfusion (group IV), the left ureter was ligated, and a bladder catheter connected to a femoral vein as in group II to allow reinfusion of urine from the right, nonobstructed kidney. Rats with unilateral ureteral ligation were also prepared but insufficient urine flow was obtained from left kidney to conduct satisfactory collecting duct studies. Subsequently, animals of the four groups were not allowed food or water and lost 6–10% of body weight before the experiment.

24 h after surgical preparations, rats were anesthetized with Inactin (Promonta, Hamburg, W. Germany) (10 mg/100 g body wt, i.p.), a tracheostomy was performed, and a jugular vein and femoral artery were cannulated for infusion and for blood pressure measurement and sampling, respectively. After a priming dose of 1.5 ml of Ringer's solution, a constant intravenous infusion (1.5 ml/h) was maintained. The left kidney was exposed and mobilized through a flank incision, and placed in a Lucite cup. The left ureter was cannulated and incised on its upper surface as close as possible to the pelvis to expose the papilla tip. Continuous gentle suction on the ureteral catheter was used to collect urine, with the sides of the opened ureter forming a small leakproof "well". This method of urine collection has been previously shown not to be associated with significant loss of urine or aspiration of tissue fluid (6). [³H]Inulin was now added to the constant infusion to deliver 150

μCi over the course of the experiment. A 40-min equilibration was allowed before beginning the collection of urine and samples of collecting duct fluid. In both groups III and IV the patency of the ureterovenous shunt was confirmed before and after the experiment by injection of small volumes of dyed saline into the reinfusion line. Several rats could not be used for experiments because urine flow in the reinfusion line had stopped during the previous 24 h. However, cessation of urine flow in the bladder-femoral vein (reinfusion) line did not occur during any of the experiments. Urine was collected from the left kidney for 7–10 consecutive 20-min periods and arterial blood samples (0.05 ml) were taken at the midpoint of each period.

The technique of sampling of collecting duct fluid was as described previously (6, 7). Briefly, fine polyethylene catheters (outside diameter 16–40 μm) were inserted to varying distances into different collecting ducts via the previously exposed papilla tip. It was occasionally possible to obtain paired samples in the same collecting duct system from near the papillary tip and deep in the medulla. In each animal an average of 12 samples of fluid (range 5–16) were obtained with controlled suction just sufficient to overcome the tip resistance of the catheter (6). Previous experiments have shown that deliberate alterations in flow dynamics in the catheterized duct, by increasing or decreasing suction, could produce detectable changes in reabsorption, but such changes were not statistically significant and did not obscure net sodium and water secretion in the medullary duct during intravenous fluid loading (7). The depth of insertion of the catheter was measured during withdrawal with a micrometer and was related to medullary length obtained from a post-mortem sagittal section of the kidney.

Sodium and potassium concentrations in plasma and urine were determined by flame photometry and [³H]inulin determination was by liquid scintillation counting in a toluene-based scintillant. Urinary excretion of sodium ($U_{Na}V$) and potassium (U_KV) were calculated, as was glomerular filtration rate. Sodium and potassium concentration in portions (10 nl) of tubular fluid were determined in an Aminco helium glow photometer (American Instrument Co., Travenol Laboratories Inc., Silver Spring, Md., and [³H]inulin (30 nl) by liquid scintillation counting. A Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, N. Y.) was used to measure total solute concentration in tubular fluid and urine samples. For each tubular fluid sample, the fluid to plasma concentration ratio of inulin (TF/P_{in}) was calculated, as were the fractions of filtered sodium ($[TF/P_{Na}]/[TF/P_{in}]$) and potassium ($[TF/P_K]/[TF/P_{in}]$) remaining at the collection site. Linear regression and *t* test analyses were used for statistical evaluation of data.

RESULTS

Clearance data. The clearance results and the excretion of solute, sodium, potassium, and water in the four experimental groups are shown in Table I. Glomerular filtration rate was markedly decreased in the two postobstructive groups (groups II and IV, bilateral ureteral ligation and unilateral ureteral ligation with urine reinfusion), when compared to the sham-operated controls (group I) and to the urine-reinfused rats (group III). Urine flow rate and sodium excretion rate were similarly increased in groups II, III, and IV, fractional sodium excretion being significantly higher in the

TABLE I
Clearance Results, Water, Electrolyte Excretion, and Plasma Data in Experimental Groups

| | GFR* | V | U _{Na} V | TRF _{Na} | U _K V | TRF _K | U/P _{In} | U _{osm} | BP | P _{Na} | P _K | Hct |
|---|---------------|---------------|-------------------|-------------------|------------------|------------------|-------------------|------------------|-------|-----------------|----------------|------|
| | ml/min gkw | μl/min gkw | neq/min gkw | | neq/min gkw | | | mosmol/ liter | mm Hg | meq/liter | meq/liter | % |
| Group I, sham-operated (n = 7) | | | | | | | | | | | | |
| Mean | 0.94 | 6.45 | 367 | 0.0027 | 437 | 0.108 | 161 | 673 | 118 | 143 | 4.3 | 44.4 |
| SE | 0.04 | 0.91 | 106 | 0 | 83 | 0.02 | 20 | 34 | 3 | 0.7 | 0.05 | 0.6 |
| Group II, bilateral ureteral ligated (n = 5) | | | | | | | | | | | | |
| Mean | 0.16 | 34.9 | 3,801 | 0.153 | 1,258 | 1.04 | 4.77 | 448 | 131 | 151 | 7.6 | 44.9 |
| SE | 0.02 | 5.7 | 668 | 0.015 | 207 | 0.10 | 9.28 | 9 | 2 | 2 | 0.2 | 0.3 |
| Group III, urine reinfusion (UR, n = 5) | | | | | | | | | | | | |
| Mean | 0.56 | 37.8 | 2,680 | 0.030 | 2,966 | 1.08 | 15.0 | 641 | 117 | 155 | 5.0 | 43.1 |
| SE | 0.03 | 3.2 | 655 | 0.006 | 121 | 0.03 | 0.9 | 64 | 5 | 3 | 0.2 | 2.1 |
| Group IV, unilateral ureteral ligation and urine reinfusion (n = 5) | | | | | | | | | | | | |
| Mean | 0.12 | 26.7 | 2,720 | 0.164 | 1,049 | 1.07 | 4.35 | 414 | 121 | 153 | 8.2 | 43.5 |
| SE | 0.02 | 3.9 | 397 | 0.017 | 187 | 0.08 | 0.41 | 6 | 4 | 2 | 0.2 | 1.0 |
| Statistical significance: | | | | | | | | | | | | |
| I:II | <0.001 | <0.001 | <0.001 | <0.001 | <0.01 | <0.001 | <0.001 | <0.02 | <0.05 | <0.01 | <0.001 | NS |
| I:III | <0.001 | <0.001 | <0.01 | <0.01 | <0.001 | <0.001 | <0.001 | NS | NS | <0.01 | <0.02 | NS |
| I:IV | <0.001 | <0.001 | <0.01 | <0.001 | <0.01 | <0.001 | <0.001 | <0.01 | NS | <0.01 | <0.001 | NS |
| II:III | <0.001 | NS | NS | <0.001 | <0.001 | NS | <0.001 | <0.05 | <0.05 | NS | <0.001 | NS |
| III:IV | <0.001 | NS | NS | <0.001 | <0.001 | NS | <0.001 | <0.01 | NS | NS | <0.001 | NS |

* GFR, glomerular filtration; V, urine flow rate; U_{Na}V, U_KV, sodium and potassium excretion rate; TRF_{Na}, TRF_K, tubular rejection fraction sodium and potassium; U/P_{In}, urine to plasma inulin concentration ratio; U_{osm}, urine osmolality; BP, arterial blood pressure; P_{Na}, P_K, plasma sodium and potassium; Hct, hematocrit.

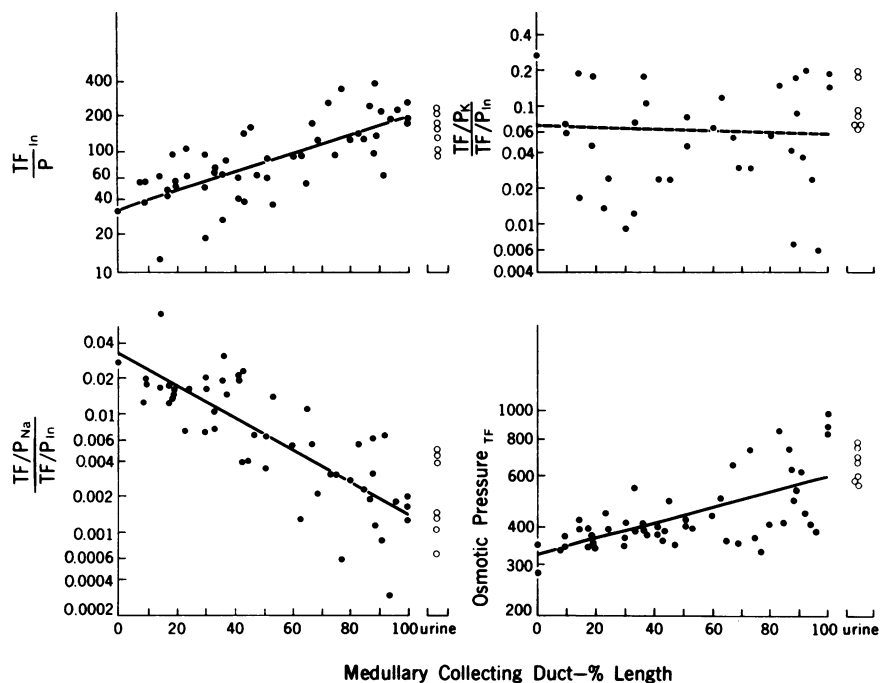


FIGURE 1 Change in collecting duct fluid with medullary length in sham-operated normal rats, showing tubular fluid to plasma inulin concentration ratio (TF/P_{In}), fraction of filtered sodium remaining [(TF/P_{Na})/(TF/P_{In})], fraction of filtered potassium remaining [(TF/P_K)/(TF/P_{In})], and osmotic pressure of tubular fluid (TF). Average urinary values from experimental kidneys of individual animals in each group are given (open circles). Regression lines of log of concentration ratio with length are indicated.

two postobstructive groups. Potassium excretion was higher in the urine-reinfused group III (with a higher glomerular filtration rate), while fractional potassium excretion was similar in groups II–IV. Urine osmolality and urine-to-plasma inulin concentration ratio were significantly lower in both of the postobstructive groups (II and IV). The results of measurements of mean blood pressure, serum sodium and potassium concentration, and hematocrit in the four groups are also shown in Table I.

Medullary collecting duct function. The results of determinations of tubular fluid to plasma concentration ratios of inulin, sodium and potassium, and of tubular fluid osmolality in collecting duct samples from the sham-operated control rats (group I) are shown in Fig. 1 and do not differ from results in previously studied hydropenic controls (6). The tubular fluid-to-plasma inulin concentration ratios along the length of the medullary collecting duct in the other three groups (II–IV) are compared in Fig. 2. There was significant fluid reabsorption along the collecting duct in the sham-operated (group I) or urine-reinfused (group III) rats. In the postobstructive groups there was slight addition of fluid (group II) or no change (group IV). Analysis of slopes of regression lines for the four groups (Table II) indicates a significant difference between the

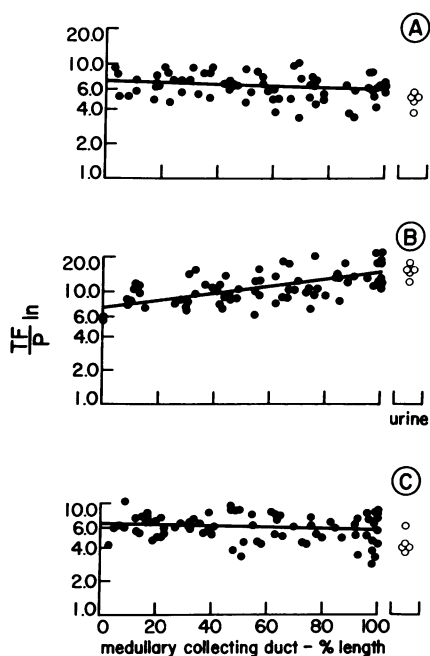


FIGURE 2 Change of collecting duct fluid to plasma concentration ratio of inulin with medullary length in rats studied. A, group II, after relief of 24-h bilateral ureteral ligation; B, group III, after 24-h of total urine reinfusion intravenously; C, group IV, after 24-h unilateral ureteral ligation with urine reinfusion from the contralateral normal kidney. Symbols and explanations as in Fig. 1.

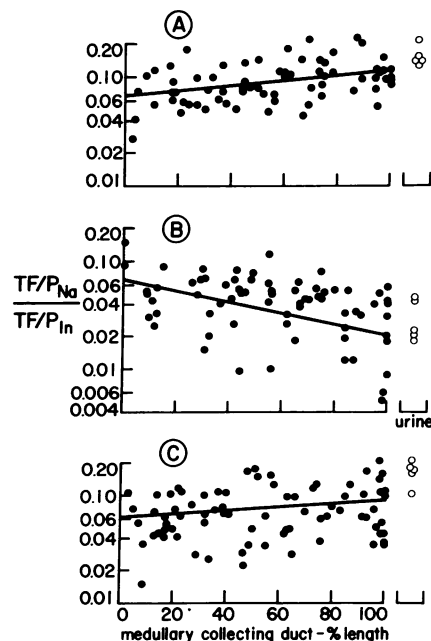


FIGURE 3 Fraction of filtered sodium remaining at different lengths of medullary collecting duct in groups II, III, and IV (A, B, and C). Symbols and explanations as in Fig. 1.

sham-operated or urine-reinfused groups and both post-obstructive groups ($P < 0.01$). The results in individual rats were consistent with the results for the group, in that the slope of the regression line for water reabsorption was negative in five out of five and four out of five postobstructive rats (groups II and IV, respectively), while the slope was positive in all seven sham-operated and all five urine-reinfused animals.

The fraction of filtered sodium remaining along the collecting duct (sodium to inulin tubular fluid to plasma concentration ratio) is shown in Figs. 1 and 3 and indicates continued reabsorption in sham-operated and urine-reinfused rats (groups I and III). However, there was a significant increase in the fraction of filtered sodium remaining along the collecting duct in the postobstructive groups. The changes in sodium reabsorption in individual rats were consistent with the results for the groups, in that five out of five and four out of five post-obstructive rats (group II and IV, respectively) showed an increase in the fraction of filtered sodium remaining, while all seven sham-operated and four of the five urine-reinfused rats showed continuing reabsorption along the duct.

Paired collecting duct samples, obtained in random order, were available from near the beginning and from near the papilla tip of the same duct system in several animals of each experimental group (Table III). In paired samples from group II, the mean $(TF/P_{Na}) / (TF/P_{In})$ increased from 0.078 ± 0.011 (1 SEM) at the

TABLE II
Regression Lines and Statistical Significance for Collecting Duct Data

| Group | | <i>r</i> | <i>P</i> | | | |
|--|--|----------|----------|--------|-------|--------|
| Group I, sham | $\ln \text{TF}/\text{P}_{\text{In}} = 1.23 + 0.01788(\%1)$ | 0.730 | <0.01 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{Na}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.15 - 0.03038(\%1)$ | 0.803 | <0.01 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{K}}}{\text{TF}/\text{P}_{\text{In}}} \times 10 = -0.381 - 0.00186(\%1)$ | 0.319 | NS | | | |
| | $\text{TF}_{\text{osm}} = 294 + 3.38(\%1)$ | 0.616 | <0.01 | | | |
| Group II, BUL | $\ln \text{TF}/\text{P}_{\text{In}} = 1.94 - 0.00205(\%1)$ | 0.237 | <0.05 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{Na}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.86 + 0.00577(\%1)$ | 0.404 | <0.01 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{K}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.85 + 0.00330(\%1)$ | 0.319 | <0.01 | | | |
| | $\text{TF}_{\text{osm}} = 414 + 0.49(\%1)$ | 0.300 | <0.05 | | | |
| Group III, UR | $\ln \text{TF}/\text{P}_{\text{In}} = 2.03 + 0.00605(\%1)$ | 0.574 | <0.01 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{Na}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.87 - 0.00930(\%1)$ | 0.413 | <0.01 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{K}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.93 + 0.00287(\%1)$ | 0.406 | <0.01 | | | |
| | $\text{TF}_{\text{osm}} = 459 + 1.50(\%1)$ | 0.579 | <0.01 | | | |
| Group IV, UUL + UR | $\ln \text{TF}/\text{P}_{\text{In}} = 1.86 - 0.00158(\%1)$ | 0.184 | NS | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{Na}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.72 + 0.00335(\%1)$ | 0.213 | <0.05 | | | |
| | $\ln \frac{\text{TF}/\text{P}_{\text{K}}}{\text{TF}/\text{P}_{\text{In}}} \times 100 = 1.71 + 0.00342(\%1)$ | 0.382 | <0.01 | | | |
| | $\text{TF}_{\text{osm}} = 398 + 0.25(\%1)$ | 0.254 | <0.05 | | | |
| <i>Difference between Slopes</i> | | | | | | |
| | Group I:II | I:III | I:IV | II:III | II:IV | III:IV |
| $\ln \text{TF}/\text{P}_{\text{In}}$ | <0.01 | <0.01 | <0.01 | <0.01 | NS | <0.01 |
| $\ln (\text{TF}/\text{P}_{\text{Na}}/\text{TF}/\text{P}_{\text{In}}) \times 100$ | <0.01 | <0.01 | <0.01 | <0.01 | NS | <0.01 |
| $\ln (\text{TF}/\text{P}_{\text{K}}/\text{TF}/\text{P}_{\text{In}}) \times 100$ | NS | NS | NS | NS | NS | NS |
| TF_{osm} | <0.01 | <0.01 | <0.01 | <0.01 | NS | <0.01 |

beginning to 0.120 ± 0.014 at the end of the duct ($P < 0.01$). The sodium concentration of collecting duct fluid, as indicated by the tubular fluid-to-plasma sodium concentration ratio, also increased from 0.50 ± 0.04 to 0.62 ± 0.03 in bilateral uretal-ligated rats ($P < 0.05$). Conversely in sham-operated rats (group I) or rats with 24-h urine reinfusion (group III), the fraction of filtered sodium remaining decreased normally along the duct, from 0.018 ± 0.007 to 0.004 ± 0.001 and from 0.064 ± 0.011 to 0.032 ± 0.006 , respectively. The sodium concentration showed similar changes. In unilateral obstruction

with urine reinfusion (group IV), the paired samples showed an increase in the fraction of filtered sodium remaining and in the sodium concentration which, however, did not achieve statistical significance ($n = 11$, $P < 0.10$).

Fractional potassium reabsorption along the collecting duct is shown in Fig. 4. No change was noted in sham-operated normal rats, while similar net addition of potassium to collecting duct fluid occurred in the other three groups, with the regression lines as indicated in Table II.

TABLE III
Tubular Fluid to Plasma Sodium Concentration Ratio and Fraction of Filtered Sodium Remaining in Paired Samples from the Same Collecting Duct System

| | Early | | | Late | | | Early | | | Late | | |
|-------------------------------|--|-------------------------------|----|--------------------|-------------------------------|-----|--|-------------------------------|----|--------------------|-------------------------------|-----|
| | TF/P _{Na} | $\frac{TF/P_{Na}}{TF/P_{In}}$ | %1 | TF/P _{Na} | $\frac{TF/P_{Na}}{TF/P_{In}}$ | %1 | TF/P _{Na} | $\frac{TF/P_{Na}}{TF/P_{In}}$ | %1 | TF/P _{Na} | $\frac{TF/P_{Na}}{TF/P_{In}}$ | %1 |
| Group I (sham) | | | | | | | Group II (bilateral obstruction) | | | | | |
| | 0.67 | 0.0041 | 45 | 0.86 | 0.0032 | 73 | 0.51 | 0.074 | 18 | 0.57 | 0.110 | 96 |
| | 0.32 | 0.0036 | 51 | 0.19 | 0.0009 | 91 | 0.36 | 0.050 | 30 | 0.55 | 0.096 | 88 |
| | 0.91 | 0.0729 | 14 | 0.17 | 0.0012 | 89 | 0.41 | 0.056 | 11 | 0.60 | 0.110 | 78 |
| | 1.28 | 0.0134 | 18 | 0.51 | 0.0020 | 87 | 0.49 | 0.061 | 18 | 0.46 | 0.054 | 95 |
| | 1.23 | 0.0142 | 37 | 0.64 | 0.0065 | 88 | 0.70 | 0.076 | 31 | 0.63 | 0.078 | 94 |
| | 1.16 | 0.0191 | 41 | 0.45 | 0.0070 | 92 | 0.40 | 0.055 | 27 | 0.71 | 0.101 | 75 |
| | 1.06 | 0.0168 | 14 | 0.38 | 0.0029 | 80 | 0.82 | 0.178 | 23 | 0.82 | 0.227 | 87 |
| | 0.58 | 0.0040 | 43 | 0.22 | 0.0006 | 77 | 0.54 | 0.098 | 27 | 0.55 | 0.085 | 74 |
| | 0.72 | 0.0126 | 8 | 0.62 | 0.0112 | 65 | 0.51 | 0.062 | 35 | 0.52 | 0.067 | 74 |
| | | | | | | | 0.63 | 0.128 | 37 | 0.70 | 0.201 | 89 |
| | | | | | | | 0.52 | 0.103 | 8 | 0.70 | 0.143 | 66 |
| | | | | | | | 0.34 | 0.041 | 4 | 0.61 | 0.137 | 75 |
| | | | | | | | 0.26 | 0.027 | 3 | 0.62 | 0.150 | 97 |
| Mean | 0.88 | 0.0178 | 30 | 0.45 | 0.0039 | 82 | 0.50 | 0.078 | 21 | 0.62 | 0.120 | 84 |
| ±SE | 0.11 | 0.0071 | 5 | 0.08 | 0.0010 | 3 | 0.04 | 0.011 | 3 | 0.03 | 0.014 | 3 |
| $\frac{TF/P_{Na}}{TF/P_{In}}$ | <i>P</i> < 0.10 (<i>n</i> = 9) | | | | | | <i>P</i> < 0.01 (<i>n</i> = 13) | | | | | |
| TF/P _{Na} | <i>P</i> < 0.01 | | | | | | <i>P</i> < 0.05 | | | | | |
| | Group III (24-h urine reinfusion) | | | | | | Group IV (unilateral obstruction with urine reinfusion) | | | | | |
| | 0.57 | 0.070 | 31 | 0.52 | 0.048 | 74 | 0.64 | 0.169 | 48 | 0.53 | 0.097 | 100 |
| | 0.50 | 0.068 | 30 | 0.43 | 0.053 | 85 | 0.36 | 0.044 | 62 | 0.35 | 0.044 | 99 |
| | 0.45 | 0.052 | 9 | 0.50 | 0.053 | 56 | 0.30 | 0.041 | 22 | 0.36 | 0.056 | 99 |
| | 0.44 | 0.051 | 46 | 0.52 | 0.040 | 95 | 0.18 | 0.028 | 29 | 0.35 | 0.050 | 97 |
| | 0.21 | 0.015 | 31 | 0.25 | 0.012 | 84 | 0.37 | 0.056 | 32 | 0.43 | 0.061 | 93 |
| | 0.30 | 0.026 | 42 | 0.16 | 0.009 | 100 | 0.31 | 0.041 | 17 | 0.66 | 0.152 | 57 |
| | 0.64 | 0.089 | 15 | 0.10 | 0.006 | 99 | 0.26 | 0.029 | 47 | 0.38 | 0.062 | 92 |
| | 0.84 | 0.148 | -2 | 0.10 | 0.005 | 98 | 0.57 | 0.109 | 23 | 0.50 | 0.097 | 63 |
| | 0.56 | 0.057 | 13 | 0.61 | 0.050 | 56 | 0.39 | 0.064 | 55 | 0.55 | 0.165 | 93 |
| | 0.47 | 0.041 | 37 | 0.54 | 0.044 | 70 | 0.40 | 0.047 | 50 | 0.58 | 0.210 | 98 |
| | 0.59 | 0.084 | 43 | 0.44 | 0.033 | 84 | 0.36 | 0.054 | 18 | 0.52 | 0.140 | 98 |
| Mean | 0.51 | 0.064 | 27 | 0.38 | 0.032 | 82 | 0.38 | 0.062 | 37 | 0.47 | 0.103 | 90 |
| ±SE | 0.05 | 0.011 | 5 | 0.06 | 0.006 | 5 | 0.04 | 0.013 | 5 | 0.03 | 0.017 | 5 |
| $\frac{TF/P_{Na}}{TF/P_{In}}$ | <i>P</i> < 0.02 (<i>n</i> = 11) | | | | | | <i>P</i> < 0.10 > 0.05 (<i>n</i> = 11) | | | | | |
| TF/P _{Na} | <i>P</i> > 0.10 | | | | | | <i>P</i> < 0.10 > 0.05 | | | | | |

Changes in the ratio of tubular fluid to plasma osmolality along the collecting duct are shown in Fig. 5. There was a greater increase in concentration along the collecting duct of group I and III compared to either group II or IV, a finding confirmed by the regression lines (Table II).

The relationship between the filtered load of water, sodium, and potassium, and the amount reabsorbed, in absolute terms, in the medullary collecting duct is shown in Table IV. Filtered loads were calculated from the glomerular filtration rate and the plasma level of sodium or potassium and compared with sham-operated normal hydropenic rats. The amount remaining at the "beginning" and "end" of the medullary collecting duct was calculated from the filtered load and the regression line. The results indicate that there was slight water addi-

tion or no net water reabsorption along the collecting duct in the kidney undergoing postobstructive diuresis (group II and IV, respectively), while marked water reabsorption was present in sham-operated and in urine-reinfused rats (group I and III).

Significant net sodium addition to the medullary collecting duct occurred in both postobstructive groups (II and IV), although sodium entry was greater in group II. Sodium reabsorption occurred normally along the duct in sham-operated controls and in urine-reinfused animals. Net entry of sodium occurred in the postobstructive groups despite a marked reduction (over 50%) in the load delivered to the beginning of collecting duct. In contrast, when the load delivered was increased, as in the urine-reinfused animals (group III), the abso-

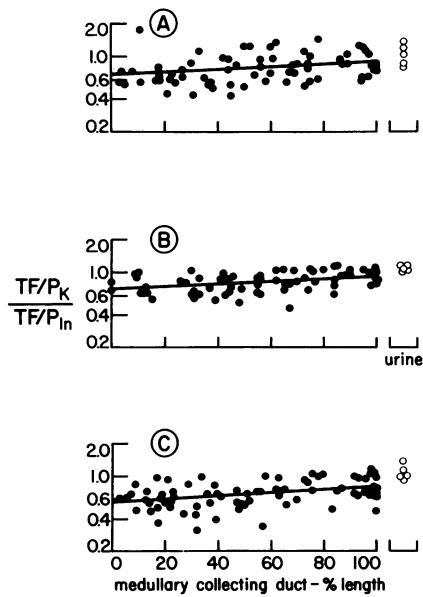


FIGURE 4 Fraction of filtered potassium remaining at different levels of medullary collecting duct in groups II, III, and IV (A, B, and C). Symbols and explanations as in Fig. 1.

lute reabsorption of sodium was similar to sham-operated controls.

Net addition of potassium to the medullary collecting duct was observed in the postobstructive or urine-reinfused rats (groups II-IV), while no statistically signifi-

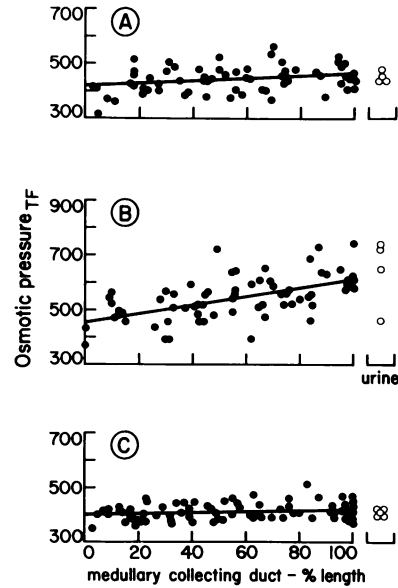


FIGURE 5 Change of osmotic pressure in collecting duct fluid with medullary length in groups II, III, and IV. Regression lines of osmolality with length are indicated.

cant change occurred in the sham-operated controls. The potassium load delivered to the duct was also increased in groups II-IV.

DISCUSSION

Medullary collecting duct function was markedly altered in the rat undergoing postobstructive diuresis after re-

TABLE IV
Tubular Load and Reabsorption of Ions and Water

| Flow rate | Medullary Collecting Duct | | | |
|---|---------------------------|-----------|-------|----------|
| | Filtered | Beginning | End | Δ |
| Flow rate, $\mu\text{l}/\text{min}/\text{gkw}$ | | | | |
| Group I, sham | 940 | 27.3 | 4.6 | -22.7† |
| Group II, BUL | 160 | 22.4 | 27.2 | +4.8* |
| Group III, UR | 560 | 76.3 | 39.1 | -37.2† |
| Group IV, UUL + UR | 116 | 17.8 | 20.2 | +2.4 |
| Sodium excretion, $\text{neq}/\text{min}/\text{gkw}$ | | | | |
| Group I, sham | 134,420 | 4,207 | 202 | -4,005† |
| Group II, BUL | 24,160 | 1,667 | 2,875 | +1,208† |
| Group III, UR | 86,800 | 5,816 | 2,604 | -3,212† |
| Group IV, UUL + UR | 17,748 | 1,109 | 1,588 | +479* |
| Potassium excretion, $\text{neq}/\text{min}/\text{gkw}$ | | | | |
| Group I, sham | 4,042 | 276 | 229 | -47 |
| Group II, BUL | 1,216 | 815 | 1,094 | +279† |
| Group III, UR | 2,800 | 1,960 | 2,604 | +644† |
| Group IV, UUL + UR | 1,009 | 569 | 806 | +237† |

BUL, bilateral uretal ligation; UR, urine reinfusion; UUL, unilateral uretal ligation.

* Statistically significant changes, $P < 0.05$, based on correlation coefficients.

† Statistically significant changes, $P < 0.01$, based on correlation coefficients.

relief of 24-h bilateral ureteral ligation. Previous micropuncture studies using a similar experimental model have shown decreased fractional reabsorption in the proximal, and particularly in the distal, tubule of surface nephrons, and have suggested that there was disproportionate damage to deeper nephrons (1-3). Interestingly, the only study in which sodium reabsorption in the late distal tubule was determined (2) indicated that the fraction of filtered sodium in the final urine was higher than the fraction remaining at the end of the surface distal tubules. Heterogeneity of nephron function made conclusions about collecting duct function tentative. The present study directly demonstrates abnormal function of the medullary collecting ducts during postobstructive diuresis. Evidence of net addition of water and sodium to the collecting duct, presumably due to decreased reabsorption and increased back diffusion, was obtained. This conclusion is based on the marked differences in the direction of the regression lines for the fraction of filtered sodium remaining along the collecting ducts in the two postobstructive groups (II and IV), compared to the sham-operated or urine-reinfused groups (I and III), as shown in Figs. 1 and 3. These differences were consistently present in individual animals of each group. Moreover, the paired samples available from the beginning and end of the same collecting duct system also indicated net entry of sodium in the postobstructive groups, compared to the other two groups (Table III).

Fractional reabsorption of sodium and water proximal to the collecting duct was decreased in the postobstructive kidney, as expected from micropuncture studies of surface nephrons (1-3). However, the associated fall in glomerular filtration rate resulted in an actual reduction by more than 50% of the delivery of tubular fluid to the beginning of the medullary collecting ducts, when compared to hydropenic controls (Table IV). Despite this reduction in delivery of fluid to the collecting ducts, the urinary output of salt and water by the postobstructive kidney was greatly increased above normal. We therefore conclude that the medullary collecting duct was probably the critical nephron segment affected by 24-h complete ureteral obstruction.

Moreover, since the technique of collecting duct microcatheterization obtains samples that represent cortical nephrons from all levels, it is not necessary to postulate that disproportionate impairment of deep nephron function is a major factor in postobstructive diuresis. Other evidence indicates that redistribution of nephron filtration rates (9) and intrarenal blood flow (3) are not characteristic of the kidney undergoing postobstructive diuresis.

Changes in sodium and water reabsorption in the medullary collecting duct during postobstructive diu-

resis could represent an intrinsic transport defect or could be secondary to changes in blood composition associated with urine retention. When urine was reinfused intravenously for 24 h to produce similar retention of urea and other natriuretic factors (group III), the function of the collecting ducts was clearly different from the postobstructive kidney (group II). There was normal absolute reabsorption of sodium and water in this nephron segment, despite an increase in delivery from the more proximal nephron due to decreased fractional reabsorption (Table IV). Natriuretic factors have been demonstrated by urine reinfusion (4) and by cross-circulation techniques (5) in the blood of rats with 24-h bilateral ureteral ligation, and in the serum (10, 11) or urine (12) of humans with chronic uremia. The present experiments indicate that such factors, although probably significant in postobstructive diuresis as discussed below, cannot account for the observed net addition of sodium and water to the collecting duct.

Further evidence that collecting duct dysfunction was a direct result of ureteral obstruction is provided by the results in rats with unilateral ureteral ligation and urine reinfusion (group IV). These animals demonstrated a similar qualitative abnormality in collecting duct function to that seen in bilateral ureteral obstruction, with significant net addition of sodium along the duct, although the addition of water did not reach statistical significance (Table IV, Figs. 2 and 3). Thus, both bilateral ureteral obstruction and unilateral obstruction with urine reinfusion may result in net addition of sodium to the medullary collecting duct. Since animals with urine reinfusion alone do not show this phenomenon, the defect in collecting duct function appears to be a direct result of ureteral obstruction.

The mechanisms responsible for the intrinsic defect in collecting duct function during postobstructive diuresis have not been established. Presumably, a marked decrease or absence of reabsorption is present, together with normal or enhanced back diffusion. Sonnenberg (7) has described similar net addition of sodium and water to the collecting duct after extracellular volume expansion with Ringer's solution, but volume expansion was not responsible for the present observations since the rats received no fluid for 24 h before study and lost 6-10% of their body weight. Numerous factors could interact to produce the observed changes in salt and water reabsorption in the medullary collecting duct, including alterations in membrane permeability, changes in hydrostatic pressure or solute concentration in the medulla, and changes in hormonal influences on the duct.

McDougal and Wright (2) have described increased permeability of the proximal and distal tubule to inulin and mannitol 14 h after the relief of 24-h bilateral ureteral ligation in the rat. It is possible that a similar de-

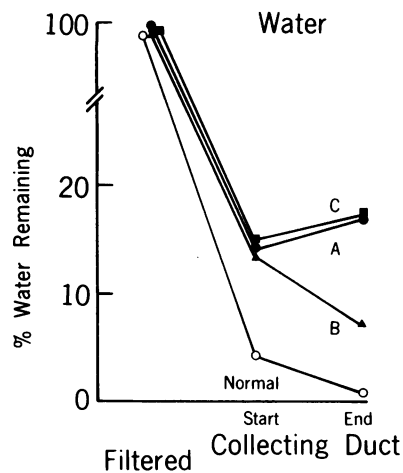


FIGURE 6 Percent of filtered water remaining at beginning and end of medullary collecting duct in groups II, III, and IV (A, B, and C) and in sham-operated normal rats (group I).

fect exists in the collecting duct but it is unlikely that this abnormality would be quantitatively large enough to account for the differences noted in the final urine that indicate net addition of water. Moreover, the significant increase in sodium concentration along the collecting duct in the pooled data from in groups II and IV ($P < 0.01$), and which was also less directly indicated by previous data (2), cannot be explained by abnormal losses of inulin from this nephron segment.

Increased intrapelvic and intratubular pressure during obstruction (1) could result in increased permeability of the collecting duct to both water and sodium. Histologic studies indicate that dilatation of the medullary collecting ducts is a prominent finding after 24 h of ureteral obstruction (13, 14). To explain net addition of sodium, it would be necessary to postulate that the return of hydrostatic pressure to normal after relief of obstruction occurred more rapidly in the medullary collecting ducts than in the surrounding medullary interstitium.

Previous studies have indicated a loss of the concentration gradient for urea and sodium in the inner medulla and papilla after brief periods of ureteral obstruction (15, 16). This change may partially result from abnormal permeability of the collecting duct and also undoubtedly contributes to decreased fluid reabsorption from the duct. In addition, if the permeability of the collecting duct epithelium was more markedly altered by ureteral obstruction than that of the distal convoluted tubule (as it might be if increased intrapelvic pressure was responsible), then the high urea concentration in the tubular fluid entering the medullary collecting duct, in the presence of a marked decrease in the medullary interstitial solute concentration, could enhance the movement of fluid into the duct.

Changes in the hormonal regulation of collecting duct transport could also contribute to net sodium entry. The lack of responsiveness of postobstructive diuresis to antidiuretic hormone (1) is not likely to be responsible since antidiuretic hormone tends to increase back diffusion into the collecting duct (17). Aldosterone has been shown to both increase active sodium reabsorption and decrease back diffusion of sodium into the medullary collecting duct (18, 19). Accordingly, if collecting duct sodium transport is unresponsive to aldosterone, as demonstrated in experimental and clinical postobstructive diuresis (1, 20, 21), then this change could contribute to net sodium addition to the duct. Finally it has been recently suggested that the local release of prostaglandins in the renal medulla, perhaps determined by changes in interstitial volume, may modulate collecting duct sodium transport (22).

The demonstration of collecting duct dysfunction in the postobstructive kidney does not establish that this abnormality is solely responsible for the phenomenon of postobstructive diuresis. In the three groups of postobstructive and/or urine-reinfused rats (groups II, III, and IV), there were identical decreases in fractional reabsorption of both water and sodium between the glomerulus and the beginning of the medullary duct, compared to normal animals (Figs. 6 and 7).

The importance of circulating natriuretic factors in this inhibition of reabsorption is indicated by the results in rats with urine reinfusion alone (group III) and in rats with unilateral ureteral ligation and urine reinfu-

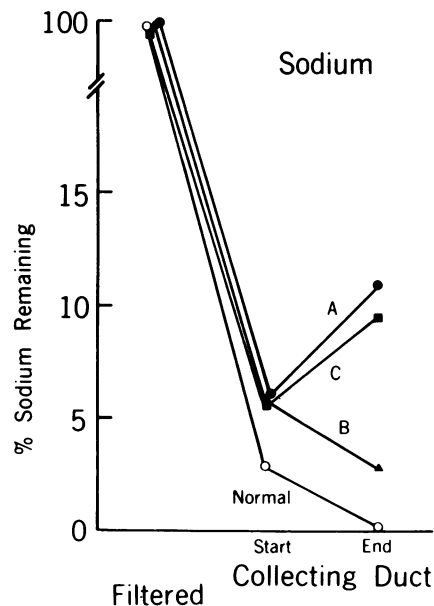


FIGURE 7 Percent of sodium remaining at beginning and end of medullary collecting duct in groups II, III, and IV (A, B, and C) and in sham-operated normal rats (group I).

sion (group IV). In urine-reinfused rats, the natriuresis-diuresis was due to increased delivery of fluid to the medullary collecting duct because of decreased reabsorption in the more proximal segments (Table IV). It has been previously shown that there was no postobstructive diuresis after relief of 24-h unilateral ureteral ligation (1, 23, 24), that is, no absolute increase in salt and water excretion, but rather a decrease compared to the normal contralateral kidney. Circulating natriuretic factors, which might inhibit reabsorption proximal to the collecting duct, could not be demonstrated in rats with unilateral ureteral ligation (4, 5). In fact, enhanced reabsorption in the proximal and distal tubule of surface nephrons has recently been demonstrated after relief of unilateral ureteral ligation (24, 25). Coupled with the reduction in filtration rate, enhanced or even normal fractional reabsorption would result in a marked decrease in delivery of sodium and water to the collecting duct system, sufficient to overcome any secretory component in this nephron segment and prevent postobstructive diuresis. In the present study, attempts to determine collecting duct function directly in this experimental model were unsuccessful due to extremely low urine flow rates from the experimental kidney of unilaterally obstructed rats. In contrast, when animals with unilateral ureteral ligation also received urine reinfusion from the normal contralateral kidney for 24 h (group IV), there was decreased fractional reabsorption proximal to the collecting duct and a natriuresis-diuresis similar to that observed after relief of bilateral ureteral ligation (Table I). The studies of Harris and Yarger (4) and recent cross-circulation experiments (5) also indicate that changes in blood composition, especially urea accumulation, may be responsible for this inhibition of fractional reabsorption proximal to the collecting duct, which is probably essential for the development of postobstructive diuresis.

Potassium, in contrast to sodium, was added to the collecting duct fluid in each of the three experimental groups (groups II-IV), a change not seen in normal rats (Table IV). Net addition of potassium was not the result of an intrinsic defect in collecting duct function since it occurred in group II rats with only urine reinfusion. Potassium addition occurred in the presence of an increase in the absolute load delivered to the collecting duct (groups II-IV) and in each case the fraction of filtered potassium remaining at the beginning of the collecting duct, or the potassium load per nephron, was increased (Fig. 8). It appears that potassium secretion by the collecting duct was determined by an increase in serum potassium level or by the potassium load per nephron. Potassium secretion may or may not be related to the increased sodium load per nephron also observed. Bank and Aynedjian (26) have previ-

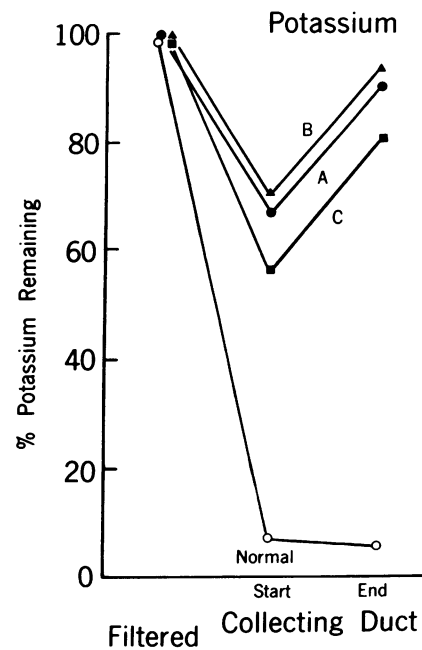


FIGURE 8 Percent of potassium remaining at beginning and end of medullary collecting duct in groups II, III, and IV (A, B, and C) and in sham-operated normal rats (group I).

ously noted that the collecting duct was responsible for the enhanced potassium excretion per nephron that occurred with acute or chronic reduction in renal mass or with potassium loading in normal rats.

In summary, three principal conclusions may be drawn from our experiments concerning the phenomenon of postobstructive diuresis. First, both physical and physiological anuria for 24 h results in inhibition of fluid and sodium reabsorption proximal to the medullary collecting duct. This inhibition is probably due to changes in blood composition. Second, ureteral obstruction per se, in addition to reducing subsequent filtration rate, produces an intrinsic abnormality in collecting duct function that may result in net addition of sodium and water to this nephron segment. Third, the medullary collecting duct appears to be the critical nephron segment affected in the postobstructive kidney, since net entry of sodium occurred in the presence of reduced absolute delivery of sodium to the duct. The factors responsible for these changes in sodium and water transport in the medullary collecting duct of the postobstructive kidney require further study.

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