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

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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 (PK) 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 usually measured with unidirectional isotopic fluxes.

We have recently shown that fluid reabsorption can affect 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 PK 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 PK value used can be shown to be independent of fluid flux rates. Experiments where fluid flux has been altered usually depend on imposed transtubular osmotic gradients, which have 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 PK 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 i.p; 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]inulin 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⁺] gradients between the tubule lumen and peritubular plasma. For the perfusate containing 4.5 mmol/l KCl, 10 mMCl/ml of [⁶⁸RbCl] was added ([Rb⁺] < 90 µmol/l). For each animal, perfusate osmolality was adjusted to 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]inulin and [⁶⁸Rb] in the perfusate 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 (Centrifree 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

\[ Jᵥ = V_p(1 - \ln p/\ln n)p/L \]

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

\[ Jx = V_p[(Cxp - Cxp(ln p/ln n))/L \]

where Jx is net ion transport (pmol·mm⁻¹·min⁻¹), and Cxp and Cce are concentrations of substance x in the perfusion solution and the collected fluid, respectively.

Initial and final potassium concentration gradients (Δ[K⁺]) and Δ(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 = 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). A positive value for fluxes and gradients indicates a reabsorptive direction, whereas a negative value indicates a secretory direction.

Apparent permeabilities were estimated from the slope of net potassium (or [⁶⁸Rb] flux vs. mean potassium (or [⁶⁸Rb] concentration gradient (assuming a tubule diameter of 28 μm; Ref. 17). In addition, the tracer permeability for [⁶⁸Rb] (in cm/s) was estimated for individual tubules using the following formula for tubules with zero net volume flux (24)

\[ P = (V_p/2πrL) \times \ln (c1/c2) \]

where, r is tubular radius (14 μm); L is length of perfused segment of tubule; and c1 and c2 are the concentrations of [⁶⁸Rb] 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 of Jᵥ on Jx within the data sets with (present study) and without raffinose added (Ref. 14), Jx was regressed against Jᵥ. The residual variations in Jx (i.e., the Jᵥ-independent variations in Jx) 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]inulin recovery are presented as means ± SE throughout the text, where n = number of tubules unless otherwise stated.

**RESULTS**

Mean values for percentage recoveries of [³H]inulin, tubule length, and net ion and fluid flux rates for the three series are presented in Table 1. In no series were

<table>
<thead>
<tr>
<th>Tubule Length</th>
<th>[³H]inulin Recovery, %</th>
<th>Jᵥ, nl·mm⁻¹·min⁻¹</th>
<th>Jₓ, pmol·mm⁻¹·min⁻¹</th>
<th>Jₚ, pmol·mm⁻¹·min⁻¹</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>
</tr>
<tr>
<td>Series 2</td>
<td>5</td>
<td>2.20 ± 0.03b</td>
<td>2.18 ± 0.16</td>
<td>98.2 ± 2.2</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>
</tr>
</tbody>
</table>

Values are means ± SE; N = no. of animals; n = no. of tubules. Jᵥ, net fluid flux; Jₓ, net flux of ion x. Values with different superscripts are significantly different from each other (P < 0.01).
Mated for potassium under identical conditions (i.e., in min) revealed no significant dependence of this relationship slopes or elevations. In addition, ANCOVA analysis significantly different from each other with respect to their in the original study). The two lines were not signifi-
cant differences in net Na\textsuperscript{+} fluxes were observed be-
tween 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 $16.8 \pm 0.9 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1} \cdot \text{mmol}^{-1} \cdot \text{l}$, equivalent to a permeability of $31.9 \pm 1.7 \times 10^{-5} \text{ cm/s}$. 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 ($P_K = 34.4 \pm 2.0 \times 10^{-5} \text{ cm/s}$; 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 $J_V$ 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 mean gradient for $^{86}$Rb. The apparent permeability for $^{86}$Rb ($20.2 \pm 5.6 \times 10^{-5} \text{ cm/s}$) 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 $J_Rb$ 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 $26.5 \pm 1.7 \times 10^{-5} \text{ cm/s} (n = 14)$.

**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$^{-1}$·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 kid-

In the present study, if we assume that the concentration of $^{86}$Rb in peritubular capillaries is zero, then the

\[
\begin{align*}
\text{Normal } J_V \\
y &= 18.1(x) - 5.1 \\
n &= 49, r = 0.928 \\
P_K &= 34.4 \times 10^{-5} \text{ cm/s}
\end{align*}
\]

\[
\begin{align*}
\text{Zero } J_V \\
y &= 16.8(x) - 6.0 \\
n &= 33, r = 0.961 \\
P_K &= 31.9 \times 10^{-5} \text{ cm/s}
\end{align*}
\]
net $^{86}$Rb flux is, by definition, also the unidirectional flux from lumen to capillary (20). Our apparent permeability estimate for $^{86}$Rb should therefore also be representative of the unidirectional tracer permeability from lumen to capillary. The similarity of this value and the tracer permeability derived from Eq. 3 is therefore perhaps not surprising. In addition, the tracer permeability value for $^{42}$K of 17.8 $\times$ 10$^{-5}$ cm$^2$/s for the rat proximal tubule (from Refs. 2 and 24) translates to a value almost identical to our $^{86}$Rb apparent permeability estimate, when converted to the same units (20.3 $\times$ 10$^{-5}$ cm$^2$/s; assuming a tubule diameter of 28 $\mu$m).

In conclusion, we have demonstrated that 1) eliminating fluid reabsorption does not affect the apparent permeability of the PCT to potassium and 2) 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 either the arithmetic or geometric mean).

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