Indomethacin Blocks Enhanced Paracellular Backflux in Proximal Tubules

CARLA R. RAMSEY, THERESA J. BERNDT, and FRANKLYN G. KNOX
Nephrology Research, Departments of Internal Medicine and Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Abstract. Renal interstitial hydrostatic pressure (RIHP) is a link between increased arterial BP and natriuresis. The mechanism whereby increases in RIHP inhibits sodium and water transport across the mammalian proximal tubule epithelium may involve changes in flux across the tight junction of the proximal tubule. The purpose of this study was to determine the effects of increases in RIHP and inhibition of cyclooxygenase on paracellular backflux of an extracellular marker from the renal interstitium into the proximal tubule of the rat. During in vivo microperfusion of proximal tubules, the extracellular tracer of paracellular flux, lanthanum (La), was infused directly into the renal interstitium via a chronically implanted matrix. The net paracellular interstitium-to-lumen lanthanum backflux was measured before and after direct renal interstitial volume expansion (DRIVE) in the absence and presence of indomethacin. DRIVE significantly increased RIHP by 37% (Δ1.8 ± 0.2 mmHg) and interstitium-to-lumen La backflux by 32% (Δ40.2 ± 16.6 pg/min per mm), and it significantly decreased proximal reabsorption by 27% (Δ−7.7 ± 3.8 nl/min; n = 6). In indomethacin-treated rats (n = 6), DRIVE again significantly increased RIHP by 40% (Δ1.9 ± 0.2 mmHg), but it did not increase La backflux (Δ−39.0 ± 24.4 pg/min per mm) or significantly decrease proximal reabsorption (Δ1.2 ± 2.3 nl/min). These results demonstrate that increased RIHP increases paracellular backflux of lanthanum from the renal interstitium to the proximal tubule lumen in association with decreases in proximal reabsorption. Furthermore, indomethacin blocks the effects of increased RIHP on proximal reabsorption and paracellular backflux of lanthanum through the intercellular tight junctions of the proximal tubule epithelium.

Renal interstitial hydrostatic pressure (RIHP) is a link between increased arterial BP and natriuresis (1–3). The elevation of renal perfusion pressure results in increased RIHP. Increases in RIHP result in decreased proximal sodium reabsorption and an increase in fractional sodium excretion (4). Increases in RIHP during increases in mean arterial BP, during acute saline volume expansion and during direct renal interstitial volume expansion are associated with an inhibition of proximal tubule reabsorption and a natriuresis (1–8). Although several studies have demonstrated a relationship between changes in RIHP and proximal tubule reabsorption, the mechanism whereby increases in RIHP inhibit sodium and water transport across the mammalian proximal tubule epithelium is unknown.

Further studies have also examined the role of cyclooxygenase inhibition on proximal tubule sodium or water reabsorption during increases in RIHP. Selectively increasing RIHP by renal interstitial volume expansion was shown to increase PGE2 excretion (9). Inhibition of cyclooxygenase by indomethacin administration during increases in renal perfusion pressure and during direct renal interstitial volume expansion attenuates the effects of increased RIHP on proximal tubule reabsorption and sodium excretion (2,7,10). These studies have demonstrated that the presence of intact cyclooxygenase system is necessary for increases in RIHP to inhibit proximal tubule reabsorption. However, the mechanism whereby inhibition of cyclooxygenase modulates the effect of RIHP on sodium and water transport across the mammalian proximal tubule epithelium is also unknown.

The proximal tubule is characterized as having highly permeable intercellular tight junctions, allowing fluid and solutes to cross this nephron segment not only transcellularly but also through the paracellular pathway. However the contribution of paracellular transport to fluid reabsorption by the proximal tubule is controversial. Recent in vivo studies used aquaporin-1 knockout mice to determine the role of aquaporin-1–mediated transcellular water transport in proximal tubule fluid reabsorption. The water permeability of the proximal tubule was decreased by approximately 80% in the knockout mice, whereas fluid reabsorption was decreased by 50%. These studies demonstrated that the transcellular fluid reabsorption due to aquaporin-1 accounts for approximately 50% of fluid reabsorption and suggest that the non-aquaporin-1–mediated water reabsorption due to osmotic driving forces may use the paracellular pathway (11,12). Thus, fluid absorption through the intercellular tight junctions of the proximal tubule epithelium likely contributes to fluid reabsorption. The mechanisms of epithelial fluid transport have recently been reviewed (13). According to the most recent models of epithelial transport, fluid and ions move through the tight junctions with solute backleak, esti-
mated to be 15 to 20%, from the lateral intercellular space back into the tubule lumen. The present studies used lanthanum as a marker to determine the effect of increasing RIHP on backflux from the interstitial compartment into the proximal tubule lumen. Recent studies from our laboratory have demonstrated that tight junctions of the rat proximal tubule are permeable to lanthanum in vivo and, furthermore, that acute volume expansion increases the paracellular backflux of lanthanum from the interstitium to the proximal tubule lumen (14).

The intercellular tight junctions of the proximal tubule are sites where RIHP and cyclooxygenase products may influence sodium and water transport. It has been shown that the presence of permeable intercellular tight junctions in the proximal tubule facilitates paracellular backflux from the renal interstitium to the proximal tubule lumen (15,16). A hypertonic osmotic gradient within the lateral intercellular space of the proximal tubule could provide a driving force for backflux of ions into the proximal tubule lumen (17,18). Increased interstitial pressure might enhance backflux of solute and dissipate the osmotic gradient adjacent to the tight junctions and decrease the paracellular lumen-to-interstitium transport of water and solute.

The purpose of this study was to determine the effects of direct increases in renal interstitial hydrostatic pressure on paracellular backflux from the renal interstitium into the proximal tubule in the presence and absence of a cyclooxygenase inhibitor. Lanthanum is an extracellular marker that permeates the proximal tubule epithelium paracellularly via the tight junctions and not via a transcellular pathway (19). In the present study, the paracellular backflux of the interstitially infused, extracellular marker, lanthanum, from the renal interstitium to the proximal tubule lumen was determined during selective increases in RIHP of the magnitude observed during changes in renal perfusion pressure. Furthermore, additional experiments were performed during cyclooxygenase inhibition, because it is known that cyclooxygenase inhibitors blunt the effect of increases in RIHP on proximal tubule reabsorption.

Materials and Methods

The experiments in this study were designed to determine the net backflux of lanthanum across the proximal tubule epithelium before and after direct increases in RIHP both in the presence and absence of a cyclooxygenase inhibitor.

Experimental Methods

Direct renal interstitial volume expansion, by infusion of 2.5% albumin through a chronically implanted renal matrix, was used to directly increase RIHP. A second chronically implanted renal matrix was used to continuously measure RIHP throughout the experiment. Therefore, 2 to 3 wk before the acute experiment two matrices were chronically implanted in the left kidney of each 200 to 250 g Sprague-Dawley rat as described previously (7).

Rats were fed normal rat chow containing 0.1 mEq Na/g and had free access to water. On the day of the acute experiment, rats were anesthetized with an intraperitoneal injection of 100 mg/kg body wt of 5-s-butyl-5-ethyl-2-thiobarbituric acid (Inactin; Byk Gulden, Konstanz, Germany) and placed on a heated table to maintain body temperature. After a tracheostomy, both jugular veins were cannulated for the infusion of 3% inulin in isotonic saline at 2% body wt/h and for injections of lissamine green dye. The left carotid artery was cannulated to obtain blood samples and to measure arterial pressure, and a PE-50 catheter was inserted into the left ureter. The left kidney was exposed by a flank incision and placed in a kidney holder and soaked in heated mineral oil for microperfusion. One hour after surgery, the tubing leading from one of the implanted interstitial matrices was connected to a syringe and the continuous infusion of a 2% LaCl solution at a rate of 5 μl/min was initiated. The tubing from the second matrix was then connected to a pressure transducer (156PC05GWL, Microswitch, Freeport, IL) for the continuous measurement of RIHP.

Paracellular transport of ions through the intercellular tight junctions of the proximal tubule epithelium was determined in microperfusion experiments by the measurement of net lanthanum flux from the renal interstitium to the proximal tubule lumen as follows.

Sixty minutes after the lanthanum infusion and RIHP measurements were initiated, superficial proximal tubules were micropерfused as described previously (8,20). Briefly, a micropipette (outside diameter, 6 to 8 μm) containing dyed artificial tubule fluid perfusate connected to a Humpel-type microperfusion pump was positioned in an early proximal convoluted tubule. Injections of small volumes of this fluid were used to identify proximal tubules having five or more surface loops distally. A glass micropipette (outside diameter, 8 μm) containing bone wax was inserted into the second loop of the identified proximal tubule. Wax was forced into the tubules by a hydraulic microdrive to obstruct the glomerular flow. Contamination of perfusion fluid by glomerular filtrate is thus avoided. However, a fistula to other nephrons or capillaries may be produced; therefore inulin was systemically infused to aid in the detection of such contamination. Data analyses were restricted to collected perfusates in which inulin concentration was negligible. A hole made by the perfusion pipette on the first loop allowed the glomerular filtrate to escape onto the surface of the kidney. A perfusion micropipette containing artificial tubule fluid perfusate without dye was then positioned in the third loop to perfuse the proximal tubules with a Ringer-like solution: 147 mmol/L NaCl; 148.6 mmol/L Cl−; 5 mmol/L KCl; 5 mmol/L NaHCO3; 1.8 mmol/L CaCl2; 1 mmol/L MgSO4; 1 mmol/L NaHPO4; 5.5 mmol/L glucose; 5 mmol/L urea. The perfusion rate of the pump was set at approximately 55 nl/min in all experiments.

Timed complete collection of fluid reaching the last surface loop of the proximal tubule was obtained in a mineral oil-filled pipette. After the micropipette was inserted into the collection site, an oil block stained with Sudan black B was placed distal to the collection site. The rate of fluid collection was adjusted to maintain a constant position of the oil droplet and a constant luminal diameter. After control collections, either 100 μl of 2.5% albumin in saline (DRIVE) or 100 μl of saline were injected directly into the renal interstitium via the infusion matrix, and microperfusions from the same proximal tubules used in the control collections were repeated. After completion of the experiment, the perfused segment was filled with latex (Canton Bio-Medical Products, Boulder, CO) to determine tubule length by microdissection. Proximal tubule reabsorption was calculated as the difference between the tubule perfusion rate and the fluid collection rate for each perfused segment. Tubule length, volume of collected tubule fluid, and tubule fluid lanthanum concentration were determined for calculation of net lanthanum flux from the interstitium to the proximal tubule lumen.
Experimental Protocols

Three groups of rats were studied according to the following protocols.

Group 1: Effect of Direct Increases in RIHP on the Paracellular Flux of Lanthanum from the Renal Interstitium to the Proximal Tubule Lumen (n = 5 Rats). Rats were prepared as described above in Experimental Methods. One hour after the initiation of the continuous interstitial lanthanum infusion, superficial proximal tubules were microperfused and samples were collected for control measurements of lanthanum concentration and tubular flow rate. RIHP was also recorded at the time of each collection. After these control collections, RIHP was increased by DRIVE. DRIVE was accomplished by a 100-μl bolus injection of 2.5% albumin into the renal matrix and the addition of 2.5% albumin to the continuous interstitial infusion of lanthanum.

Fifteen minutes after the initiation of DRIVE, microperfuizations were repeated from the same tubules used in the control period. RIHP was recorded at the time of each collection (21–23).

Group 2: Effect of Direct Increases in RIHP on the Paracellular Flux of Lanthanum from the Renal Interstitium to the Proximal Tubule Lumen during Inhibition of Cyclooxygenase (n = 4 Rats). This experimental protocol was identical to group 1 except that the cyclooxygenase inhibitor, indomethacin (Sigma Chemical Co., St. Louis, MO), was intravenously administered at a dose of 3 mg/kg 1 h before control microperfusion collections. This dose of indomethacin was chosen on the basis of previous studies in this laboratory, which demonstrated that the administration of indomethacin at a dose of 2 to 3 mg/kg significantly decreased prostaglandin excretion and blunted pressure natriuresis (2,9,10). Previous studies have furthermore confirmed that when used as in these experiments the intravenous administration of indomethacin at this dose is effective in inhibiting the synthesis and urinary excretion of those prostaglandins derived from the cyclooxygenase-induced metabolism of arachidonic acid for 8 h (24–26).

Group 3: Time Control for the Effects of Direct Increases in RIHP on Net Lanthanum Flux from the Interstitium to the Proximal Tubule Lumen during Inhibition of Cyclooxygenase (n = 4 Rats). This group is similar to group 2 except that saline was infused into the matrix in place of 2.5% albumin after the control collections.

Analyses

Inulin concentration in collected perfusate was determined by the microfluorometric method of Vurek and Pegram (27), and inulin concentration in plasma was determined by the anthrone method (28). The volumes of collected perfusate were measured with 1-μl constant bore capillary tubes. The length of latex casts of the perfused proximal tubule segments was determined by planimetry.

Lanthanum concentration in collected samples was measured by modification of an existing spectrophotometric assay for lanthanum (29). This method uses the change in light absorption at 652 nm, when the dye Arsenezo III (Sigma) selectively complexes with lanthanum at pH 3.1. The assay was modified using a microcuvette that has a filling volume of 1 μl and a sample volume of 100 nl (30). Specifically, 100 nl of standards in tubule perfusate, aliquots of tubule perfusate, and individual experimental collections were added to 1 μl of 0.1 mM Arsenezo III in 0.1 M sodium acetate, pH 3.1. The absorbance of the samples was then read with a Zeiss spectrophotometer at a wavelength of 652 nm. The lanthanum standards were prepared by adding lanthanum to the artificial perfusate solution described above, and the lanthanum standards and perfusate were freshly prepared and mixed before each analysis and experiment to minimize the possible formation of lanthanum radiocolloid or lanthanum phosphate/carbonate complexes. This methodology has been successfully used in a previous study from our laboratory to assess paracellular flux by the proximal tubule (14). Net lanthanum flux from the interstitium to proximal tubule lumen was calculated by dividing the product of lanthanum concentration in the collected sample and tubular flow rate at the collection site by the length of the perfused segment. Proximal tubule reabsorption was calculated as the difference between the perfusion rate and the tubule flow rate at the site of collection.

Statistical Analyses

All values are expressed as mean ± SEM. Intragroup comparisons were made by paired t test, and intergroup comparisons were made by group t test. A value of P < 0.05 was considered statistically significant using a one-tailed t test.

Results

Figure 1 and Table 1 summarize the effects of direct increases in renal interstitial hydrostatic pressure on proximal reabsorption and net lanthanum flux from the renal interstitium to the proximal tubule lumen in both the presence and absence of indomethacin. In group 1, the group with intact cyclooxygenase, DRIVE significantly increased RIHP from 4.8 ± 0.5 mmHg to 6.6 ± 0.6 mmHg (P < 0.05). This 37% increase in RIHP was associated with a significant 32% increase in net lanthanum flux from the renal interstitium to the proximal tubule lumen (127.5 ± 23.3 to 167.8 ± 32.8 pg/min per mm; P < 0.05, one-tailed t test) and a significant decrease in proximal reabsorption of 27% (26.2 ± 1.8 to 18.5 ± 2.7 nl/min; n = 6 tubules; P < 0.05, one-tailed t test). Mean arterial pressure (MAP) was unchanged during DRIVE. In the control period, MAP was 136 ± 4 mmHg; during DRIVE, MAP was 134 ± 3 mmHg.

In the indomethacin-treated group, DRIVE again significantly increased RIHP by 40% from 5.0 ± 0.6 mmHg to 7.0 ± 0.8 mmHg (P < 0.05). However, in the presence of indomethacin, the increase in RIHP did not change, either net lanthanum flux (136.6 ± 26.9 to 97.6 ± 18.5 pg/min per mm) or reabsorption by the proximal tubule (22.3 ± 2.6 to 23.5 ± 1.5 nl/min; n = 6 tubules). Furthermore, when compared with group 1, the effect of DRIVE on proximal reabsorption (∆1.2 ± 2.3 versus ∆−7.7 ± 3.8 nl/min; P < 0.05, one-tailed t test) and lanthanum flux (∆−39.0 ± 24.4 versus ∆40.2 ± 16.6 pg/min per mm; P < 0.05, one-tailed t test) was significantly decreased with indomethacin. In the presence of indomethacin, MAP was 145 ± 4 during the control period and 139 ± 4 during DRIVE.

In group 3, the time control group with indomethacin alone, there were no changes in either net lanthanum flux (∆0.14 ± 10.3 pg/min per mm) or proximal reabsorption (∆1.6 ± 3.2 nl/min; n = 5 tubules). MAP was also unchanged in this group of rats (126 ± 5 versus 124 ± 6 mmHg).

Discussion

The results of this study demonstrate that the inhibition of proximal tubule reabsorption during direct increases in RIHP is associated with an increase in the paracellular backflux of
lanthanum from the renal interstitium to the proximal tubule lumen. This increase in the backflux of interstitially infused lanthanum occurred during increases in RIHP of the magnitude measured during increases in mean arterial pressure or during extracellular fluid volume expansion (2,5,6). Moreover, the advantages of the direct renal interstitial volume expansion technique allowed the effects of increases in RIHP to be specifically determined without the influence of changes in systemic hormonal and physical factors and without alterations in GFR or renal blood flow.

Figure 1. Changes in lanthanum flux (La flux) and proximal tubule reabsorption in response to direct renal interstitial volume expansion (DRIVE) in the presence and absence of prostaglandin synthesis blockade with indomethacin (INDO). *, significant increase in lanthanum flux or decrease in proximal reabsorption \( (P < 0.05, \text{paired } t \text{ test}) \). #, significant difference between indomethacin + DRIVE versus DRIVE alone \( (P < 0.05, \text{one-tailed, unpaired } t \text{ test}) \).

Table 1. Effect of increasing RIHP on proximal reabsorption and lanthanum backflux in the absence and presence of indomethacin

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 6))</th>
<th>Indomethacin ((n = 6))</th>
<th>Indomethacin ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>DRIVE</td>
<td>C</td>
</tr>
<tr>
<td>Proximal Reabsorption</td>
<td>26.2 ± 1.8</td>
<td>18.5 ± 2.7(^b)</td>
<td>22.3 ± 2.6</td>
</tr>
<tr>
<td>(nl/min per mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lanthanum Conc (µM)</td>
<td>44.1 ± 7.4</td>
<td>46.1 ± 7.5</td>
<td>54.4 ± 9.9</td>
</tr>
<tr>
<td>Lanthanum backflux (pg/min per mm)</td>
<td>127.5 ± 23.3</td>
<td>167.8 ± 32.8(^b)</td>
<td>136.6 ± 26.9</td>
</tr>
</tbody>
</table>

\( ^a \) DRIVE, direct renal interstitial volume expansion; \( n \), number of perfused proximal tubules.

\( ^b \) Significant difference; \( P < 0.05 \) paired \( t \) test.

The finding that DRIVE increases the paracellular backflux of lanthanum could support the hypothesis that increased RIHP enhances paracellular backflux of solutes and thereby dissipates the local osmotic gradient across tight junctions. This in turn would decrease the paracellular component of fluid reabsorption. However, if DRIVE were to decrease reabsorption by another mechanism, such as inhibition of active transport, the results could be similar. In this case, decreased reabsorption would result in increased tubule flow rate and facilitate the diffusion of lanthanum down its concentration gradient from interstitium to tubule lumen. Thus, increased backflux of lanthanum could reflect the cause of decreased reabsorption, dissipation of a local osmotic gradient across the tight junction, or the result of decreased reabsorption, enhanced tubule fluid flow.

This study confirms the results of previous studies showing that DRIVE has no significant effect on proximal tubule reabsorption in the presence of cyclooxygenase inhibitors, indicating an important role of cyclooxygenase metabolites in the effect of increases of RIHP on proximal tubule reabsorption.
(2,7,31). Furthermore, in the presence of indomethacin, increases in RIHP did not increase the interstitium-to-lumen paracellular flux of lanthanum.

As above, one mechanism whereby indomethacin may block the effects of increased RIHP on proximal tubule transport is by preventing the dissipation of the local osmotic gradient that drives paracellular reabsorption. This possibility is supported by several lines of evidence. Prostaglandins, the main cyclooxygenase metabolites, have been demonstrated to be present in proximal tubular fluid as well as renal interstitial fluid (32,33). Activation of prostaglandin E2 receptor EP3 has been shown to increase paracellular flux of mannitol in a tubular epithelial cell line, perhaps through activation of the GTPase RhoA (34). Other mediators of prostaglandins, such as cAMP, may also regulate fluid and solute flux through tight junctions in the proximal tubule (35). In the meantime, urinary excretion of PGE2 is increased by DRIVE, which can be blocked by indomethacin (9). Therefore, it is possible that DRIVE increases prostaglandins, leading to increased paracellular backflux of solutes, dissipation of the local osmotic gradient, and decreased total fluid reabsorption in the proximal tubule. Indomethacin blocks the increase in prostaglandins induced by DRIVE and, therefore, blocks the effect of DRIVE on the paracellular backflux and the proximal tubule reabsorption. Indomethacin did not alter the paracellular flux in the absence of DRIVE, suggesting that baseline levels of prostaglandins might not be sufficient to elicit these effects on proximal tubule transport, although the present study was not optimally designed to examine the effect of indomethacin on baseline proximal tubule transport.

It is also possible that the increase of paracellular flux induced by DRIVE is the result, rather than the cause, of the decreased proximal tubule reabsorption; therefore, alteration of the transcellular transport may be an alternative mechanism by which indomethacin blocks the effect of DRIVE on the paracellular backflux. Indeed, pressure natriuresis has been shown to associate with rapid suppression of the function of sodium transporters in the proximal tubule (36), although the exact mediator for this effect is unclear. On the other hand, indomethacin was shown to increase Na+/K+-ATPase activity in rat proximal tubules, whereas PGE2 decreased it (25). Thus, indomethacin could block or counteract the inhibitory effect of DRIVE on the transcellular transport in the proximal tubule, which abolishes the effect of DRIVE to increase paracellular backflux. Indeed, in the presence of indomethacin, DRIVE actually tended to decrease lanthanum backflux.

This study did not rule out possible roles of other indomethacin-inhibitable pathways in mediating the effect of DRIVE on the paracellular backflux and the proximal tubule reabsorption. One of the promising candidates is the cytochrome P450 pathway of arachidonic acid metabolism that is present in the proximal tubule and has been shown to play a significant role in the regulation of the proximal tubule transport and pressure natriuresis (37–39). Studies using specific manipulations of these pathways are needed to delineate their roles in the response of the proximal tubule transport to increases in RIHP.

We conclude that increased RIHP increases paracellular backflux of lanthanum from the renal interstitium to the proximal tubule lumen in association with decreases in proximal reabsorption. Furthermore, indomethacin blocks the effects of increased RIHP on proximal reabsorption and paracellular backflux of lanthanum through the intercellular tight junctions of the proximal tubule epithelium.

Acknowledgments
This research was supported by National Institutes of Health grant HL-55594 and by the Mayo Foundation. The authors gratefully acknowledge the assistance of Dr. Mingyu Liang, John Haas, and Marcy Ongs-Meyer and the secretarial assistance of Joanne Zimmerman.

References


