Secretion of salt and water into the medullary collecting duct of Ringer-infused rats

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Sonnenberg, H. Secretion of salt and water into the medullary collecting duct of Ringer-infused rats. Am. J. Physiol. 228(2): 565-568, 1975.—Using a microcatheterization technique, the contribution of the collecting duct to the renal response to extracellular fluid volume expansion was studied in anesthetized rats. During intravenous infusion of Ringer solution (0.25 ml/min per 100 g body wt), urinary excretion of fluid, sodium, and potassium was 365 μl/min per g kidney wt (V), 52.6 μeq/min per g kidney wt (U NaV), and 3.86 μeq/min per g kidney wt (U K V), representing 23, 24, and 63% of filtered load, respectively. Analysis of collecting duct fluid from cortex and outer medulla indicated continued net reabsorption of ions and water in these nephron segments; in contrast, in inner medulla net secretion of Na, K, and fluid into the collecting duct was demonstrated. Addition of sodium and water was equivalent to approximately 10% of filtered load. It is concluded that under the stress of extreme intravenous fluid loading, tubular secretion of salt and water into the inner medullary collecting duct contributes importantly to diuresis and natriuresis. The mechanism of such secretion remains undetermined.

METHODS

Male Sprague-Dawley rats (weight range: 220–400 g) were maintained on water and Purina laboratory chow. They were anesthetized with Inactin (10 mg/100 g body wt, ip) and kept at a body temperature near 38° C on a heated operating table. Following cannulation of trachea and jugular vein, Ringer solution (NaCl = 130 mM, KCl = 5 mM, NaHCO3 = 20 mM, CaCl2 = 5.8 mM) was infused intravenously at a rate of 0.25 ml/min per 100 g body wt. The right ureter was cannulated through an incision in the left flank and connected to a femoral vein catheter, thus returning any urine produced by the right kidney to the circulation. The left, experimental kidney was mobilized through the same incision, placed in a Lucite cup, and covered to prevent drying and cooling of the surface. A femoral artery was cannulated for blood sampling and pressure recording. On completion of operative procedures, inulin-3H was added to the infusate to deliver approximately 125 μCi over the course of the experiment. Collections of tubular fluid and urine were begun 60 min later. Total time of infusion before collections varied between 1.5 and 2.5 h, as did the duration of the subsequent experiment. Urine from the experimental kidney was collected quantitatively at 20-min intervals; urine from the control kidney was collected in the middle of each interval by briefly opening the ureterovenous shunt. Arterial blood samples (0.05 ml) were taken at 10-min intervals. Methods of obtaining samples of collecting duct fluid and urine from the experimental kidney were described in detail previously (9). Briefly, the left ureter was dissected to the pelvis and cannulated. The papilla tip was exposed by a small incision in the upper surface of the ureteral wall, and urine was collected by gentle continuous suction. Fine polyethylene catheters (OD = 16–40 μm) were inserted to varying distances into different collecting ducts, and samples of fluid were obtained by controlled suction. Measured distance of insertion was related to medullary length taken from a postmortem sagittal section of the kidney. Sodium and potassium concentrations in plasma and urine were determined by flame photometry, and inulin-3H was determined by liquid scintillation counting in a toluene-based scintillator. Urinary excretions of sodium (U Na V) and potassium (U K V) were calculated for each kidney, as was glomerular filtration rate (GFR). Sodium and potassium concentrations in aliquots (10 nl) of tubular fluid were determined by an Amicon helium-glow photometer; inulin-3H (30 nl) was determined by liquid scintillation counting. To assess the possibility of variable quenching, equal volumes of diuretic urine from the same animals were added to 1 of 2 aliquots of 42 tubular fluid samples. The average difference in counts was 0.3 ± 3.9 (SD), indicating no effect of the added urine. A Clifton nanoliter osmometer was used to measure total solute concentration in tubular fluid and urine samples. Plasma protein concentration was estimated by refractometry. Precision of measurements was as described previously (9). Plasma inulin concentration
RESULTS

Intravenous infusion of Ringer solution resulted in average decreases of hematocrit and plasma protein concentration to 39.0 ± 0.6 (SE)% and 3.3 ± 0.1 (SE) g/100 ml, respectively, compared to values of 47.5 ± 1.6 % and 6.1 ± 0.1 g/100 ml for nondiuretic rats (ref. 9 and unpublished data). The decreases of both hematocrit and protein concentration were apparent at the beginning of collections and showed no further consistent variation throughout the experimental period. The stability of the preparation was also indicated by the constancy of arterial blood pressure (average = 121 ± 5 (SE) mmHg).

A comparison of average function of control and experimental kidneys of each animal is shown in Table 1. Despite the differences in urine collection times and methods, no statistically significant difference in function of the two kidneys was observed. Renal function tended to remain constant throughout each experiment.

Changes along the collecting duct system of intratubular inulin concentration and fractional remainder of filtered sodium and potassium are plotted in Fig. 1. The relationship between the percentage scales of collecting duct length in cortex and outer and inner medulla corresponds to absolute length differences of these segments (cortex = 2.0 ± 0.035 SE, outer medulla = 2.9 ± 0.1, inner medulla = 5.6 ± 0.1 mm). After inspection of the data, separate regression analyses were performed for points in cortical and outer medullary segments and those in inner medulla, respectively. Statistically significant decrease of inulin concentration and fractional remainder of filtered sodium declined significantly from cortex through outer medulla (T = 0.55, P = 0.01), indicating continued Na reabsorption from this part of the nephron. Corresponding correlations for inulin and potassium, although suggesting similar reabsorption, were not statistically significant.

Since the variability of the data might cast doubt on the physiological reality of the demonstrated secretion, in two animals deliberate attempts were made to collect samples from the beginning of the inner medullary duct, followed immediately by a collection from the same duct system close to the papilla tip. Results of such “paired” collections are depicted for sodium in Fig. 2. There was a rise in the intratubular amount of Na between the beginning and end of each inner medullary duct system tested. Changes for inulin and potassium were similarly consistent. To determine whether alteration in flow dynamics in the catheterized duct could account for this apparent secretion, in three additional animals ducts were catheterized with pipettes that could be wedged either at the beginning or end of the inner medullary duct. A set of four samples was then collected from a given duct system as follows: two samples each were collected at the beginning and end of the duct, with the suction set at either 10 mmHg lower or 10 mmHg higher than the previously determined optimum. Sequence of collections was varied for each duct system. Results are shown in Table 2. Using the paired t test, statistically significant secretion of sodium into this tubular segment. In contrast, the fraction of filtered sodium declined significantly from cortex through outer medulla (r = 0.55, P = 0.01), indicating continued Na reabsorption from this part of the nephron. Corresponding correlations for inulin and potassium, although suggesting similar reabsorption, were not statistically significant.

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ions and water was found whether flow was artificially reduced or increased. Not unexpectedly, partial obstruction reduced tubular content, presumably by enhancing up-stream reabsorption. This reduction was not statistically significant, however.

In two rats that were allowed to excrete urine from both kidneys while receiving a doubled intravenous infusion, 14 pairs of samples from beginning and end of inner medulla showed an increase from 0.233 ± 0.020 to 0.297 ± 0.010 (SE) (*P < 0.01) in the fraction of filtered sodium remaining in the duct, indicating that urine reinfusion is not essential to the secretion.

In contrast to the change in intratubular amount of sodium, concentration of the ion remained unaltered in any segment of the collecting duct system, with an average of 134 ± 11 (SD) meq/liter. Similarly, tubular fluid osmolality remained constant at 291 ± 21 (SD) mosmol/liter, while potassium concentration tended to fall along the inner medullary duct (**T/F/F_R = inner medulla = 3.14 - 0.0067 X % length; r = 0.42, P < 0.01). Absolute tubular loads of ions and water were calculated from filtered load and remaining fraction at beginning and end of the inner medullary duct system (Table 3). Corresponding data from antidiuretic rats (9) are included for comparison. It is evident that with Ringer infusion a larger proportion of the enhanced filtered load reaches the duct system at the border between inner and outer medulla. In addition, however, secretion of both sodium and water equivalent to about 10% of filtered load and 40% of total urinary excretion in inner medulla of infused rats is contrasted to continued reabsorption in antidiuretic animals.

**Discussion**

Comparison of infusion rate and urinary volume produced by the single excreting kidney shows that these animals excreted on average three-fourths of the infused fluid. Since hematocrit and plasma protein concentration did not change over the experimental period, extrarenal routes of loss, including visible intraperitoneal fluid, contributed to maintenance of fluid balance. However, as in an earlier series with comparable infusion rates (10), expansion of intravascular volume by about 20% is indicated by the initial decrease in hematocrit. Interestingly, fractional excretion of sodium and water in the previous group in which both kidneys were functioning was one-half of that found in the present experiments.

Direct (9, 11) as well as indirect (4, 6) evidence indicates that the medullary collecting duct plays a decisive role in the regulation of urinary excretion. However, secretion of sodium and water into collecting duct fluid has not been previously demonstrated, although such Na entry was suggested as a mechanism of natruresis in DOCA-escaped rats during intravascular expansion (8). The possibility that the observed secretion was an artifact due either to the surgical treatment of the experimental kidney or to the microcatheterization technique itself is deemed unlikely.

**Table 2. Effect of increased and reduced suction on collecting duct transport at beginning and end of inner medulla**

<table>
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<tr>
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<th>Iner Suction—Begin</th>
<th>Deer Suction—End</th>
<th>Deer Suction—Begin</th>
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<td>(TF/F)R</td>
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\( \% 1 = \) percent length. \( *+ \) Significant difference (\( P < 0.01, P < 0.02 \)) between average values at beginning and end of inner medulla during increased or decreased suction.

**Fig. 2. Fractions of filtered sodium in paired collections of fluid from beginning and end of inner medullary duct. Urinary values corresponding in time with collection pairs are represented by open circles.**
for the following reasons: first, comparison of control and experimental kidneys (Table 1) showed no significant difference in either filtration or excretion, indicating that both kidneys were functioning similarly. Second, while increasing interference of the microcatheter with tubular flow in progressively smaller ducts might cause artificially increased reabsorption at the deeper sampling sites, such an effect was not evident in samples taken even farther upstream in the outer medulla. In addition, deliberate increase or decrease in intratubular flow rate (Table 2) did not disrupt the secretory pattern in the inner medulla. Furthermore, even when samples with enhanced suction at the beginning of the duct were compared with those during partial obstruction at the end of the duct (Table 2), statistically significant (P < 0.01) Na secretion remained. It may be concluded, therefore, that secretion of salt and water into the collecting duct system of the inner medulla is a feature of the renal response to massive Ringer infusion, contributing in the present experiments about 40% of total urinary excretion (Table 3). The quantitative aspects of this secretion must be interpreted with caution, however, since it is obvious that alterations of intratubular pressure may affect upstream transport (Table 2). It should also be pointed out that lower infusion rates resulted in inhibition, but not reversal, of the normal Na and fluid reabsorption in this nephron segment (9).

The present results are in conflict with those of Diczi et al. (1), who observed reabsorption of sodium equal to 2% of filtered load per millimeter of exposed papillary collecting duct in young, saline-infused rats. While the high intravenous infusion rates in the present experiments preclude direct comparison of duct function, it seems unlikely that the difference in infusion rate alone could account for maximal reabsorption on the one hand and actual secretion on the other. It has been found that exposure of the papilla and alteration of surrounding fluid composition may affect intratubular fluid composition (7). Since in the present experiments on adult rats the papilla remained within the body of the kidney, and central ducts were cannulated, the apparent discrepancy could be explained on this basis.

The mechanism of the observed secretion is unclear. Diffusion of sodium into the lumen of the collecting duct has been suggested as a possible contributing factor to natriuresis (12). Since total solute as well as sodium concentrations along the duct remained unaltered in these experiments, however, it is difficult to visualize simultaneous diffusion of sodium and water in the same direction. Based on the increase of interstitial hydrostatic pressure with extracellular fluid volume expansion (3), secretion of salt and water into the collecting duct might be due to bulk flow of solution from medullary interstitium to duct lumen. In the absence of information on the filtration coefficient of the duct epithelium and the magnitude of pressure gradient, such an explanation remains speculative. Active transport of sodium into the lumen followed by diffusion of water offers an alternate explanation for the results. However, active Na transport in opposite directions at the same tubular site has not been found previously under any experimental conditions. Finally, active tubular secretion of a substance with consequent diffusion of Na and fluid in the same direction could explain the present findings. Such fluid secretion was shown to be associated with PAH transport in the straight portion of the isolated proximal tubule (2). The mechanism by which sodium and fluid may be added to collecting duct fluid in the inner medulla therefore requires further investigation.

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