Understanding the Role of Paracellular Transport in the Proximal Tubule

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Fluid and solute reabsorption by the proximal tubule is the result of both transcellular and paracellular flux. The role of transcellular transport has been extensively studied, but the importance of paracellular flux has not been as thoroughly investigated. The purpose of this review is to update concepts about the contribution of paracellular transport for reabsorption by the proximal tubule.

Why, decades after the first studies of paracellular transport, is there so much uncertainty regarding the importance of the paracellular pathway for reabsorption by the proximal tubule of the kidney? Numerous studies have conclusively established the prominent role of the proximal tubule in fluid and electrolyte transport in the kidney. Approximately 65% of the water and sodium filtered at the glomerulus is reabsorbed by the proximal tubule. Like many types of epithelia that transport large amounts of fluid and electrolytes, the proximal tubule is characterized as having highly permeable intercellular tight junctions. Because the proximal tubule epithelium has “leaky” tight junctions, fluid and solutes can cross this nephron segment not only transcellularly but also through the paracellular pathway (4, 15) (Fig. 1).

Although relatively few studies have been performed to determine the regulation of paracellular transport by the proximal tubule, a great deal has been learned about the structure, cell biology, and regulation of intercellular tight junctions in other tissues. These studies in other tissues, such as the intestine and the gallbladder, have demonstrated the importance of paracellular flux in transepithelial transport (1, 2, 15). The regula-
tion of tight junctions in other tissues includes influences of pressure, hormones, and cyclic nucleotides, all potentially relevant to the permeability and permselectivity of the tight junction of the proximal tubule.

In the past decades, enormous strides have been made in the understanding of transcellular transport by the proximal tubule through investigation of brush-border and basolateral membrane transporters and cotransporters. However, little attention has been paid to the paracellular component of net fluid flux and its regulation by the proximal tubule. This is, in part, because of the lack of experimental techniques for the direct measurement of tight junction permeability and, until recently, a lack of understanding of the molecular structure and organization of the tight junction. The purpose of this review is to update concepts about the role of the paracellular pathway in reabsorption by the proximal tubule.

Transport by the proximal tubule

The proximal tubule transports large quantities of fluid in near-isosmotic proportions. The mechanism of this transport is thought to be local osmosis. Salt transport causes differences in osmolality in restricted fluid compartments of the epithelium, such as the lateral intercellular spaces, and water transport occurs in response to these differences in osmotic pressure (15). Ion transport by the proximal tubule is driven by active transport and passive paracellular electrochemical diffusion. Solvent drag of ions, defined as solutes that are entrained in osmotically induced paracellular water flow, is still controversial when applied to the mammalian tubule.

What is the magnitude of paracellular transport in the proximal tubule?

Several investigators, using various techniques, have estimated osmotic flow through the paracellular pathway to be as little as 2% (14) or >50% (6) (Fig. 1). Many investigators question high values of paracellular flow because of the limited area of the intercellular junction between the cells. It has been stated, “…the paracellular pathway comprises less than 1% of the epithelial surface area even when one considers the most generous interspace dimensions” (3). On the other hand, it has also been estimated that only a small fraction of the whole epithelial area needs to be available for water transport to account for the observed water flow in the prox-
imal tubule regardless of whether that transport is transcellular or paracellular.

Significant paracellular water flow has also been questioned because the high water permeability of the proximal tubule cell membranes could account for all the transepithelial water movement. The high water permeability of the apical and basolateral membranes is thought to be due to the water-selective membrane channels, aquaporin 1-CHIP. Recently, the abundance of the aquaporin-CHIP water channel in the rat proximal tubule was measured (13), and it may be sufficient to explain the majority of the osmotic water permeability in the proximal tubule. The maximum epithelial osmotic permeability that could be accounted for by aquaporin-CHIP was calculated to be 2,070 µm/s, whereas reported values for the transepithelial water permeability of the proximal tubule ranged from ~1,000 to 4,000 µm/s.

In the absence of techniques for direct measurement of paracellular permeability, tight junctional water permeability has been estimated indirectly from several different types of in vivo and in vitro experiments. Preisig and Berry (14) estimated the contribution of the paracellular pathway to proximal tubule transepithelial osmotic water permeability in in vivo microperfused rat proximal tubules. Because the tight junction surface area in the proximal tubule is limited, these investigators reasoned that the tight junction must contain large pores to give a sufficiently high paracellular osmotic permeability to contribute to proximal tubule transport. Therefore, the permeability of the proximal tubule to two large nonelectrolytes, mannitol and sucrose, was determined. These permeabilities were then used with the Renkin equation and Poiseuille’s law to calculate the osmotic permeability of the paracellular pathway. In addition, in separate experiments, the total transepithelial osmotic water permeability of the proximal tubule was determined. The transepithelial osmotic water permeability, which is the sum of transcellular and paracellular permeability, was determined to be 1,200 µm/s. The osmotic permeability of the non-electrolyte-permeable pores of the proximal path was 18 µm s⁻¹, a value <2% of the transepithelial osmotic water permeability. Thus these authors concluded that the tight junction non-electrolyte-permeable pathway is not a major route of transepithelial osmotic flow in the rat proximal tubule. These results have been interpreted to suggest that the tight junctions account for <2% of transepithelial water flow in the rat proximal tubule.

It is important to note that the above study assumed that the tight junctions were simple, uncharged cylindrical pores or slits that were freely permeable to large nonelectrolyte molecules. However, others have suggested that the tight junction may be composed of a charged glycoprotein mesh that could restrict the passage of sucrose or mannitol but would be highly permeable to water and small electrolytes (7). Thus it is possible that the calculated paracellular permeability of the proximal tubule was significantly underestimated if the tight junction has a structure that would preferentially allow the permeation of small electrolytes and water.

The most clear demonstration of an important role of the paracellular pathway as a route for water transport was performed by Carpi-Medina and Whittembury (6). They determined the transcellular (apical and basolateral permeabilities) and transepithelial osmotic water permeabilities by measuring changes in cell volume of isolated perfused rabbit proximal straight tubules in response to osmotic gradients. To determine apical cell membrane permeabilities, proximal straight tubules were perfused, first with an isosmotic and then with an anisosmotic solution while the isosmotic bath was replaced by oil. For basolateral membrane permeability determinations, tubules were perfused with oil while the bath was changed from an isosmotic to an anisosmotic solution. To measure the transepithelial permeability, oil drops injected in the tubular lumen were alternated with isosmotic perfusion solution. The bath was exchanged from isosmotic to anisosmotic, and changes in the fluid column between the oil drops was measured to determine transepithelial permeability. To stop tubular fluid absorption due to active transport, experiments were performed at 25°C.

Carpi-Medina and Whittembury (6) determined that the transcellular permeability was less than the transepithelial osmotic permeability (~1,800 vs. 4,300 µm/s) indicating that the difference was due to the paracellular permeability (~2,500 µm/s). They also investigated the contribution of the paracellular permeability to the transepithelial permeability when both apical and basolateral membrane permeabilities were inhibited with parachloromercuribenzenesulfonate (pCMBS). In the presence of pCMBS, the apical permeability was reduced to 23% of its control value and the basolateral permeability to 8% of its control value, but the transepithelial permeability was only reduced to 50% of the control value (from 4,300 to 2,200 µm/s). This leaves a transcellular permeability of 335 µm/s and a paracellular contribution of 1,850 µm/s, which is similar to the value observed under control conditions.
These findings indicate that at least 50% of the total transepithelial water flow occurs through the paracellular route in the proximal tubule and that inhibition of the transcellular permeability may not affect the paracellular permeability.

**Importance of paracellular transport in solute reabsorption by the proximal tubule**

If significant water flow occurs via the paracellular route, it is expected that paracellular water flow would be coupled to the paracellular flux of ions through solvent drag. However, not all studies show solute-solvent coupling. Jacobson et al. (10) determined the significance of solvent drag in the reabsorption of sodium chloride and sodium bicarbonate in in vitro microperfused rabbit proximal tubules. The effects of osmotically induced water flow on the net transport of chloride and bicarbonate were measured. An osmotic gradient of raffinose resulted in increases in water flow of 1.26 nl · min⁻¹ · mm⁻¹. However, there were no changes in net chloride and bicarbonate reabsorptions associated with the increases in water reabsorption. Therefore, either the reflection coefficient of the tight junction for these salts is close to unity or the volume of water transported through the paracellular space represents an insignificant fraction of total transepithelial water flow.

On the other hand, subsequent studies did find evidence for solvent drag. Bomsztyk and Wright (5) determined the effects of changes in transepithelial water flux on sodium, chloride, calcium, and potassium transport in in vivo microperfused rat proximal tubules. Osmolarity of the tubule perfusate was adjusted with the addition of mannitol. Addition of mannitol produced osmotically induced changes in water flow of 4.5 nl · min⁻¹ · mm⁻¹. Parallel changes in net transepithelial sodium, chloride, calcium, and potassium transport were associated with the changes in water flow. Thus these results demonstrated that transepithelial solute fluxes are affected by transepithelial fluid flux. Solvent drag through the tight junction is the most likely mechanism contributing to the dependence of solute flux on volume transport observed in these in vivo studies.

Thus evidence supporting solvent drag through the tight junctions has been seen in in vivo studies of the rat proximal tubule but not in in vitro studies of the rabbit proximal tubule. At least two explanations for this discrepancy may exist. First, species differences could exist in ion permeabilities of the rabbit compared with the rat proximal tubule. Second, the baseline solute and water fluxes as well as the changes in volume flow were much greater in the in vivo studies compared with the in vitro studies. Therefore, it is possible that the osmotically induced transepithelial fluid fluxes were not large enough in the in vitro studies to elicit measurable changes in ion fluxes.

**Regulation of paracellular transport in the mammalian proximal tubule**

Studies have demonstrated that transport through the paracellular pathway can be modulated by physical factors such as the peritubular protein concentration and renal interstitial hydrostatic pressure or by factors such as prostaglandins and adenosine 3′,5′-cyclic monophosphate (cAMP).

Increasing the peritubular protein concentration increases fluid reabsorption by the proximal tubule through altering the balance of Starling forces. Imai et al. (8) tested the hypothesis that the protein concentration gradient exerts a direct effect on fluid reabsorption across the proximal tubular epithelium. They found that changing the bath from isoncotic to hyponcotic solution inhibits fluid reabsorption and net sodium flux. Urea permeability did not change, but sucrose permeability from bath to lumen increased. When they increased the bath protein concentration, fluid reabsorption and net sodium flux increased. The authors concluded that the protein concentration gradient can exert direct effects on proximal tubule reabsorption. Because sucrose but not urea permeability was affected, it was suggested that oncotic pressure may act by altering flux through the paracellular pathway.

Changes in renal interstitial hydrostatic pressure (RIHP) also alter proximal tubule reabsorption. Directly increasing RIHP in the rat kidney, by increasing renal interstitial volume (injection of a 2.5% albumin-saline solution directly into the interstitium), decreases proximal tubule reabsorption and increases the fractional excretion of sodium (11). Furthermore, an intact renal prostaglandin system is necessary for increased RIHP to decrease proximal tubule reabsorption. Cyclooxygenase inhibition with indomethacin completely abolished the effect of increased RIHP on proximal tubule transport. Increases in RIHP may affect proximal tubule transport by altering the paracellular backflux of fluid and solutes from the interstitium to the proximal tubule. Prostaglandins could mediate the effect of RIHP on the proximal tubule by regulating the permeability of the paracellular pathway. In support of this hypothesis, preliminary studies have demonstrated that increased RIHP increases the paracel-
lular backflux of the extracellular marker lanthanum (12). In addition, inhibition of prostaglandin synthesis completely abolished the effect of increased RlHP on the paracellular backflux of lanthanum.

The effect of prostaglandins on proximal tubule transport could be mediated by the intracellular second messenger cAMP. The effect of cAMP on paracellular sucrose permeability has been studied in the isolated rabbit proximal tubule (9). When cAMP was added to the bath, basal fluid reabsorption decreased 27% and transepithelial potential difference decreased 25%, whereas sucrose permeability increased 97%. To investigate if the increase in sucrose permeability induced by cAMP was dependent on decreased fluid reabsorption, proximal tubules were bathed with hypertonic solution to increase reabsorption. During the addition of cAMP in the presence of hypertonic bath, sucrose permeability was still increased, whereas fluid reabsorption was increased and transepithelial potential difference decreased. Jacobson (9) also examined the effect of active transport on the paracellular permeability when the sodium-potassium-adenosinetriphosphatase was inhibited with ouabain. In the presence of ouabain, fluid reabsorption and transepithelial potential difference decreased. In contrast, ouabain did not change the paracellular pathway. These findings indicate that the paracellular sucrose permeability was modulated specifically by cAMP.

Ultrastructure organization of the tight junction

There have been very few studies of the molecular structure and regulation of the tight junction specifically in the proximal tubule. However, results from studies examining the tight junctions in other tissues have shown that the actin cytoskeleton plays an important role in maintaining the function of the tight junction. Recent progress has been made in identifying the proteins that form the tight junction (Fig. 2) and those that connect the actin cytoskeleton with it (1, 2). Several proteins from the tight junction have been identified, and the most-investigated proteins are occludin and the ZO-1/ZO-2 multiprotein complex.

In freeze-fracture micrographs, tight junctions appear as complex meshlike strands embedded in the cell membrane. The number of tight junction strands correlates with the tight junction resistance. It is suggested that each strand of the tight junction is composed of large aggregates of occludin molecules. Occludin is a transmembrane protein in which cytoplasmic and extracellular parts can be distinguished. The cytoplasmic segment is linked to the ZO-1 protein, and this segment may play an important role in orga-
nizing and regulating the paracellular seal. The extracellular segment of occludin is composed of two highly hydrophobic loops. Their amino acid composition suggests that they can create a tight paracellular barrier through hydrophobic contacts with loops from adjacent cells.

The second most-investigated protein is the ZO-1 phosphoprotein. On the basis of sequence homology, ZO-1 and the closely associated ZO-2 phosphoprotein belong to the membrane-associated guanylate kinase homologue family. However, it is unlikely that these proteins convert GMP to GDP because both proteins lack specific residues required for ATP and GMP binding. ZO-1 binds the cytoplasmic tail of occludin, spectrin, cingulin, and the 7H6 antigen. ZO-1 is also the most likely molecule to link the actin cytoskeleton to the membrane junctional complex. Thus ZO-1 protein could participate in the regulation of occludin through stimuli that modify the actin cytoskeleton.

Although the details of how intracellular signals may influence these proteins are not understood, many signaling messengers, including prostaglandins, cAMP, and protein kinase C, have been shown to regulate the actin cytoskeleton in epithelial cells (1). Therefore, the actin cytoskeleton may regulate paracellular flux by altering the tension applied to the membrane-associated junctional complex. In addition, ZO-1, ZO-2, cingulin, and p130 are phosphoproteins, which has led to speculation that these proteins may be targeted by kinases or phosphatases associated with many intracellular messenger cascades. Changes in the phosphorylation state of these components of the tight junction multiprotein complex could induce conformational changes in occludin and alter the permeability of the tight junction (2).

In conclusion, the importance of paracellular transport in regulation of reabsorption by the proximal tubule has been controversial because of the difficulties in direct measurement of tight junction permeability. Recent studies suggest that the paracellular pathway plays an important role in reabsorption by the proximal tubule and that this pathway is actively regulated.

The authors gratefully acknowledge Joanne Zimmerman for expert secretarial assistance.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-55394.

References


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