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Review

Epithelial Impermeability to Water: A Second Look

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Loop of Henle • Renal Collecting Ducts • Water Permeability • Countercurrent Mechanisms

Abstract
Impermeability of the ascending limb of the Henle loop for water is traditionally regarded as essential for countercurrent multiplication in the kidney. Similar claims have been made about permeability properties of the collecting duct and some other epithelia. It is not clear, however, how a structure based on phospholipid bilayers can be water-impermeant if phospholipid bilayers themselves have measurable permeability. The presence of two membranes separated by the cytoplasm may only account for a several-fold reduction in permeability compared to a single bilayer. By analyzing published data, we conclude that these tubules do have a finite water permeability, especially the collecting duct. Although the results on isolated ascending limbs vary among authors, osmotic shock experiments clearly indicate that both the collecting duct and the ascending Henle loop are sufficiently water-permeable to observe volume regulation effects. We conclude that these epithelia by themselves do not display unusual resistance to water flow; it can be estimated that 20-50% of the fluid entering the tubules can be reabsorbed into a strongly hypertonic medulla. It is possible, however, that unstirred layers in the intact kidney may contribute to the apparent low permeability of the tubules.

Introduction

The belief that the ascending loop of Henle and unstimulated collecting duct are impermeable to water is widely shared by physiologists; it is stated as a matter of course in most textbooks [1-3] and in scientific literature [4-6]. The biological significance of water-impermeant epithelia is that while ions are being removed from the filtrate by various transporters, water is unable to follow ions by osmosis, and the fluid becomes hypotonic. A similar process is responsible for the production of hypotonic saliva [7].

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But even pure lipid membranes are permeable to water to some extent, with osmotic permeabilities P_f in the range of 10 – 160 μm/s [8, 9] (see [10] for the explanation of permeability); how can it be that some epithelia are not? This question has drawn interest from researchers, who pointed out the potential importance of membrane asymmetry, integral proteins, and mucins [11-13]. To that, we would like to add a few additional considerations.

1. *Single membrane vs. epithelium.*

When comparing the permeability of a phospholipid bilayer with that of a simple epithelium, we must account for the presence of two membranes in the latter (apical and basolateral) separated by cytoplasm. For simplicity, we can assume that both membranes are identical and separated by the distance h = 7 μm [14]; the diffusion coefficient of water D_w in the cytoplasm has been estimated at 400 μm^2/s [15-17]. For a single membrane, the steady state water flux is expressed as

$$\Phi_m = P_f(C_0 - C_i)$$

In the case of a three-layer epithelium, the expression changes to

$$\Phi_e = \frac{P_f D_w}{P_f h + 2D_w}(C_0 - C_i)$$

which can be derived by equating water fluxes through each of the three compartments or by using the rule for calculating the combined permeability of membranes in series [18]:

$$\frac{1}{P_{total}} = \sum_i \frac{1}{P_i}$$

Therefore, the additional barriers present in the epithelium slow down water transport by the factor of

$$\frac{\Phi_m}{\Phi_e} = 2 + h\frac{P_f}{D_w}$$

For the values of P_f listed above, this ratio ranges from 2.2 to 4.8. Although this may partly explain the difference between a single membrane and an epithelium (for example, higher values in vesicles isolated from the medullary thick ascending limb (MTAL) than in intact MTAL [18]), most data on kidney permeability have been obtained on isolated tubules.

2. *What values of permeabilities would qualify a membrane as water-impermeant?*

The rate of water permeation through a tube with length L and radius R and subjected to an osmolarity gradient ΔΠ is

$$\Phi_{\text{out}} = P_f \cdot 2\pi RL \cdot v_w \cdot \Delta\Pi$$

where v_w is the molar volume of water equal to 18 cm^3/mol. The typical value of P_f for the medullary collecting duct (MCD) in the absence of vasopressin is 20 μm/s [18-20]; some authors have obtained slightly higher [21] or slightly lower [22, 23] values. The permeability of 20 μm/s is regarded as low: "P_f greater than 0.01 cm/sec (at 25–37°C) is considered to be high and suggests the involvement of molecular water channels, whereas P_f less than 0.005

cm/sec is consistent with water diffusion through the lipid portion of a membrane" [24]. However, assuming R = 12 µm, $L = 10^4$ µm [25], and $\Delta\Pi$ = 0.5 mol/L during diuresis [26], we find the rate of water removal by a single collecting duct on the order of 6 nl/min, which is comparable to the estimated flow rate Φ_{in} = 14 nl/min [25]. In other words, almost half of the fluid entering the MCD and destined for elimination is expected to be reabsorbed, even in the "water-impermeable" state of the duct.

The permeability of MTAL has also been a focus of much research. Rocha and Kokko [27] found its permeability close to zero; however, the standard error of their measurements was 4-6 µm/s. The results of Sasaki and Imai [28] were similar. The accuracy of those estimates may have been compromised by using fast perfusion rates [29]. Other measurements have produced a wide range of values from 0.07 µm/s [30] to 5 µm/s [23] or between 6 µm/s and 23 µm/s (cited in [29]).

Using the conservative estimate P_f = 1 µm/s and the parameters R = 15 µm, $L = 2{\cdot}10^4$ µm, and $\Delta\Pi$ = 1 mol/L, we find that the leak through the walls would amount to 2 nl/min, or about a quarter of the total flow of 8 nl/min [25]. Here, once again, epithelial impermeability does not directly follow from the data.

3. *Cell volume regulation*.

A separate body of work has focused on cell volume regulation - restoration of cell water content following either osmotic swelling (regulatory volume decrease, or RVD) or osmotic shrinkage (regulatory volume increase, RVI). The RVI and RVD are secondary responses to osmolarity changes caused by the activation of membrane channels for ions or organic osmolytes [31]; but to initiate these responses, the membrane must be permeable to water in the first place.

The numerous reports of volume regulatory responses in the collecting duct [19, 32-35] and in the ascending loop [36 – 40] provide compelling evidence that these cells are sufficiently permeable to water, as are all other mammalian cells. A single publication claiming the lack of swelling of cheek epithelial cells in hypotonic solutions [41] may have resulted from unnoticed rapid RVD that develops and subsides within a minute (according to our unpublished observations).

Conclusion

The presented brief review suggests that "water-impermeable" kidney epithelia do not possess watertight properties significantly beyond those expected from phospholipid bilayers. Their permeabilities are indeed two orders of magnitude less than the permeability of the thin descending loop [42, 43], which can be due to the lack of water channels, but are similar to those of many other cell types [8, 18, 44-48]. When comparing P_f values for epithelia and isolated lipid layers, the presence of an additional membrane and the cytoplasm should be taken into account; additionally, the effects of unstirred layers can be significant even in perfused tubule preparations [18, 43] and are expected to be particularly prominent in the interstitium of the kidney [49]. Indeed, a several-fold difference between *in vivo* and *in vitro* permeabilities of the salivary duct has been reported [50]. Conceivably, slow convection and dissipation of osmolality gradients can be a factor in water retention.

The main question, however, is whether the diluting function of the nephron and the effective diuresis require that MTAL and unstimulated MCD be strictly water-impermeable. It seems that the existing mathematical models assume zero permeabilities of both the thin and thick ascending loops [51-55], and the possibility of deviations from zero have not been considered. That would be an interesting question to investigate.

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Disclosure Statement

The authors have no conflicts of interest.

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