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The Paracellular Shunt of Proximal Tubule

A.M. Weinstein, E.E. Windhager
Department of Physiology and Biophysics, Weill Medical College of Cornell University, 1300 York Ave., New York, NY, USA

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Hans Ussing's conceptual contributions form the basis of our current understanding of epithelial ion and water transport. It was he who introduced the concept of a polarized epithelial cell in which the topographic arrangement of specific ion permeabilities and pumps leads to the net transport of ions and water (Koefoed-Johnson & Ussing, 1958). He pioneered in the application of radioisotopes to studies of ion fluxes across epithelia, and introduced the concept of exchange diffusion (Ussing, 1947). Ussing established firm criteria for the definition of active transport (Ussing & Zerahn, 1951). He was the first to speak about solvent drag (Anderson & Ussing, 1957), and to demonstrate an intercellular or paracellular shunt path for transepithelial ion movement (Ussing & Windhager, 1964). He had come to the conclusion that ion movement between neighboring cells could contribute significantly to transepithelial net transport from measurements of ion fluxes in frog skins treated on the apical side with hyperosmotic solutions, and microelectrode studies essentially confirmed this view (Ussing & Windhager, 1964). Subsequently, numerous authors, working on a multitude of mammalian and non-mammalian epithelia, have explored the quantitative significance of intercellular movement of ions and of water. Whereas studies of paracellular ion movement are relatively easy to assess by the combination of isotope fluxes and electrophysiological measurements, the quantitative significance and putative control of the paracellular shunt of water remains to be established.

The tight junction of the proximal tubule was securely established as a low-resistance pathway between the luminal fluid and the lateral intercellular space (Windhager, Boulpaep & Giebisch, 1967, Frömter, Muller & Wick, 1971). In comparison with other epithelia, the high solute permeability of these junctions correlated with the relatively sparse fibrillar structure seen on freeze fracture (Claude & Goodenough, 1973), and with the permeation of lanthanum (Whittembury & Rawlins, 1972; Martinez-Palomo & Erlij, 1973; Tisher & Yarger, 1973). These junctional properties qualified the proximal tubule as a typical "leaky" epithelium, and encouraged speculation as to the function of the tight junction in isotonic water transport. A number of mathematical models of proximal tubule, incorporating a permeable tight junction, were fashioned to try to reveal some role for the paracellular pathway in solute-solvent coupling (Spring, 1973; Huss & Marsh, 1975; Sackin & Boulpaep, 1975), but these and subsequent models could not provide a rationalization as to why electrical leakiness should correlate with the ability to transport isotonically.

The fraction of reabsorptive water flow that actually traverses the proximal tubule tight junction is unknown. Arguments in favor of transjunctional water flow have included: substantial solvent drag of ionic species (Frömter, Rumrich & Ullrich, 1973; Schafer, Patlak & Andreoli, 1975; Hierholzer et al., 1980), the appearance of streaming potentials with the application of an impermeant osmotic agent (Frömter & Gessner, 1974; Tripathi and Boulpaep, 1988); and ionic permeabilities roughly in proportion to their mobility in free solution (Kottra & Frömter, 1983). None of these findings, however, were direct proof of tight-junctional water flow, and concerns were difficult to dispel that solute polarization within the lateral interspace could be responsible for these observations. This particular question, whether one could construct a model of proximal tubule with just the right solute polarization to yield realistic reflection coefficients as well as a proper water permeability (L_p) was addressed in model calculations (Weinstein, 1987). With respect to analysis of rat data from Frömter et al. (1973), all of the acceptable in-

Correspondence to: E.E. Windhager; email: ewindhag@med.cornell.edu

terspace models required substantial tight junction convective chloride flux.

More direct evidence for tight junctional water flux in rabbit tubules came from Whittembury and his associates (Gonzalez, Carpi-Medina & Whittembury, 1982; Carpi-Medina, Gonzalez & Whittembury, 1983; Whittembury et al., 1985), whose estimate of the water permeability of the peritubular cell membrane indicated a transcellular water permeability less than the overall epithelial L_p. In the rat, strong evidence for tight junction water flow was the observation of convective entrainment of sucrose, despite relatively small diffusional flux (Whittembury et al., 1988). Nevertheless, Rector and Berry had resisted ascribing substantial water flux to the tight junction based upon pore-theoretic calculations, which indicated that the junctions were not large enough to allow anything but a small fraction of transepithelial water flow (Rector & Berry, 1982; Berry, 1983b). Preisig and Berry (1985) measured the permeation of sucrose and mannitol across the rat proximal tubule. Applying the Renkin equations to their data, they computed the dimensions of the "sucrose pore", and indicated that it could be responsible for at most 2% of the tubule water permeability. An important contribution to this discussion came with the suggestion of Fraser and Baines (1989) that the tight junction might be more realistically represented as a fiber matrix, rather than as a collection of pores. The critical feature of the fiber matrix equations is that, for a given solute permeability, the water permeability can be substantially greater than that predicted from the Renkin equations. This formulation was compatible with the permeabilities of rat proximal tubule, although it is a phenomenologic equation and not based upon the fine structure of junctional strands.

Once the tight junction had been established as a route for reabsorptive solute flux, an hypothesis was advanced that the junction might be an important site for the regulation of proximal sodium reabsorption. Lewy and Windhager found that in rats, both with and without acutely elevated renal venous pressure, there was a direct correlation between single-nephron filtration fraction and proximal tubule sodium reabsorption (Lewy & Windhager, 1968). Given that lower filtration fractions would result in reduced protein oncotic pressure within the peritubular capillaries, they surmised that this would lead to reduced capillary uptake of fluid from the renal interstitium and lateral intercellular space, and, hence, elevated interspace pressure. This, in turn, would result in backflux of the sodium already transported into the interspace, that is, backflux across the tight junction into the lumen. Prior to this proposal, Dirks, Cirksena & Berliner (1965) had shown that proximal tubule sodium reabsorption was depressed during extracellular volume expansion. In the intact dog, the ability to reverse natriuresis with infusion of hyperoncotic albumin indicated that peritubular oncotic pressure could influence sodium reabsorption and Earley and his associates (Earley, Martino & Friedler, 1966; Martino & Earley, 1967) had proposed that renal interstitial pressure might be an intermediate variable. Subsequent micropuncture experiments in the rat demonstrated that the depression of proximal tubule sodium reabsorption, which occurs with saline infusion, could be reversed by perfusion of the efferent arteriole with a solution whose protein concentration is comparable to that of control conditions (Spitzer & Windhager, 1970; Brenner, Troy & Daugharty, 1971). In *Necturus*, the elimination of peritubular colloid caused sodium reabsorption to fall by 40% (Grandchamp & Boulpaep, 1974). This occurred in association with a 60% increase in the electrical conductivity of the epithelium but no perceptible change in the cell membrane conductance, i.e., an effect on the tight junction.

It is a tenet of the backflux hypothesis that the action of peritubular protein is mediated through an effect on renal interstitial pressure and, hence, pressures within the lateral intercellular spaces. Morphologic examination of the proximal tubule of *Necturus* (Bentzel, 1972; Maunsbach & Boulpaep, 1980) and of the rat (Caulfield & Trump, 1962; Bengele & Evan, 1975) has shown progressive dilation of the lateral intercellular space, as well as opening of the interspace at the tubule basement membrane, with increments of saline volume expansion. Indeed, some widening of the tight junction (Bentzel, 1972) or disruption of junctional strands (Humbert et al., 1976) has been reported in proximal tubules of *Necturus* undergoing saline expansion, although no discernible changes occurred in the rat (Evan, Baker & Bengele, 1976). The precise mechanism of the "backflux" of sodium across the tight junction is uncertain. One possibility is that with increased interstitial pressure there is junctional widening and back-diffusion of sodium from interspace to lumen. Evidence from several sources has documented increased junctional permeability with volume expansion, both in *Necturus* (Boulpaep, 1972), and in the rat (Seely, 1973). Perhaps the strongest objection to the view that there is net backflux of sodium across widened junctions is that, at least in rat proximal tubule, due to a lumen-positive electrical potential difference, the electrodiffusive force on sodium is likely to be in the reabsorptive direction. A second possibility is that backflux of sodium across the tight junction occurs by convective flow. The tight junctions of the leaky epithelia are very sensitive to hydrostatic pressures applied from the contraluminal side. In *Necturus* proximal tubule (Hayslett, 1973; Grandchamp & Boulpaep, 1974), contraluminal pressures drive substantially greater volume flow across the epithelium than an equal luminal pressure,

and volume expansion was found to decrease the proximal tubule NaCl reflection coefficient (Bentzel & Reczek, 1978). In these experiments, it is likely that the junctional structure was distorted, forming relatively large pores with negligible sieving of solute, as both salt and water return to the lumen. Convective backflux across the tight junction of rat proximal tubule has been invoked by Ramsey, Berndt & Knox (1998) to explain their observation that the luminal appearance of lanthanum deposited within the renal interstitium is enhanced during saline volume expansion.

In view of these considerations, a mathematical model of the paracellular pathway of rat proximal tubule was developed, which included a compliant tight junction (Weinstein, 1990). In this case, "compliance" signified that both tight junctional salt and water permeability increase and the salt reflection coefficient decreases in response to small pressure differences from lateral interspace to tubule lumen. Although these compliance properties were empirical, they provided a model in which a decrease in peritubular protein concentration (which increased interspace hydrostatic pressure) could open the tight junction, and produce a secretory salt flux. This backflux was a combination of both diffusive and convective terms and did not specifically require either component to dominate. In this model of the tight junction, once the interspace pressure falls below that of the lumen, the junction is closed and junctional properties are fixed. The consequence of junction closure is that beyond a certain value of peritubular protein, one may expect little influence of peritubular Starling forces on volume reabsorption. Consistent with this view are the observations by Ott et al. (1975) that in the dog, hyperoncotic albumin infusion increased proximal reabsorption only in the previously volume-expanded group. In the individual tubule in vivo, a number of investigators found no significant influence of peritubular protein on proximal reabsorption (Holzgreve & Schrier, 1975; Conger, Bartoli & Earley, 1976; Bank & Aynedjian, 1984), and in the isolated perfused proximal tubule of the rabbit, several reports have indicated a lack of effect of peritubular bath protein on paracellular permeability (Berry & Rector, 1978; Berry, 1983a; Lapointe, Laprade & Cardinal, 1984). Pirie and Potts (1983) explored the influence of pressure gradients in this preparation. They found that elevations in intraluminal hydrostatic pressures abolished the effect of peritubular protein to enhance sodium reabsorption. In view of these findings, it is tempting to speculate that with higher luminal pressures, the tight junction always stayed closed.

In sum, the recognition that the tight junction could be a route for solute flux has provided, over four decades, rich and still active inquiry into proximal tubule transport physiology. Although electrodiffusive solute permeation has been well defined, the major limitation of this inquiry has been the lack of an accurate method for determining transjunctional water flow. Even the most basic question of whether there is any substantial water flow remains controversial. If such water flow were confirmed, it will still be necessary to develop the theoretical models to rationalize this flow with the known fine-structure of the junction. In particular, this means an accurate representation of junctional strands, along with a hydrodynamic model of water flow through breaks and between the strands. Beyond this static picture of the junction, there remains the task of characterizing the regulation of transjunctional fluxes. This includes modulation of the permeation pathway by ambient physical factors, as well as by cytosolic signals modifying the structural components. Finally, tight junctional pathology has been hypothesized to help explain the distorted transport of the ischemic kidney. In this regard, one may ask for a quantitative characterization of tight junctions in disease, as a prelude to modulation for therapeutic gain.

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