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Water transport in neonatal and adult rabbit proximal tubules

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Abstract

We have recently demonstrated that although the osmotic water permeability (P_f) of neonatal proximal tubules is higher than that of adult tubules, the P_f of brush-border and basolateral membrane vesicles from neonatal rabbits is lower than that of adults. The present study examined developmental changes in the water transport characteristics of proximal convoluted tubules (PCTs) in neonatal (9–16 days old) and adult rabbits to determine whether the intracellular compartment or paracellular pathway is responsible for the maturational difference in transepithelial water transport. The permeability of *n*-butanol was higher in the neonatal PCT than the adult PCT at all temperatures examined, whereas the diffusional water permeability was identical. Increasing the osmotic gradient increased volume absorption in both the neonatal and the adult PCT to the same degree. The P_f was not different between the neonatal and the adult PCT at any osmotic gradient studied. To assess solvent drag as a measure of the paracellular transport of water, the effect of the osmotic gradient on mannitol and chloride transport were measured. There was no change in chloride or mannitol transport with the increased osmotic gradient in either group, indicating that there was no detectable paracellular water movement. In addition, the mannitol permeability of the neonatal PCT was found to be lower than that of the adult PCT with the isotonic bath (8.97 ± 4.01 vs. 40.49 ± 13.89 $\mu\text{m/s}$, $P < 0.05$). Thus the intracellular compartment of the neonatal PCT has a lower resistance for water transport than the adult PCT and is responsible for the higher than expected P_f in the neonatal PCT.

Keywords

proximal convoluted tubules; development; butanol permeability; diffusional water permeability; in vitro microperfusion

WATER TRANSPORT ACROSS CELLULAR membranes is a fundamental biological process that occurs either by diffusion through the lipid bilayer or by movement through specific water channels (aquaporins) (3, 11, 28). There is also evidence that water can traverse across other membrane transport proteins (12). Transport of water through the lipid

bilayer is characterized by a high activation energy ($>9 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{degree}^{-1}$) and resistance to inhibition by mercury, whereas water movement through aquaporins is characterized by a low activation energy ($\sim 4\text{--}5 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{degree}^{-1}$) and is inhibited by mercury for most aquaporin isoforms (28).

The proximal tubule of the mammalian kidney is responsible for reabsorbing the bulk of the glomerular ultrafiltrate (24). This process is nearly isosmotic because the proximal tubule has a very high water permeability, which is thought to be due to the constitutive presence of the water channel aquaporin-1 (AQP1). The importance of AQP1 to water transport by this nephron segment is evidenced by the fact that mice lacking AQP1 have a much lower rate of fluid transport than wild-type mice (25). Previous studies in the adult kidney have also indicated that most of the water transport through the proximal tubule is transcellular, not paracellular, and that the intracellular compartment may be responsible for more than one-half of the resistance to water flow (4, 16).

During renal development, there is a maturational increase in proximal tubule AQP1 expression (6, 21, 22). However, we found that the osmotic water permeability (P_f) in the neonatal rabbit proximal tubule was actually equal to or greater than that of the adult proximal tubule (20). Further examination of water transport in this epithelium demonstrated that the apical and basolateral membranes of the neonatal tubules had a lower P_f and lower expression of AQP1 than the adult membranes (21, 22). Thus the reason for the higher P_f in the neonatal tubules was unclear. This must be due to either a higher fraction of water traversing the paracellular pathway in the neonatal tubules than the adult tubules or the intracellular compartment of the neonatal tubules offering less resistance to the movement of water than the adult tubules. Because the apical and basolateral membrane water permeabilities and surface areas of the adult and neonatal proximal tubules are known (10), examining and modeling the developmental changes in water transport could facilitate our understanding of neonatal epithelial water transport.

The purpose of the present study was to determine whether there are differences between the neonatal and adult proximal tubule intracellular compartments or paracellular pathways that explain the high neonatal transepithelial water permeability despite the low P_f of the apical and basolateral membranes.

METHODS

Juxtamedullary proximal convoluted tubules (PCTs) from adult and neonatal (9–16 days old) New Zealand White rabbits were perfused in vitro as previously described (7, 18). Briefly, PCTs were dissected in cooled (4°C) modified Hanks' solution containing (in mM) 137 NaCl, 5 KCl, 0.8 MgSO_4 , 0.33 Na_2HPO_4 , 0.44 KH_2PO_4 , 1 MgCl_2 , 10 Tris-HCl, 0.25 CaCl_2 , 2 glutamine, and 2 L-lactate. This solution was bubbled with 100% O_2 and had a pH of 7.4. Tubules were then transferred to a 1.2-ml thermostatically controlled bathing chamber and perfused with concentric glass pipettes. The perfusion solution simulated late proximal tubule fluid and contained (in mM) 140 NaCl, 5 KCl, 4 Na_2HPO_4 , 1 CaCl_2 , 1 MgCl_2 , and 1 Na butyrate (20). The bathing solution was identical, except it also contained 6 g/dl of albumin. All solutions were bubbled with 100% O_2 and had a pH of 7.4.

Osmolalities of the perfusion and bathing solutions were adjusted to 295 mosmol/kgH₂O by the addition of water or NaCl. The bathing solution was exchanged at a rate of 0.5 ml/min to keep the osmolality and pH constant. For determining the effect of increased osmolality in the bath, raffinose was added to the bathing solution to increase the osmolality.

Volume absorption (J_v ; in nl·min⁻¹·mm⁻¹) was measured as the difference between the perfusion and collection rates and normalized per millimeter of tubule length. These experiments were performed at 37°C. The collection rate was determined by timed collections by using a constant-volume pipette. Exhaustively dialyzed methoxy-[³H]inulin (New England Nuclear) was added to the perfusate at a concentration of 50 µCi/ml so that the perfusion rate could be calculated. The perfusion rate was between 25 and 30 nl/min for these experiments.

The P_f was calculated from the following equation (1)

$$P_f = - \frac{\dot{V}_0 C_0}{A \bar{V}_w} \left[\frac{C_0 - C_L}{C_0 C_b C_L} + \frac{1}{(C_b)^2} \ln \frac{(C_L - C_b) C_0}{(C_0 - C_b) C_L} \right]$$

where $\dot{V}_0$ is the perfusion rate; C_0 , C_L , and C_b are the osmolalities of the perfusate, collected fluid, and bathing solution, respectively, and L is the tubule length; A is the tubule inner surface area; and $\bar{V}_w$ is the molar volume of water. C_L is determined from the relationship $\dot{V}_0 C_0 = \dot{V}_L C_L$, where $\dot{V}_L$ is the collection rate measured by using a constant-volume pipette.

[¹⁴C]mannitol was added to the perfusate to measure the effect of bath osmolality on the flux of mannitol. The flux of mannitol was calculated as

$$J_{\text{mannitol}} = \frac{\dot{V}_0 C_0^* - \dot{V}_L C_L^*}{L} \cdot \frac{C_0}{C_0^*}$$

and the permeability to mannitol was calculated from the following equation (16, 19)

$$P_{\text{mannitol}} = \frac{\frac{\dot{V}_0 C_0^* - \dot{V}_L C_L^*}{L}}{\frac{C_0^* - C_L^*}{\ln \left(\frac{C_L^*}{C_0^*} \right)}}$$

where $\dot{V}_0$ and $\dot{V}_L$ represent the perfusion and collection rates, respectively, C_0^* and C_L^* represent the ¹⁴C counts in the perfusate and collected fluid, respectively, and C_0 represents the mannitol concentration in the perfusate. Mannitol permeability was normalized to the inner surface area of the tubule by using the inner diameter. The tubule length and inner diameter were measured with an eyepiece micrometer.

The transport of chloride was measured by determining the chloride concentration in the perfusate and collected fluid by using the Ramsey technique as previously described by our laboratory (23, 26). The absorption of chloride was calculated as

$$J_{\text{Cl}} = \frac{\dot{V}_0 C_0 - \dot{V}_L C_L}{L}$$

where C_0 and C_L represent the chloride concentration in the perfusate and collected fluid, respectively.

In a separate set of experiments, *n*-butanol permeability was measured at 40, 32, 25, and 20°C by adding ^{14}C -labeled *n*-butanol to the perfusate at a concentration of 15–20 $\mu\text{Ci/ml}$. The diffusional water permeability (P_{DW}) of the tubule was measured by adding $^3\text{H}_2\text{O}$ to the perfusate at a concentration of 50 $\mu\text{Ci/ml}$. These permeabilities were calculated by the following equation

$$P_x = \left(\frac{\dot{V}_L}{A} \right) \cdot \ln \left(\frac{X_0}{X_L} \right)$$

where P_x represents the diffusional permeability of butanol or water, and X_0 and X_L represent the counts for butanol or water in the perfusate and collected fluid, respectively.

All data are expressed as means $\pm$ SE. Comparisons were made by using unpaired analysis (Student's *t*-test and linear regression where appropriate), and significance was taken to be $P < 0.05$. Calculations were made with SigmaStat software (Jandell Scientific).

RESULTS

P_f . Figure 1 shows the effect of increasing bath osmolality on the rate of adult and neonatal PCT volume absorption. As can be seen, the volume absorption rate increased with an increase in bath osmolality for both the adult ($n = 33$) and the neonatal tubules ($n = 31$). There was no difference between the rates of volume absorption in the adult and neonatal tubules.

The P_f of adult and neonatal tubules is shown in Fig. 2. At each osmotic concentration gradient examined, there was no difference between the adult ($n = 18$) and neonatal ($n = 20$) PCT P_f .

***n*-Butanol permeability and P_{DW}**

To assess the contribution of the intracellular compartment to the movement of water, the diffusive permeability to *n*-butanol and water were measured simultaneously. *n*-Butanol is a lipophilic substance that has been previously used to examine the contribution of the intracellular compartment to the resistance to water flow (4). As seen in Fig. 3, the permeability of the neonatal tubules ($n = 7$) to *n*-butanol was higher than that of the adult tubules ($n = 6$) at all temperatures examined except for 20°C. Thus the resistance of the intracellular compartment to the movement of water is lower in the neonatal tubules than the adult tubules at physiological temperatures.

The P_{DW} of adult and neonatal tubules were the same at each temperature studied (Fig. 4). This is identical to our previous study in the development of proximal tubule water transport (20). We then applied the analysis of Berry (4) to examine the P_{DW} of the membrane component. The P_{DW} of the membrane [$P_{DW(\text{membrane})}$] was calculated from the following equation

$$P_{DW(\text{membrane})} = \left\{ \left(\frac{1}{P_{DW(\text{tubule})}} \right) - \left[\frac{\left(\frac{D_{NB}}{P_{DNB(\text{tubule})}} \right)}{D_W} \right] \right\}^{-1}$$

where D_{NB} and D_W are the free diffusion constants of *n*-butanol and water, respectively (15, 29).

The $P_{DW(\text{membrane})}$ of neonatal tubules ($n = 7$) was lower than that of the adult PCTs ($n = 6$), as shown in the Arrhenius plot in Fig. 5. In addition, the slope of the Arrhenius plot was higher for the neonatal tubules than for the adult tubules. Thus the activation energy for $P_{DW(\text{membrane})}$ was higher in the neonatal tubules than in the adult membranes (5.92 ± 0.77 vs. 3.02 ± 1.08 kcal-degree⁻¹·mol⁻¹, $P < 0.05$). These findings are in agreement with our previous findings of a lower P_f in neonatal than adult brush-border and basolateral membranes (21, 22).

Paracellular pathway

We next examined the paracellular pathway to determine whether it is a contributing factor in explaining the high transcellular P_f in the neonatal tubules despite the low $P_{DW(\text{membrane})}$. Paracellular transport of water is difficult to measure directly (27). One approach is to induce osmotic water flow with an impermeable solute and measure the solvent drag of small particles. The assumption is that if water moves between the cells, it will carry small particles with it (27). We measured the flux of mannitol as a nonionic solute and chloride as a small ion and determined their flux in response to an increasing osmotic gradient. As shown in Fig. 6, the transport of mannitol was the same in both adult ($n = 33$) and neonatal ($n = 31$) tubules except for the isotonic bath (300 mosmol/kgH₂O). In both the adult and the neonatal tubules, the transport of mannitol did not increase as the bath osmolality was increased. This would argue against solvent drag. The fact that the adult transport rate of mannitol was higher than the neonatal transport rate with the isotonic bath indicated that the mannitol permeability in the neonatal tubule was lower than that of the adult (Fig. 7).

We also examined the osmotic dependence of chloride transport to further determine characteristics of the paracellular pathway. As seen in Fig. 8, there was no difference in the rate of chloride transport between the adult ($n = 26$) and neonatal ($n = 25$) tubules. There was also very little change in the chloride transport rate as the bath osmolality increased, suggesting that there is almost no solvent drag. This is similar to previous studies in adult proximal tubules (13).

Cell height

The height of the proximal tubule cells was determined by measuring the inner and outer diameters of the tubules with an eyepiece reticle. The neonatal proximal tubule cell height ($8.6 \pm 0.5 \mu\text{m}$) was significantly smaller than the adult cell height ($11.0 \pm 0.3 \mu\text{m}$, $P < 0.005$). These values are very similar to previously reported values (2, 10). The ratio of the adult to neonatal cell heights (1.27) is identical to the ratio of the neonatal to adult *n*-butanol permeabilities at 25°C (1.26). This suggests that the primary determinant for the diffusion of butanol is the cell interior and is directly proportional to the path length through the cell.

DISCUSSION

The proximal tubule reabsorbs the bulk of the glomerular ultrafiltrate by a process that is nearly isosmotic because of its high transepithelial P_f (24). Hypotonicity of the luminal fluid due to active transport of solute from the lumen is thought to be the driving force for this water movement (24). Water can move through the epithelium via the transcellular pathway, which is made up of the apical membrane, the cellular compartment, and the basolateral membrane, or the paracellular pathway. In adult proximal tubules, transepithelial water transport is predominantly, if not solely, transcellular (16); however, this remains somewhat controversial (8, 24). Recently, additional evidence for the transcellular route for water transport comes from mice that lack the water channel AQP1 (9, 25). The water permeability in proximal tubules from these mice was significantly lower than in wild-type mice, indicating that most water transport must be transcellular and mediated by AQP1.

In the present study, we found that the P_f of the neonatal PCT was the same as that of the adult. These results are qualitatively similar to our previous measurements of the P_f in adult and neonatal tubules at these perfusion rates (20). However, in our previous study, the maximum P_f for each tubule was determined by increasing the perfusion rate and extrapolating to an infinite perfusion rate. This approach was not feasible in the present study because of the use of multiple bath osmolalities for each tubule. Thus the methodology for calculating the P_f was different and may have a direct effect on the calculated values (3). In the present study, the P_f of the neonatal tubules was not statistically different from the adult tubules, as was found in our previous study (20) at an infinite perfusion rate.

Because the transcellular route for water movement consists of the apical and basolateral membranes and the intracellular compartment, any differences in transepithelial water permeability must be due to differences in one or more of these components. We have previously demonstrated that the apical and basolateral membranes of the neonatal tubule have a lower P_f than the adult tubule (21, 22). The expression of AQP1 in the neonatal membranes was also found to be significantly lower than that of the adult membranes. In the present study, the P_{DW} of the tubule membranes was shown to be lower in the neonatal tubules than the adult tubules. In addition, the $P_{\text{DW(membrane)}}$ in the neonatal tubules had a higher activation energy than the adult tubules that our laboratory has previously found in the apical membrane (22). Thus the water permeabilities of the cellular membranes of the proximal tubule would contribute to a lower P_f for the neonatal tubule than the adult tubule.

The remaining component of transcellular water movement is the intracellular compartment. The cytoplasmic compartment is a complex unstirred layer (5) and may account for >50% of the transcellular resistance to water movement (4). Thus small changes in the cellular compartment may significantly affect transepithelial water permeability. We found that the cell height of the neonatal proximal tubule was significantly lower than that of the adult proximal tubule, as previously described by our laboratory and others (2, 10). This suggests that the path length for water movement through the cell is much shorter in the neonatal proximal tubule than the adult. To assess this more directly, we measured the permeability of *n*-butanol, a lipophilic small molecule that would have a very high permeability through the membranes (4). The *n*-butanol permeability was higher in the neonatal tubules, thus offering evidence that the intracellular pathway of water transport in the neonatal tubule has less resistance to the movement of water than the adult tubule. Interestingly, the ratio of the adult to neonatal cell heights was equal to the ratio of the neonatal to adult *n*-butanol permeability. Thus the higher than expected transepithelial P_f in neonatal proximal tubules may be due to developmental changes in the intracellular compartment.

The remaining component of the transepithelial pathway for water movement to be examined is the paracellular pathway. This component is difficult to measure directly (27); thus we made several indirect measurements to characterize the paracellular pathway in the adult and neonatal tubules. First, we found that the neonatal tubules had a lower mannitol permeability than the adult tubules. This is contrary to previous studies that indirectly determined the mannitol permeability of the neonatal proximal tubule to be higher than that of the adult tubule in the guinea pig (14). However, this is in agreement with developmental changes in permeabilities of other solutes. We have previously shown that the permeabilities of bicarbonate and chloride are also lower in the neonatal tubule than the adult tubule (17, 26). Second, there was no change in the flux of mannitol when an osmotic gradient was imposed on the tubules. Thus there is no evidence for the solvent drag of mannitol, a small nonionic solute, in either the neonatal or the adult tubules. Third, there was also no influence of the osmotic gradient on the flux of chloride in either the adult or the neonatal tubules. This is similar to the results obtained by Jacobson et al. (13) in adult tubules. Thus there was no solvent drag for chloride, a small ion. Taken together, these data argue against any appreciable water movement through the paracellular pathway in the neonatal or the adult tubules.

The differences between the adult and neonatal proximal tubules are illustrated in Fig. 9. We have previously shown that the apical and basolateral membranes of the neonatal proximal tubule have less AQP1 and a lower P_f than the adult membranes (21, 22). The present study shows that the intracellular compartment of the neonatal tubules provides less resistance to the movement of water than that of the adult tubule. The low water permeability of the neonatal tubule cell membranes is offset by the high permeability of the cell compartment, making the overall epithelial water permeability the same as that of the adult tubule. Thus the intracellular compartment of the proximal tubule plays a significant role in the overall water permeability of the tubule.

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Fig. 1.

Osmotic gradient dependence of neonatal and adult proximal convoluted tubule (PCT) volume absorption. Tubules were perfused with a solution adjusted to 300 mosmol/kgH₂O. Bathing solution was identical except for 6 g/dl of albumin and raffinose to increase the osmolality. As can be seen, there was no difference in volume absorption between the adult and neonatal tubules.

Fig. 2. Osmotic gradient dependence of osmotic water permeability (P_f) in adult and neonatal PCT. Data from Fig. 1 were used to calculate the P_f . There was no significant difference between the P_f of the adult and neonatal tubules at any bath osmolality.

* $p < 0.05$ Neonatal vs Adult PCT

** $0.05 < p < 0.10$ Neonatal vs Adult PCT

Fig. 3.

Neonatal and adult PCT butanol permeability (P_{DNB}). Tubules were perfused with a solution containing ^{14}C -labeled *n*-butanol and $^3\text{H}_2\text{O}$. P_{DNB} of the neonatal tubules was higher than that of the adult tubules at each temperature except for 20°C.

Fig. 4.
Temperature dependence of neonatal and adult PCT diffusional water permeability (P_{DW}).
There was no difference between the adult and neonatal tubule P_{DW} at any temperature.

Fig. 5.

P_{DW} of the membrane [$P_{DW(membrane)}$]. P_{DW} of the tubule membrane was calculated from the simultaneous measurement of P_{DW} and P_{DNB} . $P_{DW(membrane)}$ of the neonatal tubule was lower than that of the adult at all temperatures studied. Slope of the Arrhenius plot is also greater for the neonatal tubules, indicating that the neonatal membranes have a higher activation energy for water transport than the adult.

Fig. 6.

Osmotic gradient dependence of mannitol transport (J_{mannitol}). Tubules were perfused with a perfusion solution as in Fig. 1 containing [^{14}C]mannitol. There was no change in J_{mannitol} as the bath osmolality was increased.

Fig. 7.

Developmental changes in mannitol permeability (P_{mannitol}). Tubules were perfused with [^{14}C]mannitol to measure the permeability of mannitol with an isosmotic bath. P_{mannitol} was significantly lower in the neonatal PCT compared with the adult PCT.

Fig. 8.

Osmotic gradient dependence of chloride transport. Tubules were perfused as in Fig. 1, and chloride transport was measured. There was a small decrease in chloride transport in the neonatal tubules at the highest osmotic gradient examined.

Fig. 9.

Model of proximal tubule water transport. Transcellular transport of water is depicted as the apical and basolateral membranes in series with the intracellular compartment. AQP1, aquaporin-1.