Role of Prostaglandins in Proximal Tubule Sodium Reabsorption: Response to Elevated Renal Interstitial Hydrostatic Pressure

Yoshikazu Kinoshita and Franklyn G. Knox

Previous studies have shown that the elevation of renal interstitial hydrostatic pressure by the direct expansion of renal interstitial volume increases urinary sodium excretion. The objective of the present study was to investigate whether proximal tubules respond to the elevated renal interstitial hydrostatic pressure and whether the inhibition of prostaglandin synthesis would alter the effect of elevated renal interstitial hydrostatic pressure on proximal sodium reabsorption. Expansion of renal interstitial volume by injecting 100 µl of 2.5% albumin solution through a chronically implanted matrix increased renal interstitial hydrostatic pressure similarly in control rats (n=8) and in indomethacin (n=8) or meclofenamate-treated (n=7) rats.

In the absence of prostaglandin synthesis inhibition, renal interstitial volume expansion significantly increased the fractional delivery of sodium at the superficial late proximal tubules from 56.5±6.1 to 67.0±6.5% (p<0.01) with an accompanying increase in fractional excretion of sodium from 2.1±0.5 to 3.0±0.4% (p<0.01). In the presence of indomethacin or meclofenamate, renal interstitial volume expansion failed to augment the fractional delivery of sodium and the fractional excretion of sodium. In summary, these studies demonstrate that the synthesis of prostaglandins plays a role in the regulation of sodium reabsorption by the proximal tubules in response to elevated renal interstitial hydrostatic pressure. (Circulation Research 1989;64:1013-1018)

Although the acute elevation of renal perfusion pressure is well known to increase urine flow and sodium excretion, the mechanisms underlying these phenomena still remain to be elucidated. Renal interstitial hydrostatic pressure is reported to increase in response to increases in renal perfusion pressure1,2 and has been proposed to be involved in the pressure natriuresis phenomenon.3 Indeed, elevation of renal interstitial hydrostatic pressure by various procedures results in an increase in urine sodium excretion, suggesting an important role of renal interstitial hydrostatic pressure in the regulation of sodium handling by the kidney.4-8

Granger et al9,10 recently showed that direct expansion of the renal interstitial volume by an injection of 2.5% albumin solution through a polyethylene matrix implanted in the kidney interstitium resulted in an increase in renal interstitial hydrostatic pressure and urinary sodium excretion. Micropuncture studies revealed that the fractional delivery of sodium at the late proximal tubules was increased in response to direct renal interstitial volume expansion and indicated that proximal tubules were involved in the natriuresis caused by elevated renal interstitial hydrostatic pressure.11 Recent studies in our laboratory have disclosed that renal interstitial volume expansion also increases urinary prostaglandin E2 excretion and that the inhibition of prostaglandin synthesis blunts the natriuretic effect of elevated renal interstitial hydrostatic pressure.12 These data suggested the involvement of prostaglandins in the natriuresis caused by elevated renal interstitial hydrostatic pressure. However, at present, little information is available regarding the role of prostaglandins production in the attenuated sodium reabsorption by the proximal tubules. Therefore, the present study was designed to test the hypothesis that prostaglandins are involved in the decreased sodium reabsorption by the proximal tubules in response to the elevated renal interstitial hydrostatic pressure.
Materials and Methods

Male Munich-Wistar rats were used for the experiments. The rats were fed normal rat chow containing 0.1 meq sodium/g and had free access to water.

Matrix Implantation

The matrices, which are a microadaptation of renal interstitial capsules used in dogs, were made from material with a pore of 70 μm (Bel-Art Products, Pequannock, New Jersey). The matrices for measuring renal interstitial hydrostatic pressure were cylinders measuring 2.0 mm in diameter and 3.0 mm in length with polyethylene tubing (PE-50) glued to one end. The matrices for infusing isoncotic albumin solution into the renal interstitial space were also cylindrical in shape and measured 1.5 mm in diameter and 2.0 mm in length with PE-10 tubing glued to one end. Two to three weeks before the experiment, two small polyethylene matrices were implanted in the renal interstitium of the left kidney. The rats were anesthetized with an intramuscular injection of 100 mg/kg body wt of 5-sec-butyl-5-ethyl-2-thiobarbituric acid (Inactin, Byk Gulden, Konstanz, West Germany) and placed on a heated table to maintain body temperature at 36-38° C. After a 10-minute equilibration period, the interstitial hydrostatic pressure at the center of the ventral surface of the kidney approximately 4 mm away from the infusion matrix. After the abdominal cavity was washed and heparinized saline, the open end of the tubing was sealed and a small piece of mesh tubing was pushed into the kidney through the incision and glued to one end. The matrices for infusing isoncotic albumin solution into the renal interstitial space were also cylindrical in shape and measured 1.5 mm in diameter and 2.0 mm in length with PE-10 tubing glued to one end. Two to three weeks before the experiment, two small polyethylene matrices were implanted in the renal interstitium of the left kidney. The rats were anesthetized with an intramuscular injection of 100 mg/kg body wt of a solution of equal volumes of 20 mg/ml xylazine (Miles Laboratories, Shawnee, Kansas) and 100 mg/ml ketamine hydrochloride (Parke-Davis, Morris Plains, New Jersey). The left kidneys were exposed via a left paramedian abdominal incision. A small incision was made near the center of the ventral side of the kidney by inserting a conical pipette tip at the corticomedullary junction. A matrix for infusing albumin solution was pushed into the kidney through the incision and flushed with heparinized saline. The open end of the tubing was then sealed and a small piece of mesh (RM53, Ethicon, Somerville, New Jersey) was introduced around the catheter and placed on the kidney. A matrix for measuring interstitial hydrostatic pressure was implanted in the upper part of the kidney approximately 4 mm away from the infusion matrix. After the abdominal cavity was washed with saline to remove any residual blood, the incision was closed and 15,000 units of penicillin G were injected intramuscularly.

Micropuncture Study

All animals were fasted 14–18 hours prior to the micropuncture study. The rats (186–269 g body weight) were anesthetized with an intraperitoneal injection of 100 mg/kg body wt of 5-sec-butyl-5-ethyl-2-thiobarbituric acid (Inactin, Byk Gulden, Konstanz, West Germany) and placed on a heated table to maintain body temperature at 36–38° C. After a tracheostomy, catheters were inserted in both jugular veins for infusions, a carotid artery for blood sampling and blood pressure monitoring, and the left ureter for urine collection. Six percent inulin in isotonic saline was infused at a rate of 1.5 ml/hr during the entire experimental period. In addition, 0.1 meq sodium/g and had free access to water.

The experimental protocols were designed to determine the effects of prostaglandin synthesis inhibition on the renal interstitial volume expansion-induced alteration of sodium reabsorption by the renal tubules. The experimental protocols were designed to determine the effects of prostaglandin synthesis inhibition on the renal interstitial volume expansion-induced alteration of sodium reabsorption by the renal tubules.
proximal tubules. Four groups of rats were studied according to the following protocols:

**Group I (RIVE): Effect of renal interstitial volume expansion on sodium reabsorption by the superficial proximal tubules (n=8).** After the stabilization of urine flow (approximately 3 hours after the initiation of inulin infusion), the vehicle for indomethacin and meclofenamate (3 mM sodium carbonate in isotonic saline, 0.25 ml/100 g body wt) was administered intravenously. Forty minutes later, one 30-minute control clearance period was taken during which measurements of mean arterial pressure (MAP), renal interstitial hydrostatic pressure, glomerular filtration rate (GFR), and fractional excretion of sodium (FE\textsubscript{Na}) were made. During the clearance period, micropuncture samples were also taken to determine the fractional delivery of sodium (FD\textsubscript{Na}) at the superficial late proximal tubules. After the control period, renal interstitial volume expansion was achieved by an injection of 100 \mu l of 2.5\% albumin solution into the renal interstitium via the implanted matrix. Five minutes after the injection, a second 30-minute clearance was performed, and measurements and recollection micropunctures were repeated. At the midpoint of the second clearance period, another 100 \mu l of 2.5\% albumin solution was injected into the renal interstitium to keep the interstitial hydrostatic pressure elevated.

**Group II (IND+RIVE): Effect of renal interstitial volume expansion on sodium reabsorption by the superficial proximal tubules in the presence of indomethacin (n=8).** To determine whether the inhibition of prostaglandin synthesis attenuates the effect of elevated renal interstitial hydrostatic pressure on the sodium reabsorption by the proximal tubules, indomethacin (2 mg/kg body wt, Sigma Chemical Co, St. Louis, Missouri) was administered intravenously 40 minutes before the control clearance period. This dose of indomethacin has been shown to effectively inhibit the renal interstitial volume expansion-induced prostaglandin synthesis.\textsuperscript{12} The experiment protocol was otherwise the same as in Group I.

**Group III (MCL+RIVE): Effect of renal interstitial volume expansion on sodium reabsorption by the superficial proximal tubules in the presence of meclofenamate (n=7).** Instead of indomethacin as in Group II, meclofenamate (Warner-Lambert Co, Ann Arbor, Michigan) at a dose of 5 mg/kg body wt was used as an inhibitor of prostaglandin synthesis. This dose of meclofenamate was also shown to be sufficient to inhibit the renal synthesis of prostaglandin.\textsuperscript{12} The experimental protocol was otherwise the same as in Group I.

**Group IV (Saline): Time control study (n=8).** Isotonic saline was injected into the renal interstitium instead of 2.5\% albumin solution. The remainder of the experimental protocol was the same as in Group I.

### Analysis

Inulin concentrations in the plasma and tubule fluid were determined by the microflurometric method of Vurek and Pegram.\textsuperscript{15} The concentrations of inulin in urine were measured by the anthrone method.\textsuperscript{16} Sodium concentrations in tubular fluid were determined by atomic absorption spectrophotometry, and those in plasma and urine were measured with a Beckman E-2A electrolyte analyzer. The volumes of tubule fluid were measured with 1 \mu l constant bore capillaries. All values are expressed as mean±SEM. Comparisons were made using paired \(t\) tests and unpaired \(t\) tests as appropriate. A value of \(p<0.05\) was considered statistically significant.

### Results

Table 1 shows the effects of interstitial volume expansion on renal interstitial hydrostatic pressure at three different sites of the kidney. The distance between those sites and the infusion matrix is also given in Table 1. Although the increase of interstitial hydrostatic pressure was largest at the center of the kidney (2.4±0.3 mm Hg) and smallest at the lower pole (1.5±0.3 mm Hg), renal interstitial volume expansion with a single injection of 2.5\% albumin solution significantly increased the interstitial hydrostatic pressure at all three sites. These data show that the increase of interstitial hydrostatic pressure produced by renal interstitial volume expansion through an implanted matrix is transmitted fairly well throughout the kidney. The areas selected for micropuncture, which are located between the center and the lower pole of the kidneys, are exposed to the increased renal interstitial hydrostatic pressure.

The effects of renal interstitial volume expansion on GFR, urine volume, FE\textsubscript{Na}, and the renal interstitial hydrostatic pressure measured via measurement matrices are shown in Table 2. Renal interstitial volume expansion of the left kidney with the 2.5\% albumin solution increased renal interstitial hydrostatic pressure, urine volume, and FE\textsubscript{Na} significantly. Although renal interstitial volume expansion increased renal interstitial hydrostatic pressure.
significantly, it failed to augment urine volume and FE_{Na} in the presence of indomethacin or meclofenamate. When isotonic saline was injected into the renal interstitium, there was no change in interstitial hydrostatic pressure, urine volume, and FE_{Na}.

Recollection micropuncture at the late proximal tubules in vehicle-infused rats demonstrated a significant decrease of tubule fluid-to-plasma inulin concentration ratio [(TF/P)_{N}] and a significant increase of FD_{Na} after renal interstitial volume expansion with albumin solution (Table 3). The pretreatment of the animals with prostaglandin synthesis inhibitors abolished the effect of renal interstitial volume expansion on (TF/P)_{N} and FD_{Na}. MAP, GFR of the left experimental kidney, single nephron glomerular filtration rate, and tubule fluid-to-plasma sodium concentration ratio [(TF/P)_{N}] remained reasonably constant before and after renal interstitial volume expansion.

Discussion

The results of our experiments demonstrate that elevation of renal interstitial hydrostatic pressure induced by renal interstitial volume expansion inhibits sodium reabsorption by the superficial proximal tubules, and this inhibition of sodium reabsorption is abolished by the pretreatment with meclofenamate or indomethacin (Figure 1).

The elevation of renal perfusion pressure, which results in increased renal interstitial hydrostatic pressure, is known to augment the urinary excretion of sodium and prostaglandin E_{2} without changing GFR. The direct elevation of renal interstitial hydrostatic pressure by selective expansion of the renal interstitial volume has also been reported to increase FE_{Na} and prostaglandin E_{2} excretion. These results suggest the possibility that the elevated renal perfusion pressure increases prostaglandin production via elevating renal interstitial hydrostatic pressure, and that the increased synthesis of prostaglandins may play an important role in inhibiting sodium reabsorption by the renal tubules. Indeed, previous studies demonstrated that the inhibition of prostaglandin synthesis blunts the pressure natriuresis phenomenon. Recent studies in our laboratory also revealed that prostaglandin synthe-

### Table 2. Effects of Renal Interstitial Volume Expansion on Renal Function of the Left Kidney in the Presence and Absence of Prostaglandin Synthesis Inhibition

<table>
<thead>
<tr>
<th>MAP (mm Hg)</th>
<th>RHP (mm Hg)</th>
<th>GFR/100 g body wt (nl/min/100 g b.w.)</th>
<th>V/100 g body wt (µl/min/100 g b.w.)</th>
<th>FE_{Na} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. RIVE C</td>
<td>123±4</td>
<td>3.0±0.3</td>
<td>0.30±0.02</td>
<td>6.7±1.6</td>
</tr>
<tr>
<td>(n=8) RIVE</td>
<td>123±3</td>
<td>5.7±0.5*</td>
<td>0.37±0.05</td>
<td>10.3±3.1t</td>
</tr>
<tr>
<td>II. IND+RIVE C</td>
<td>122±4</td>
<td>2.6±0.3</td>
<td>0.38±0.04</td>
<td>4.1±1.1</td>
</tr>
<tr>
<td>(n=8) RIVE</td>
<td>124±2</td>
<td>4.8±0.3*</td>
<td>0.35±0.04</td>
<td>4.2±0.9</td>
</tr>
<tr>
<td>III. MCL+RIVE C</td>
<td>128±2</td>
<td>2.0±0.2</td>
<td>0.37±0.07</td>
<td>5.9±1.3</td>
</tr>
<tr>
<td>(n=7) RIVE</td>
<td>128±2</td>
<td>4.7±0.7*</td>
<td>0.28±0.04†</td>
<td>5.4±1.3</td>
</tr>
<tr>
<td>IV. Saline C</td>
<td>125±5</td>
<td>2.8±0.9</td>
<td>0.34±0.03</td>
<td>5.4±1.0</td>
</tr>
<tr>
<td>(n=8) Saline</td>
<td>125±5</td>
<td>2.8±0.4</td>
<td>0.30±0.03</td>
<td>5.1±1.0</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; RHP, renal interstitial hydrostatic pressure; GFR/100 g body wt, glomerular filtration rate of left kidney per 100 g body weight; V/100 g body wt, urine volume per 100 g body weight; FE_{Na}, fractional excretion of sodium; IND, indomethacin; MCL, meclofenamate; C, control clearance; RIVE, renal interstitial volume expansion. Saline refers to infusion of isotonic saline into an implanted matrix.

*tp<0.01, significantly different from control.

### Table 3. Effects of Renal Interstitial Volume Expansion on Proximal Sodium Reabsorption in the Presence and Absence of Prostaglandin Synthesis Inhibition

<table>
<thead>
<tr>
<th>SNGFR (nl/min)</th>
<th>(TF/P)_{IN}</th>
<th>(TF/P)_{IN}</th>
<th>FD_{IN} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. RIVE C</td>
<td>30.7±4.8</td>
<td>2.07±0.23</td>
<td>1.07±0.05</td>
</tr>
<tr>
<td>(n=8) RIVE</td>
<td>30.2±5.5</td>
<td>1.73±0.17*</td>
<td>1.08±0.05</td>
</tr>
<tr>
<td>II. IND+RIVE C</td>
<td>42.3±5.4</td>
<td>1.70±0.10</td>
<td>0.98±0.03</td>
</tr>
<tr>
<td>(n=8) RIVE</td>
<td>42.7±4.6</td>
<td>1.69±0.10</td>
<td>0.94±0.03</td>
</tr>
<tr>
<td>III. MCL+RIVE C</td>
<td>37.3±6.0</td>
<td>2.30±0.46</td>
<td>1.01±0.04</td>
</tr>
<tr>
<td>(n=7) RIVE</td>
<td>39.9±8.4</td>
<td>2.15±0.34</td>
<td>0.98±0.08</td>
</tr>
<tr>
<td>IV. Saline C</td>
<td>38.2±5.3</td>
<td>1.96±0.22</td>
<td>1.04±0.04</td>
</tr>
<tr>
<td>(n=8) Saline</td>
<td>37.4±3.4</td>
<td>2.07±0.09</td>
<td>1.05±0.04</td>
</tr>
</tbody>
</table>

SNGFR, single nephron glomerular filtration rates; (TF/P)_{IN}, tubular fluid-to-plasma inulin ratio; (TF/P)_{IN}, tubular fluid-to-plasma sodium ratio; FD_{IN}, fractional delivery of sodium at late proximal tubules; IND, indomethacin; MCL, meclofenamate; C, control clearance; RIVE, renal interstitial volume expansion. Saline refers to infusion of isotonic saline into an implanted matrix.

*tp<0.01, significantly different from control.
sis inhibitors attenuate the natriuretic effect of elevated renal interstitial hydrostatic pressure.\textsuperscript{12} Taken together, these data indicate that prostaglandin synthesis plays an important role in mediating the effect of changes in renal perfusion pressure on sodium reabsorption by the renal tubules.

Although there are studies which indicate that the proximal tubule, the loop of Henle, and other distal nephron segments may be responsible for pressure natriuresis,\textsuperscript{18-21} it is still unclear which segment of the nephron is most important for this phenomenon. Among others, proximal tubules are considered to be an important link between renal interstitial hydrostatic pressure and urinary sodium excretion, since sodium reabsorption by the proximal tubules is reported to be inhibited not only by the elevated renal perfusion pressure,\textsuperscript{22-24} but also by the direct elevation of renal interstitial pressure.\textsuperscript{11} Therefore, the present study was designed to investigate whether prostaglandin production plays a role in the regulation of proximal tubular sodium reabsorption in response to the elevated renal interstitial hydrostatic pressure. When the animals were pretreated with meclofenamate or indomethacin to inhibit the renal interstitial volume expansion-induced production of prostaglandins, the elevated renal interstitial hydrostatic pressure failed to inhibit sodium reabsorption by the proximal tubules. Using proximal tubules of the Necturus kidney, Boulpaep showed that the backflux of sodium through the tight junctions of tubular cells is responsible for the reduced sodium reabsorption by the proximal tubules in response to plasma volume expansion.\textsuperscript{25,26} He proposed that the increased renal interstitial hydrostatic pressure was one of the possible factors that cause the backflux.\textsuperscript{25} Renal interstitial hydrostatic pressure was indeed reported to be elevated by the plasma volume expansion,\textsuperscript{1,27} and was elevated similarly in our experiment by the selective renal interstitial volume expansion. However, our results that the elevated renal interstitial hydrostatic pressure failed to decrease the proximal sodium reabsorption in the presence of prostaglandin synthesis inhibitors indicate that not only the elevation of renal interstitial hydrostatic pressure but also the synthesis of prostaglandins might be necessary to decrease proximal sodium reabsorption.

Proximal tubules have not been considered to be major sites of prostaglandin synthesis and several previous studies failed to demonstrate an effect of prostaglandin E\textsubscript{2} on sodium reabsorption by the proximal tubules.\textsuperscript{28,29} However, prostaglandin E\textsubscript{2} is known to attenuate the sodium reabsorption by the proximal convoluted tubules,\textsuperscript{30} and recent studies have shown that the inhibition of prostaglandin synthesis blunts the change of proximal sodium reabsorption in response to the changes in renal perfusion pressure.\textsuperscript{22} Furthermore, there is increasing evidence that prostaglandin E\textsubscript{2} influences the transporting ability and adenylate cyclase activity
of proximal tubules. Although further studies may be necessary to determine if prostaglandins directly influence the proximal sodium reabsorption and to identify the specific prostaglandin that is responsible for the inhibition of proximal sodium reabsorption, the results of the present study clarified that some cyclooxygenase products might be involved in the renal interstitial volume expansion-induced decrease of sodium reabsorption by the proximal tubules.

In summary, the present study demonstrates that prostaglandin synthesis is necessary for renal interstitial volume expansion-induced elevation of renal interstitial hydrostatic pressure to decrease proximal sodium reabsorption.

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References

**KEY WORDS** • proximal convoluted tubule • micropuncture • kidney • rat
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