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Neonatal and Adult Rabbit Renal Brush Border Membrane Vesicle Solute Reflection Coefficients

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Abstract

The interaction between solute and water in epithelial transport is represented by the solute reflection coefficient. Because the osmotic water transport process changes in the rabbit proximal tubule during maturation, there is a potential for the solute reflection coefficients to also undergo maturational changes. In the present study, we directly examined solute reflection coefficients in neonatal and adult brush border membrane vesicles (BBMV) using the stop-flow light-scattering technique. Reflection coefficients for NaCl, KCl, NaHCO₃ and urea were found to be identical in the neonatal and adult BBMV and were not different from 1. Thus, although the water transport pathway under-goes changes in the proximal tubule during maturation, there is no evidence for changes in solute and water interaction. Because the reflection coefficients are not different from 1, there is no evidence for solvent drag in the proximal tubule apical membrane in either the neonatal or adult tubule.

Keywords

Stop-flow kinetics; Urea; Transport; Proximal tubule

Introduction

The proximal tubule reabsorbs approximately 75% of the glomerular ultrafiltrate isosmotically [1, 2]. Transepithelial water movement is primarily transcellular with little paracellular movement of water [1-3]. Water molecules can pass through the cell membranes either via diffusion through the lipid bilayer or via aquaporin 1 (AQP1), the constitutively expressed water channel in the proximal tubule [4].

Solute transport through the proximal tubule may interact with water transport pathways as a result of solvent drag [5, 6]. The interaction between solutes and water is represented by the reflection coefficient, σ . When $\sigma = 1$, there is no interaction between the solute and water

(i.e. they are transported by separate pathways, and there is no solvent drag), whereas $0 < \sigma < 1$ indicates that there is some interaction between solute and water, and convective flow of water will transport the solute by solvent drag. Evidence thus far indicates that AQP1 is able to transport water, but can exclude small solutes, including urea [7]. Thus, the reflection coefficient for most solutes in the proximal tubule membranes is close to 1.

We have recently demonstrated that the apical membrane of rabbit proximal tubules has a developmental increase in AQP1 expression [8]. The change in water channel expression was reflected in characteristics of osmotic water flow. The neonatal brush border membrane vesicles (BBMV) had a greater temperature dependence and less inhibition with HgCl_2 than the adult BBMV [8]. Thus, there was a higher fraction of water transport occurring through the lipid bilayer than through the water channel in the neonatal compared to the adult membranes [8]. This may impact the route for solute transport which could be measured as a difference in the solute reflection coefficient. The purpose of the present study was to examine directly the maturation of proximal tubule apical membrane solute reflection coefficients using BBMV.

Methods

Preparation of BBMV

BBMV were prepared from adult (>12 weeks old) or neonatal (9–11 days old) New Zealand white rabbits in our laboratory as previously described [8, 9]. Briefly, kidneys were removed from freshly sacrificed animals, kept in ice-cold PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4 , 1.7 mM KH_2PO_4 , pH 7.4) and divided into 1.5- to 2-gram portions which were placed immediately in 15 ml ice-cold isolation buffer (300 mM *D*-mannitol, 16 mM HEPES, 5 mM EGTA, adjusted to pH 7.5 with Tris-base; 2 $\mu\text{g}/\text{ml}$ each of aprotinin and leupeptin and 100 $\mu\text{g}/\text{ml}$ phenylmethylsulfonyl fluoride were added immediately before use). Tissues were homogenized with 15 strokes using a Potter homogenizer at 4°C. The homogenate was added to 20 ml ice-cold water to induce hypotonic shock and 600 μl of 1 M MgCl_2 to precipitate cell debris. The homogenate was kept on ice for 20 min, vigorously shaken for approximately 10 s every 5 min and then centrifuged for 15 min at 2,500 g, 4°C. The supernatant was added to fresh centrifuge tubes containing 600 μl of 1 M MgCl_2 , which were then shaken and centrifuged as before. The subsequent supernatant was then centrifuged for 30 min at 48,000 g, 4°C. Pellets from a single adult animal or from one litter of neonates were combined and resuspended in ice-cold buffer (5 mM HEPES, Tris-base to pH 7.5, *D*-mannitol to 80 mOsm as measured on an Advanced Instruments Osmometer) with 23- and 25-gauge needles. Vesicles were resuspended at approximately 10–20 mg protein/ml, after which protein concentration was determined by BCA assay (Pierce; Rockford, Ill., USA). Alkaline phosphatase activity was measured in the crude homogenate and the BBMV preparation to assess the enrichment as previously described in our laboratory [8, 9]. There was no difference between the adult ($n = 6$) and neonatal ($n = 5$) BBMV alkaline phosphatase enrichment (fold increase: 6.1 ± 0.9 vs. 5.3 ± 0.5 , respectively, $p = \text{NS}$).

Rapid Kinetics for Reflection Coefficient Measurement

Experiments were performed using a stop-flow instrument to ensure rapid mixing of vesicles and extravesicular buffer as previously described [8]. Vesicles were loaded with a solution containing 80 mM mannitol and 16 mM Tris (pH = 7.4). The extravesicular solution was the same as the intravesicular solution to which 200 mOsm/kg water of mannitol or test solute was added for a final concentration of 280 mOsm/kg water. Because the refractive index of the solutions will affect the light-scattering results, the refractive index of each solution was corrected to that of the mannitol solution by the addition of polyvinylpyrrolidone (PVP) [10]. Figure 1 shows the effect of PVP on the refractive index. This curve was used for determining the amount of PVP to add to each solution. The final refractive index for all test solutions was 1.3386–1.3393.

The stopped flow apparatus (SFM-3, BioLogic, dead time ~7.4 ms) was set to mix 100 µl of vesicles 1:1 (final concentration of 0.3 mg protein/ml) with extravesicular buffer so that the final osmotic gradient was 100 mOsm/kg water. Experiments were performed at 25°C. Excitation was from a 75-watt xenon arc lamp via a monochromator set at 400 nm, and emission was measured via a monochromator set at 400 nm using a Photon Technology Incorporated fluorometer (PTI; Monmouth Junction, N.J., USA). Data were collected at 200 points/s for 2 s using Biokine software (Molecular Kinetics; Pullman, Wash., USA). For each experiment, 15 raw tracings were collected and averaged for subsequent analysis.

The data were normalized to an initial light-scattering intensity of 1, then fit to a double exponential curve:

$$I=c_1e^{-k_1t}+c_2e^{-k_2t}+b;$$

where I is the scattered light intensity, c_i and k_i are the coefficients and rate constants, and b is the asymptote from the experimental fit. Thus, the initial slope of the curve is then:

$$\text{slope}=c_1k_1+c_2k_2.$$

The reflection coefficient was then determined by dividing the initial slope of the curve for the test solute by the initial slope of the mannitol curve for that preparation.

Data are presented as means $\pm$ SEM. Comparisons were made by unpaired t tests. Significance was determined by a $p < 0.05$.

Results

Figures 2 and 3 show typical tracings for the neonatal and adult BBMVs. Shown are the curves for mannitol and NaCl. As can be seen, the initial slopes for both solutes are identical, indicating that the reflection coefficient for NaCl is the same as that for mannitol. The asymptote for the NaCl curve is slightly less than that for mannitol. This is likely due to the greater permeability of NaCl than mannitol resulting in a partial collapse of the osmotic gradient. This will not affect the initial slope of the curve because the water permeability of

the BBMV is much greater than the permeability of the solutes tested. Thus, the initial change in volume represents only water movement [11].

The reflection coefficients for the solutes tested are shown in table 1 and in figure 4. Although there was a tendency for the neonatal BBMV reflection coefficients of NaCl, KCl and NaHCO₃ to be slightly higher than those of the adult BBMV, there was no statistically significant difference. The reflection coefficient for urea tended to be slightly lower in the neonatal than in adult BBMV but did not reach statistical significance.

None of the reflection coefficients were significantly different from 1, indicating that the solute and water transport pathways did not interact. Thus, although there may be differences in the pathway for water movement between the neonatal and adult membranes, the pathway for solute transport appears to be independent from water in both neonatal and adult membranes.

Discussion

The present study examined solute reflection coefficients of neonatal and adult rabbit proximal tubule apical membranes. Reflection coefficients were identical in neonatal and adult BBMV and were not significantly different from 1. Thus, solute and water transport pathways have no interaction in the developing as well as the mature proximal tubule apical membrane.

Transport rates for solute and volume in the immature proximal tubule are lower than those in the adult tubule [12, 13]. Studies in the guinea pig suggested one reason for the lower transport rates was that the immature proximal tubule was more permeable to mannitol than the adult tubule [14]. Thus, the paracellular pathway was thought to be 'leaky', and the increased backleak of solutes contributed to lower net transport rates in the immature tubule. A previous study in the rabbit proximal tubule demonstrated that the neonatal tubule had a higher hydraulic conductivity than the adult tubule [15]. This suggested that volume absorption in the neonatal proximal tubule could be driven in part by a higher intratubular hydrostatic pressure and could potentially drive solute transport passively by solvent drag.

For solute transport to be driven by solvent drag, the solute reflection coefficient must be less than 1 [6]. This was thought to be the case because of the higher paracellular permeability to mannitol that was found in the neonatal tubule [14]. We have previously directly examined the reflection coefficients for NaCl and NaHCO₃ in the in vitro perfused neonatal and adult proximal tubule to determine if solvent drag could account for solute transport [16]. In both neonatal and adult tubules, the NaCl and NaHCO₃ reflection coefficients were identical and were not different from 1. Thus, we had shown that there was no evidence for solvent drag in the neonatal as well as the adult proximal tubule. The adult data were consistent with other previously published results indicating that the proximal tubule solute reflection coefficients were not different from 1 [17, 18].

While the in vitro microperfusion of tubules is a powerful technique, it has some limitations. The results obtained are transepithelial measurements and cannot distinguish differences between apical and basolateral membranes and the paracellular pathway [17]. Although

there is little or no unstirred layer effect under normal flux conditions, there may be some unstirred layer effect when large gradients are imposed across the tubule [19]. The integrity of the tubule may also be compromised when large concentration gradients of salts are imposed, especially potassium chloride [20, 21]. For these reasons, the present study was performed using BBMVs to measure the reflection coefficients of the proximal tubule apical membrane.

The technique of examining osmotic shrinkage of BBMVs using stop-flow kinetics is well established [7, 22]. Solute gradients that may be toxic to an intact cell may be examined using this technique. The primary limitations are that only one membrane surface can be studied and that the paracellular pathway cannot be examined. In addition, when BBMVs are made from the renal cortex, the membranes include proximal convoluted and proximal straight tubules. Thus, comparing results to perfused tubules may be problematic. Despite these limitations, stop-flow kinetics has become a powerful tool in studying membrane transport [7, 22].

Solute reflection coefficients in adult BBMVs have been examined in rat [10, 23] and rabbit [11, 24, 25] kidneys. The initial studies in the rat renal BBMVs demonstrated that the reflection coefficients for NaCl and KCl were 0.53 and 0.56, respectively [23]. Thus, it was thought that there was a significant interaction between these solutes and the water transport pathway which would allow for solvent drag. Subsequently, however, the investigators demonstrated that the refractive index of the solutions had a significant influence on the results. When the refractive index was adjusted with PVP, the reflection coefficient for NaCl was 1 [10]. In adult rabbit BBMVs, the reflection coefficients for NaCl and KCl were also shown to be not different from 1 [24]. The urea reflection coefficient has also been examined in adult rabbit BBMVs and was also shown to be 1 [11, 25]. Thus, the present results are in agreement with previously published results in the adult BBMVs.

In addition to the above solutes (NaCl, KCl and urea), we examined the reflection coefficient for bicarbonate. The proximal tubule reabsorbs bicarbonate preferentially over chloride so that by the end of the proximal tubule, the tubular fluid bicarbonate concentration is much lower than the chloride concentration [26]. A small difference in the reflection coefficient between NaCl and NaHCO_3 could form an effective osmotic gradient across the tubule and drive water transport [26]. We found in both the adult and neonatal BBMVs that the reflection coefficient for NaHCO_3 was identical to that of NaCl and was not different from 1. Thus, there is no evidence for the asymmetry of the chloride and bicarbonate concentrations across the epithelium to contribute to the driving force of fluid transport.

Recent evidence indicates that AQP1 may allow ion diffusion after stimulation by forskolin [27]. There has also been a suggestion that water molecules may be transported through ion channels [28]. Thus, there is a potential for either solute or solvent drag through the proximal tubule apical membrane. The present study, however, indicates that any interactions between water and ion transport must be negligible since the reflection coefficients were not different from 1.

Solute reflection coefficients have not been previously examined in the neonatal BBMV. The apical membrane of the neonatal proximal tubule has a lower expression of AQP1 than the adult proximal tubule [8]. Thus, in the neonatal membrane, the fraction of water that is transported through the water channel is lower, and the fraction of non-channel-mediated water transport is greater than in the adult membrane. The non-channel-mediated water transport appears to be simple diffusion through the lipid bilayer [7, 22]. This could potentially influence the solute reflection coefficients of the membrane. The present study demonstrated that this difference in water transport pathways does not affect the transport of solutes. The reflection coefficients in the neonatal BBMV as well as in the adult BBMV were not different from 1. Thus, there is no evidence for solvent drag through the apical membrane in the neonatal or adult proximal tubule.

In conclusion, although the process of water transport in the proximal tubule changes as the animal matures, the pathways for solute and water transport remain distinct. Thus, there is no evidence for significant solvent drag in either the neonatal or adult proximal tubule. In addition, the reflection coefficient for NaHCO_3 was identical to that of NaCl so that there was also no evidence for solute asymmetry to provide a driving force for fluid transport.

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Fig. 1. Effect of PVP on refractive index. These data were fit with a line used to adjust the test solutions to the same refractive index as the mannitol solution.

Fig. 2.

Typical tracing of neonatal BBMVs. The vesicles were exposed to a 100-mOsm/kg water osmotic gradient of mannitol or NaCl (with PVP). These curves were then fit to a double exponential to determine the initial slope.

Fig. 3.
Typical tracing of adult BBMVs. Conditions were identical to those for neonatal BBMVs in figure 2.

Fig. 4.

Solute reflection coefficients for neonatal and adult BBMV. All solutes were compared to mannitol which was assumed to have a reflection coefficient of 1. There was no difference between the neonatal and adult BBMV solute reflection coefficients. Coefficients were also not different from 1.

Table 1Solute reflection coefficients for neonatal and adult BBMV (means $\pm$ SEM)

Solute	Neonates (n = 5)	Adults (n = 6)
NaCl	1.193 $\pm$ 0.177	0.963B0.136
KCl	1.351 $\pm$ 0.314	0.989B0.086
NaHCO ₃	1.263B0.134	1.015 $\pm$ 0.174
Urea	0.842B0.125	1.024B0.198