Potassium permeability in the absence of fluid reabsorption in proximal tubule of the anesthetized rat

ROD W. WILSON, MARK WAREING, JON KIBBLE, and ROGER GREEN

1School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT; 2Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS; and 3Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, United Kingdom

Wilson, Rod W, Mark Wareing, Jon on Kibble, and Roger Green. Potassium permeability in the absence of fluid reabsorption in proximal tubule of the anesthetized rat. Am. J. Physiol. 274 (Renal Physiol. 43): F1109–F1112, 1998.—A luminal microperfusion technique was used to examine the K⁺ permeability of surface proximal convoluted tubules (PCT) in the kidney of anesthetized rats. Transtubular potassium concentration ([K⁺⁺]) gradients were varied by altering the concentration of KCl in luminal perfusates, to which 32 mmol/l of the impermeant solute raffinose was also added to prevent net fluid reabsorption. The arithmetic mean transtubular [K⁺⁺] gradient was highly predictive of net potassium flux, yielding an apparent K⁺⁺ permeability of 31.9 ± 1.7 × 10⁻⁵ cm/s in the absence of fluid reabsorption. When compared using identical calculation techniques, we found this was not significantly different from the permeability derived in a previous study when fluid reabsorption was present [J. D. Kibble, M. Wareing, R. W. Wilson, and R. Green. Am. J. Physiol. 268 (Renal Fluid Electrolyte Physiol. 27): F778–F783, 1995]. We conclude that fluid reabsorption does not affect the apparent permeability of the proximal tubule to potassium. The apparent permeability to ⁸⁶Rb, measured following its addition to luminal perfusates, was not significantly different from the value obtained for K⁺⁺, suggesting that rubidium is a useful marker for net potassium movements in the PCT of the rat.

microperfusion; potassium transport; rubidium-86; diffusion; solvent drag

THE RENAL PROXIMAL TUBULE reabsorbs some 50–70% of the filtered potassium load. However, there has been no consensus as to the mechanisms by which this transport is effected. Recently, we have attempted to address this problem by examining the potential driving forces (diffusion, convection, and active transport) involved in potassium reabsorption in the proximal convoluted tubule (PCT) of the rat (14, 24, 27).

The component of K⁺⁺ reabsorption due to diffusion is dependent on the electrochemical gradient and the epithelial permeability. Recent data from our laboratory support the view that under free-flow conditions the K⁺⁺ activity in the proximal tubular fluid exceeds that in the plasma by ~10% (15, 22). Additionally, the transepithelial potential difference is about +2 mV (lumen positive) in the S2 and S3 segments of the proximal tubule (9, 21). These data suggest that the electrochemical potential can support diffusive potassium reabsorption in the PCT providing the potassium permeability (Pₓ) is sufficiently high.

We have previously calculated an apparent potassium permeability of 22 × 10⁻⁵ cm/s for the PCT of the anesthetized rat (14). This estimate was based on the change in net potassium fluxes resulting from manipulation of the transepithelial chemical gradient for potassium and was measured in the presence of normal fluid reabsorption rates (~2.5 nl·mm⁻¹·min⁻¹). Strictly speaking, true permeability can only be derived in the absence of any driving force. Because of this, it is typically measured with unidirectional isotopic fluxes. We have recently shown that fluid reabsorption can effect significant potassium transport by solvent drag (25), which has highlighted the need to perform permeability studies for potassium in the absence of normal fluid reabsorption. Additionally, we have recently used the calculated apparent Pₓ value (14) to estimate the impact of diffusion on solvent drag (i.e., pseudo-solvent drag; Ref. 25). The reverse estimation (i.e., the impact of solvent drag on diffusion) now needs to be applied but can only be done if the Pₓ value used can be shown to be independent of fluid flux rates. Experiments where fluid flux has been altered usually depend on imposed transtubular osmolar gradients, which have been shown to alter proximal tubular ultrastructure (17, 18), that could potentially alter the permeability of the paracellular and/or transcellular pathways.

The present study had two main objectives: 1) to compare our previously derived value for apparent Pₓ with the value measured in the absence of net fluid transport and 2) to evaluate the use of ⁸⁶Rb as a marker for net potassium movements in the PCT of the anesthetized rat.

MATERIALS AND METHODS

Continuous microperfusion experiments were performed on male Sprague-Dawley rats (190–280 g). Anesthesia was induced with sodium thiopentone (Intraval sodium, 100–110 mg/kg ip; May & Baker). Once a satisfactory level of anesthesia was achieved (assessed by the absence of pinch and corneal reflexes) the animal was placed on a thermostatically controlled table set to maintain body temperature at 37°C. The animal was prepared for micropuncture as described by Green and co-workers (12). Kidneys with a proximal tubule transit time (23) greater than 12 s upon completion of surgery were rejected, as were animals with a mean arterial blood pressure below 100 mmHg. Hematocrit and plasma osmolality were determined from a blood sample (280 µl) taken from the carotid artery catheter upon completion of surgery.

Three experimental series were conducted to investigate the permeability of the proximal tubule to potassium and ⁸⁶Rb under conditions of zero net fluid transport. Methods for the continuous microperfusion of individual nephron segments have been described previously (1). In the present study, we perfused PCT with their normal peritubular blood
supply intact. Upon completion of the experiment, the perfused sections of tubule were filled with a silicone rubber solution (Microfil; Flow Tek, Boulder, CO). The micropunctured kidney was removed and stored overnight in deionized water at 4°C. A terminal blood sample (2–4 ml) was taken via the carotid artery catheter, and the animals were subsequently overdosed with anesthetic. The length of perfused nephron was determined from dissection of the silicone rubber casts as has been described previously (12).

PCT were perfused at 25 nl/min with a physiological solution containing (in mmol/l) 153 NaCl, 5.5 NaHCO₃, 0.55 CaCl₂, 32 raffinose, 0.05% erioglaucine dye, and [³H]julin in at 50 µCi/ml (gassed with 95% O₂-5% CO₂ to pH 6.8). Three different perfusate concentrations of KCl (4.5, 2.2, and 0 mmol/l) were used to create a range of [K⁺] in the perfusion fluid and peritubular plasma. For the perfusate containing 4.5 mmol/l KCl, 10 µCi/ml of [³H]julin and [⁸⁶Rb] was added. For each animal, perfusate osmolality was adjusted to be 32 mosmol/kg H₂O higher than systemic plasma, which has been shown previously to reduce net fluid reabsorption to zero (25).

The volume of the collected fluid (in nl) was measured using a calibrated constant-bore capillary tube. [³H]julin and [⁸⁶Rb] in the perfuse and collected fluids were measured by liquid scintillation counting. The concentrations of Na⁺ and K⁺ in the perfusate and collected fluids were measured by electrothermal atomic absorption spectrophotometry (Perkin-Elmer Zeeman 3030) using a previously described protocol (22). The Na⁺ and K⁺ in ultrafiltrates of plasma (Centrifer micropartition system; Amicon) were measured by flame photometry (Corning 480). Plasma and perfusate osmolalities were determined by freezing point depression (Roelbling; Camlab).

Fluid reabsorptive rate (Jᵥ, nl·mm⁻¹·min⁻¹) was calculated using the following equation

\[ Jᵥ = V_p (1 - L/n_p/n_c) / L \]

where \( V_p \) is the tubular perfusion rate (nl/min), \( n_p \) and \( n_c \) are the concentrations of [³H]julin in perfused and collected fluids, respectively, and \( L \) is tubule length (in mm). Net ion fluxes were calculated using the following equation

\[ Jxo = V_p (C_{xp} - C_{xc}(n_p/n_c)) / L \]

where \( Jxo \) is net ion transport (pmol·mm⁻¹·min⁻¹), and \( C_{xp} \) and \( C_{xc} \) are concentrations of substance \( x \) in the perfusion solution and the collected fluid, respectively.

Initial and final potassium concentration gradients (\( \Delta[K⁺] \)) were calculated by subtracting the plasma ultrafiltrate [K⁺] from the [K⁺] in the luminal perfusate and collected fluid, respectively. The [K⁺] in plasma ultrafiltrates was used in preference to whole plasma [K⁺], as the former has been shown to give a good estimate of true K⁺ activity in plasma (15, 22). However, the luminal K⁺ concentration changes along the perfused length of tubule as a result of the dissipation of any applied diffusion gradients (14). Because this dissipation is thought to be exponential, a geometric mean gradient has sometimes been used by first calculating the geometric mean for the luminal potassium concentration and then subtracting it from the ultrafilterable [K⁺] (14).

In the present study, this can give a distorted estimate of the mean luminal potassium concentration, since in some series, one of the values comprising the mean is close to zero (as in series 3 where the nominal perfusate concentration of potassium was 0). To avoid this distortion, we elected to use the arithmetic mean of the initial and final potassium concentration in calculating the average gradient along the perfused tubule (whether arithmetic or geometric means are used does not affect the conclusions, providing all data in the comparison have been calculated using identical methods).

Apparent permeabilities were estimated from the slope of net potassium (or [⁸⁶Rb]) flux vs. mean potassium (or [⁸⁶Rb]) concentration gradient (assuming a tubule diameter of 28 µm; Ref. 17). In addition, the tracer permeability for [⁸⁶Rb] (in cm/s) was estimated for individual tubules using the following formula for tubules with zero net volume flux (24)

\[ P = \frac{V_p}{2\pi r L} \times \ln \left( \frac{c_2}{c_1} \right) \]

where \( r \) is luminal radius (14 µm); \( L \) is length of perfused segment of tubule; and \( c_1 \) and \( c_2 \) are the concentrations of [⁸⁶Rb] at the beginning and end of the perfused segment, respectively.

Statistical significance among the three groups perfused with different concentrations of potassium was assessed using one-way analysis of variance followed by the Scheffé post hoc test. Statistical significance between the slopes and intercepts of linear regressions was assessed using Student’s t-test (29). However, Jᵥ was not perfectly reduced to zero in the present study. Therefore, to factor out the influence of small variations in Jᵥ on JK within the data sets (present study) and without raffinose added (Ref. 14), Jᵥ was regressed against Jᵥ. The residual variations in JK (i.e., the Jᵥ-independent variations in JK) were then analyzed by ANCOVA with the presence of raffinose as a fixed effect and K⁺ gradient as covariate. In the ANCOVA, a significant interaction term indicates a difference in the relationship between the Jᵥ-independent K⁺ flux and the K⁺ gradient that depends on the presence of raffinose (i.e., effectively a real difference in the apparent K⁺ permeability estimates). Values for ion and fluid fluxes, tubule length, and [³H]julinin recovery are presented as means ± SE throughout the text, where \( n \) is number of tubules unless otherwise stated.

### RESULTS

Mean values for percentage recoveries of [³H]julinin, tubule length, and net ion and fluid flux rates for the three series are presented in Table 1. In no series were significant differences found between the groups for these variables.

<table>
<thead>
<tr>
<th>Series</th>
<th>n</th>
<th>Initial Perfusate [K⁺]</th>
<th>Tubule Length (mm)</th>
<th>[³H]julinin Recovery (n)</th>
<th>Jᵥ</th>
<th>Jₓo</th>
<th>Jₘo</th>
<th>Jₓc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series 1</td>
<td>6</td>
<td>4.37 ± 0.02a</td>
<td>2.53 ± 0.29</td>
<td>102.1 ± 1.2</td>
<td>-0.12 ± 0.19</td>
<td>198 ± 48</td>
<td>3.1 ± 1.0b</td>
<td></td>
</tr>
<tr>
<td>Series 2</td>
<td>5</td>
<td>2.20 ± 0.01b</td>
<td>2.18 ± 0.16</td>
<td>98.2 ± 2.2</td>
<td>-0.41 ± 0.20</td>
<td>171 ± 33</td>
<td>-23.1 ± 2.3b</td>
<td></td>
</tr>
<tr>
<td>Series 3</td>
<td>5</td>
<td>0.01 ± 0.00c</td>
<td>1.78 ± 0.17</td>
<td>100.0 ± 1.8</td>
<td>-0.44 ± 0.17</td>
<td>219 ± 28</td>
<td>-50.1 ± 2.3c</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals; n = no. of tubules. Jᵥ, net fluid flux; Jₓo, net flux of ion xo. Values with different superscripts are significantly different from each other (P < 0.01).
Mated for potassium under identical conditions (i.e., in min) between series. Revealed no significant dependence of this relationship slopes or elevations. In addition, ANCOVA analysis revealed no statistically significant differences in net Na+ fluxes were observed between series.

Figure 1 shows a plot of net potassium flux vs. the arithmetic mean potassium concentration gradient for individual tubules of all three series. There was a significant linear relationship between net flux and gradient with a slope of 16.8 ± 0.9 pmol·mm⁻¹·min⁻¹·mmol⁻¹·l, equivalent to a permeability of 31.9 ± 1.7 × 10⁻⁵ cm/s. This is not directly comparable to the $P_K$ value previously calculated in the presence of normal fluid reabsorption (14). However, to allow direct comparison, the slope of the same linear regression derived from tubules with normal fluid reabsorption (using data from Ref. 14) is shown as a dotted line ($P_K = 34.4 ± 2.0 × 10^{-5}$ cm/s; calculated using the arithmetic mean transtubular concentration gradient for potassium as opposed to the geometric mean gradient used in the original study). The two lines were not significantly different from each other with respect to their slopes or elevations. In addition, ANCOVA analysis revealed no significant dependence of this relationship between $J_K$ and K⁺ gradient on the presence of raffinose ($P = 0.526$).

Figure 2 shows a similar plot for the $^{86}$Rb data from tubules in series 1. There was a significant linear relationship between the net flux and the gradient for $^{86}$Rb. The apparent permeability for $^{86}$Rb (20.2 ± 5.6 × 10⁻⁵ cm/s) was somewhat lower than that estimated for potassium under identical conditions (i.e., in the absence of fluid transport). However, there was no statistically significant difference between the regression coefficients for $J_V$ vs. K⁺ gradient and $J_K$ vs. Rb gradient ($P > 0.5$), so the apparent permeabilities for Rb and K derived from these regression relationships are not demonstrably different. For comparison, the estimated tracer permeability using Eq. 3 gave a mean $P_{Rb}$ of 26.5 ± 1.7 × 10⁻⁵ cm/s ($n = 14$).

**DISCUSSION**

The predictable linear relationship between net K⁺ fluxes and the mean transtubular gradient agrees with previous reports on rat PCT in vivo (4, 14), as well as rabbit PCT in vitro (13) and rabbit proximal straight tubule in vitro (26). The lack of effect of eliminating fluid reabsorption on the slope of net K⁺ flux vs. mean transtubular [K⁺] gradient (Fig. 1) indicates that over a physiological range of fluid fluxes (0 to 2.5 nl·mm⁻¹·min⁻¹), the apparent $P_K$ does not change significantly, even though there may be significant changes in inter- and intracellular volumes and ultrastructure (18). This justifies our use of the apparent $P_K$ in studies of solvent drag (25), in which fluid fluxes were manipulated across a similar range. It also enhances the view that potassium is one of the most permeant ions in the PCT (3, 5, 10), with the paracellular pathway being the most likely route for such a high permeation (14).

The lack of difference between the apparent permeabilities derived for $^{86}$Rb and K⁺, under identical conditions of zero fluid reabsorption, suggests that $^{86}$Rb is a reasonably valid marker for potassium in the renal proximal tubule. This is in line with previous studies demonstrating that microinjections of $^{86}$Rb into rat proximal tubules (6) gave very similar urine recoveries to that for microinjections of $^{42}$K (7). Indeed, the use of $^{86}$Rb as a tracer for potassium in clearance studies (8), as well as transport studies in rat distal tubules (16) and rabbit pars recta (28), has shown that $^{86}$Rb and K are handled similarly in various segments of the kidney.

In the present study, if we assume that the concentration of $^{86}$Rb in peritubular capillaries is zero, then the
The apparent permeability estimate for $^{86}$Rb should therefore also be representative of the unidirectional tracer permeability from lumen to capillary. We have demonstrated that eliminating fluid reabsorption does not affect the apparent permeability of the PCT to potassium and estimates for apparent potassium permeability and $^{86}$Rb tracer permeability were similar, which suggests that $^{86}$Rb may be used as a reasonable marker of potassium transport in the PCT. It should be noted that when using the potassium permeability value to predict diffusive fluxes, the value used is dependent on the method used to calculate the driving force (i.e., the mean transtubular concentration gradient for potassium as well as the arithmetic or geometric mean).

We gratefully acknowledge the financial support of the Wellcome Trust.

Address for reprint requests: R. W. Wilson, Dept. of Biological Sciences, Hatherly Laboratories, Univ. of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK.

Received 1 August 1997; accepted in final form 23 February 1998.

REFERENCES


