Pressure-Diuresis in Volume-Expanded Rats
Tubular Reabsorption in Superficial and Deep Nephrons

RICHARD J. ROMAN

SUMMARY Micropuncture experiments were performed in volume-expanded rats to better define the nephron segments in which changes in renal perfusion pressure inhibit tubular reabsorption. Neural influences on the kidney were eliminated by renal denervation, and plasma levels of vasopressin, aldosterone, corticosterone, and norepinephrine were maintained at fixed levels by i.v. infusion. Fractional excretion of sodium, chloride, and water increased markedly after renal perfusion pressure was elevated from 110 to 150 mm Hg. Renal blood flow, glomerular filtration rate, and single nephron glomerular filtration rate measured from deep and superficial nephrons were unaltered. Reabsorption of chloride and water in the proximal tubule of superficial nephrons decreased by 10% after renal perfusion pressure was elevated and contributed to the pressure-diuretic response. Changes in renal perfusion pressure also altered the reabsorption of water and chloride in juxtamedullary nephrons. The percentage of the filtered water load reaching the tip of the loop of Henle increased from 19.8 ± 2.9 to 38.1 ± 3.0% after renal perfusion pressure was elevated. Chloride delivery rose from 34.2 ± 4.3 to 65.2 ± 4.8% of the filtered load. These results support the view that alterations in medullary hemodynamics participate in the pressure-natriuretic response by inhibiting tubular reabsorption in the proximal tubule or the thin descending limb of the loop of Henle (or both) of juxtamedullary nephrons. (Hypertension 12: 177-183, 1988)

KEY WORDS • hypertension • blood pressure • kidney • vasa recta • urine concentration and dilution • papilla • rats

The mechanism of pressure-diuresis and pressure-natriuresis and the nephron segments in which changes in renal perfusion pressure (RPP) influence tubular function are poorly defined. Koch et al.1 reported that elevations in RPP inhibited proximal tubular reabsorption, perhaps by increasing peritubular capillary pressure.1-3 However, others have found that pressure-diuresis can occur in the absence of changes in renal hemodynamics4-9 or in the reabsorption of sodium in the proximal tubule5-7 or the loop of Henle of superficial nephrons.7, 10 These findings suggest that RPP influences water and electrolyte transport in deep nephrons5-6 or in the collecting duct.7, 10, 11 More recently, the natriuretic response to carotid occlusion was attributed to inhibition of reabsorption in the proximal tubule of superficial nephrons.4 These authors concluded that the release of an autocoid might be involved in this response, since renal blood flow, glomerular filtration rate (GFR), and peritubular capillary pressure were autoregulated.4

We recently characterized a model of pressure-diuresis in volume-expanded rats with neural and humoral influences on the kidney controlled.9 In the present study, micropuncture experiments were performed using this model to better define the nephron segments in which elevations in RPP inhibit tubular reabsorption.

Materials and Methods
Experiments were performed on 51 male Sprague-Dawley rats (weight, 225-300 g). The animals were purchased from Harlan Industries (Madison, WI, USA) and were studied in one of two protocols.

General Surgical Preparation
The animals were anesthetized with Inactin 100 mg/kg, and surgically prepared for study of pressure-natriuresis with neural and hormonal influences on the kidney controlled8, 9 as described in the companion article.12 [3H]Inulin (50 μCi/ml) was included in the infusion solution for measurement of GFR.

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Protocol 1: Cortical Micropuncture Experiments

The left kidney of 28 rats was prepared for micropuncture.\textsuperscript{12, 13} RPP was lowered to 110 mm Hg by tightening an aortic clamp above the renal artery, and urine and plasma samples were collected during a 60-minute control period. Tubular fluid samples were collected from three late proximal and three early distal tubules. RPP was then increased to 150 mm Hg by partially occluding the lower aorta and the mesenteric and celiac arteries, and urine and plasma samples were again collected during a 60-minute experimental period. Tubular fluid samples were recollected from the sites punctured during the control period.

During both periods, additional proximal and distal tubules were sampled for measurement of single nephron GFR (SNGFR). These samples were quantitatively collected for 2 to 5 minutes after first blocking the tubule with castor oil.

Protocol 2: Papillary Micropuncture Experiments

Experiments were performed on 23 rats prepared with an exposed papilla.\textsuperscript{12, 14, 15} RPP was lowered to 110 mm Hg, and tubular fluid samples were collected from three to five thin loops of Henle and from collecting ducts near the base of the papilla. Urine samples were also collected from the ducts of Bellini using a micropipette.

In 17 rats, RPP was increased to 150 mm Hg by partially occluding the aorta and the mesenteric and celiac arteries. Tubular fluid samples were collected from thin loops of Henle and collecting ducts that were not punctured previously, and urine samples were again collected from the tip of the papilla. Tubular fluid samples were collected after blocking the tubular lumen with castor oil stained with Sudan black. Timed collections were obtained from some of the thin loops of Henle for measurement of deep nephron SNGFR.

Six time-control experiments were performed. In these rats, the celiac and mesenteric arteries were occluded after the control period, but RPP was maintained at 110 mm Hg by tightening the aortic clamp above the kidney. Tubular fluid and urine samples were then collected during a 60-minute experimental period.

Analytical Methods

Tubular fluid sample volume was determined by measuring sample length in 1-μl capillary tubes. [1H]Inulin concentrations were determined using a liquid scintillation spectrophotometer (Model 2450, Packard Instrument, Downers Grove, IL, USA). The chloride concentrations of tubular fluid samples were measured using a microtitrator (Model F-25, World Precision Instruments, New Haven, CT, USA). Chloride concentrations of urine and plasma samples were measured using a chloridometer (Corning Instrument, Corning, NY, USA). Sodium and potassium concentrations of urine and plasma samples were measured using flame photometry.

The absolute and fractional excretions of sodium, water, and chloride, GFR, and SNGFR were calculated using standard formulas.\textsuperscript{15} The percentage of the filtered water load remaining at various points along the nephron was calculated as \( 100 \times \frac{F_{in}}{F_{a}} \), where \( F_{in} \) and \( F_{a} \) represent the concentration of inulin in the plasma and tubular fluid samples, respectively. The percentage of the filtered chloride load remaining at the puncture site was calculated as \( 100 \times \frac{F_{ca}/P_{ca}}{F_{ci}/P_{ci}} \), where \( F_{ca} \) and \( P_{ca} \) represent the concentration of chloride in the tubular fluid and plasma and \( F_{ci} \) and \( P_{ci} \) represent the concentration of inulin in the tubular fluid and plasma.

Statistical Methods

Data are presented as mean values ± 1 SE. Significance of differences in measured values was determined using a paired \( t \) test.\textsuperscript{16} A \( p \) level below 0.05 was considered statistically significant.

Results

Protocol 1: Cortical Micropuncture Experiments

Increasing RPP from 111 to 151 mm Hg produced threefold increases in urine flow and in the excretion of sodium and chloride (Table 1). Potassium excretion doubled, while GFR was not significantly altered. The percentage of the filtered chloride load delivered to the late proximal tubule rose by 14% (from 64.3 ± 4.0 to 73.2 ± 4.4%; Figure 1). The percentage of the filtered water load reaching the end of the proximal tubule increased from 50.5 ± 3.2 to 56.7 ± 3.4%.

The percentage of the filtered water load delivered to the early distal tubule increased significantly, from 19.7 ± 2.7 to 25.0 ± 2.9%, after RPP was elevated (see Figure 1). The increases in water

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Experimental</th>
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<tr>
<td>RPP (mm Hg)</td>
<td>111 ± 3</td>
<td>151 ± 3*</td>
</tr>
<tr>
<td>GFR (ml/min/g kwt)</td>
<td>1.03 ± 0.06</td>
<td>1.10 ± 0.07</td>
</tr>
<tr>
<td>Urine flow</td>
<td>24.3 ± 4.5</td>
<td>79.4 ± 9.6*</td>
</tr>
<tr>
<td>Sodium excretion</td>
<td>4.14 ± 0.77</td>
<td>13.05 ± 1.48*</td>
</tr>
<tr>
<td>Potassium excretion</td>
<td>1.17 ± 0.15</td>
<td>2.48 ± 0.23*</td>
</tr>
<tr>
<td>Chloride excretion</td>
<td>5.41 ± 0.97</td>
<td>15.19 ± 1.59*</td>
</tr>
</tbody>
</table>

Mean values ± 1 SE from 28 rats are presented. Left kidney weight = 1.46 ± 0.07 g; Body weight = 287 ± 15 g. RPP = renal perfusion pressure; GFR = glomerular filtration rate; kwt = kidney weight.

\*\( p < 0.05 \), compared with control values.
delivery to the late proximal (6.2 ± 2.5%) and early distal tubule (5.3 ± 2.5%) were similar. The percentage of filtered load of water reabsorbed in the loop of Henle was not significantly altered and averaged 30.8 ± 2.7 and 31.7 ± 2.9% at low and high RPP, respectively.

The percentage of the filtered load of chloride reaching the distal tubule doubled, from 12.7 ± 2.9 to 23.5 ± 3.7%, after RPP was elevated. The chloride concentration of this fluid increased from 71 ± 6 to 94 ± 7 mEq/L. Chloride reabsorption in the loop was 51.4 ± 2.9% of the filtered load during the control period and 49.7 ± 3.6% after RPP was elevated.

The pressure-diuretic and pressure-chloruretic responses observed in the rats studied with an intact ureter (Protocol 1) were compared with those observed in the rats with an exposed papilla (Protocol 2) and are presented in Figure 2. The increases in the fractional excretion of water and chloride were similar in the two groups, indicating that creation of the papillary window and exposure of the papilla do not alter the pressure-diuretic response.

In the time-control experiments (data not shown), the fractional excretions of water (1.7 ± 0.4%) and chloride (1.7 ± 0.3%) observed at a RPP of 110 mm Hg were similar to those observed in the cortical or papillary micropuncture experiments (see Figure 2). Fractional excretions of water and chloride rose slightly from 1.7 ± 0.4 to 2.3 ± 0.6% and from 1.7 ± 0.3 to 2.3 ± 0.5% of the filtered load, respectively, over the course of the experiment. These changes, however, were inconsequential in comparison to the responses observed in rats in which RPP was elevated during the experimental period (see Figure 2).
Protocol 2: Micropuncture Experiments

The percentage of the filtered load of water reaching the bend of the thin loop of Henle increased significantly from 19.8 ± 2.9 to 38.1 ± 3.0% after RPP was elevated (Figure 3). The percentage of the filtered chloride load delivered to this site rose from 34.2 ± 4.3 to 65.2 ± 4.8%. The chloride concentration of the loop fluid was not significantly altered and averaged 216 ± 16 and 201 ± 18 mEq/L at the low and high RPP, respectively.

The percentage of the filtered load of water reaching the base of the papillary collecting duct rose from 70 to 60% when RPP was elevated (see Figure 3). Delivery of chloride rose from 5.9 ± 0.9 to 21.2 ± 3.5% of the filtered load. During the control period, 2.65 ± 0.57 and 4.54 ± 0.98% of the filtered loads of water and chloride were reabsorbed along the papillary collecting duct. After RPP was elevated, 7.59 ± 1.62% of the filtered load of water and 12.59 ± 2.18% of the filtered load of chloride were reabsorbed in this portion of the nephron.

Autoregulation of Glomerular Filtration Rate

GFR measured in the cortical micropuncture experiments (Protocol 1) was not significantly altered by changes in RPP and averaged 1.03 ± 0.06 and 1.10 ± 0.07 ml/min/g kidney weight at pressures of 111 and 151 mm Hg, respectively (see Table 1). GFR could not be measured in the papillary micropuncture experiments (Protocol 2) because urine flow rate could not be measured after the ureter was removed.

Control SNGFRs measured proximally or distally at an RPP of 110 mm Hg were not significantly different and averaged 44.8 ± 3.3 and 47.5 ± 3.4 nl/min/g kidney weight, respectively. No significant change in either value was detected after RPP was elevated; therefore, the proximal and distal SNGFR measurements were pooled. The pooled SNGFR measured from superficial nephrons was well autoregulated and averaged 45.5 ± 2.5 and 46.1 ± 4.1 nl/min/g kidney weight at RPPs of 111 and 150 mm Hg, respectively (Figure 4). The SNGFR of juxtamedullary nephrons was 40% greater than that measured in superficial nephrons and averaged 65.9 ± 9.6 and 69.0 ± 9.8 nl/min/g kidney weight, respectively, at RPPs of 104 and 151 mm Hg.

Discussion

Effect of Renal Perfusion Pressure on the Tubular Reabsorption of Water and Electrolytes

Pressure-natriuresis can occur in the absence of measurable changes in GFR, renal blood flow, or peritubular capillary pressure. In the companion paper,12 this response was associated with changes in medullary hemodynamics and renal interstitial pressure. The present study examined whether alterations in RPP inhibit tubular reabsorption in medullary nephron segments.

The results indicate that reabsorption of water and chloride in the proximal tubule of superficial nephrons decreased by about 10% after RPP was elevated. This finding confirms previous reports1, 2, 4 indicating that RPP can influence proximal tubular function. A change in proximal tubular reabsorption of this magnitude, however, would not be expected to produce large increases in sodium and water excretion because reabsorption of chloride in the thick ascending loop of Henle is load-dependent and usually compensates for changes in fluid delivery out of the proximal tubule.17, 18 In the present experiments, tubular reabsorption of chloride in the...
chloride delivery to the end of the late proximal tubule of superficial nephrons (see Figure 1). This finding confirms recent results indicating that elevations in RPP preferentially inhibit tubular reabsorption in deep nephrons. Assuming that there are 7,500 juxtamedullary nephrons and that 70% of the fluid reaching the tip of the loop is reabsorbed in the terminal nephron, inhibition of tubular reabsorption in deep nephrons could account for the other half of the observed diuretic response.

Several studies have suggested that inhibition of tubular reabsorption in the collecting duct might mediate pressure-natriuresis. The present results indicate that absolute and fractional reabsorption of water and chloride in the papillary collecting duct increased significantly after RPP was elevated. These findings demonstrate that changes in water and chloride reabsorption in the terminal collecting duct oppose rather than mediate the pressure-natriuretic response.

Mechanism of the Pressure-Diuresis and Pressure-Natriuresis

It has been suggested that elevations in RPP may be transmitted to the peritubular capillaries, increase interstitial pressure, and inhibit proximal tubular reabsorption by altering the backleak of ions. However, many laboratories have demonstrated that pressure-natriuresis can occur in the absence of changes in peritubular capillary pressure. Moreover, it has been argued that renal interstitial pressure is probably not affected by changes in RPP since renal blood flow and GFR are nearly perfectly autoregulated. In the companion paper, renal interstitial pressure increased 5 mm Hg after RPP was elevated from 100 to 150 mm Hg in volume-expanded rats. Elevations in interstitial pressure of this magnitude are known to increase sodium excretion. Therefore, the rise in renal interstitial pressure probably accounts for the inhibition of proximal tubular reabsorption in our study.

Fluid and electrolyte delivery to the tip of the loop of Henle increased markedly after RPP was elevated. In the companion paper, the pressure-diuretic response was associated with significant increases in pressure and flow in the vasa recta circulation. The mechanisms by which changes in medullary hemodynamics could affect tubular reabsorption are unknown, but several possibilities have been considered.

Haas et al. suggested that changes in RPP might influence proximal tubular reabsorption in deep nephrons by altering renal interstitial pressure. This conclusion was based on the observation that elevations in RPP increased the renal clearance of lithium, while reabsorption in the proximal tubule of superficial nephrons was unaltered. This effect could be related to a failure of juxtamedullary nephrons to autoregulate. However, this possibility seems remote because in the present study juxtamedullary SNGFR was not significantly altered by changes in RPP (see Figure 4). Moreover, we have
obtained preliminary evidence that interstitial pressure equilibrates throughout the kidney because this organ is encapsulated. It then becomes very difficult to explain the preferential effect of RPP on proximal tubular function of deep nephrons on the basis of intrarenal differences in interstitial pressure. These findings do not exclude the possibility, however, that the proximal tubule of deep nephrons is more sensitive to changes in interstitial pressure than that of superficial nephrons.

A second mechanism for pressure-diuresis focuses on medullary hemodynamics and its effect on tubular function. Increases in papillary blood flow may wash out the medullary solute gradient, decrease water reabsorption, and inhibit passive reabsorption of sodium and chloride in the thin descending loop of Henle. Previous results indicating that the thin descending loop of Henle of the rabbit is relatively impermeable to electrolytes argue against this view. More recent studies, however, indicate that the thin descending loop of Henle of rodents is highly permeable to sodium, chloride, and potassium.

Our finding that the delivery of chloride to the tip of the loop of Henle (34.2 ± 0.7%) was half that reaching the late proximal tubule (64.3 ± 4.0%) after RPP was reduced suggests that sodium and chloride may be reabsorbed in the thin descending limb of the loop of Henle. After RPP was elevated, the delivery of chloride to the superficial late proximal tubule and delivery to the tip of the loop of Henle were equivalent. This finding suggests that chloride reabsorption in the thin descending limb was unaltered by changes in RPP. A third explanation for the pressure-diuretic response involves a role for medullary interstitial pressure. The results of the companion paper indicate that elevations in RPP are partially transmitted to the vasa recta capillaries. This rise in vasa recta pressure would be expected to inhibit fluid reuptake in this bed and increase medullary interstitial pressure. Since the kidney is encapsulated, cortical interstitial pressure would increase as well. Our finding that vasa recta capillary and renal interstitial pressure increased by the same amount after RPP was elevated supports this hypothesis. If the thin descending limb of Henle is a leaky epithelium, elevations in interstitial pressure might be expected to inhibit reabsorption of water and electrolytes by increasing the backleak of ions in this portion of the nephron as well as in the proximal tubule of superficial or deep nephrons (or both). This mechanism is consistent with preliminary results indicating that the magnitude of the pressure-natriuretic response is modulated by changes in the hydration state that alter basal renal interstitial pressure. It also fits with the recent observation that decapsulation of the kidney blocked the increase in deep cortical interstitial pressure but not the natriuretic response to an elevation in RPP. The residual response in decapsulated kidneys was associated with changes in medullary interstitial pressure.

In summary, elevations in RPP produced diuresis and natriuresis in the absence of significant changes in GFR or SNGFR in superficial and deep nephrons. Chloride and water reabsorption in the proximal tubule of superficial nephrons was reduced by about 10%. The inhibition of proximal tubular reabsorption in superficial nephrons elevated fluid and chloride delivery to the distal tubule and accounted for about half of the pressure-diuretic response. Increasing RPP also inhibited tubular reabsorption of water and chloride in the proximal tubule or thin descending loop of Henle (or both) of deep nephrons and accounted for the remaining diuretic response. The inhibition of tubular reabsorption in both superficial and deep nephrons may be related to changes in vasa recta hemodynamics or renal interstitial pressure (or both).

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