Electrical resistance of renal proximal tubule perfused in vitro

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LUTZ, MICHAEL D., JEAN CARDINAL, AND MAURICE B. BURG. Electrical resistance of renal proximal tubule perfused in vitro. Am. J. Physiol. 225(3): 729-734. 1973.—Two techniques to measure potential differences (PD) and electrical resistance in isolated renal proximal tubules of the rabbit are described. Cable analysis was used to calculate the transepithelial resistance ($R_T$). The applicability of cable analysis to the proximal tubule was examined and confirmed. In proximal convoluted tubules, mean PD was $-3.8$ mv (lumen negative) and mean $R_T$ was $964$ $\Omega$cm. In proximal straight tubules, mean PD was $-2.0$ mv and mean $R_T$ was $1,188$ $\Omega$cm. The low electrical resistance of the proximal tubule reflects the high permeability of this nephron segment to electrolytes. By comparing the calculated short-circuit current with the net Na flux, it is concluded that Na is transported actively in the proximal tubule. Ouabain, consistent with its inhibition of Na transport, eliminated the PD while simultaneously increasing the $R_T$.

potential difference; ouabain; cable analysis; short-circuit current

CONDUCTIMETRIC METHODS have long been used to characterize permeability properties of epithelial membranes. These methods are generally simpler than those requiring flux measurements with isotopes. Electrical potential differences (PD) and resistance have been measured in renal tubules in a variety of species (21). In mammalian proximal tubules, the measurement of PD, until recently (1, 4), has been hampered by the difficulty of localizing the electrode tip within the tubule lumen (8, 10). The cylindrical shape of the tubule has made electrical resistance measurements difficult because a uniform current density across the epithelium cannot be achieved using micropipets as a current source. In order to circumvent this difficulty, electric current was introduced into very short oil-blocked segments of tubule in order to achieve a uniform PD across the epithelium (7, 9) or cable analysis was applied to the measurement of intraluminal PD at different distances from the current source (3, 9, 11, 12).

Using the technique of isolated tubule perfusion, Helman, Grantham, and Burg (19) applied cable analysis in measuring resistance in the rabbit cortical collecting tubule and confirmed its validity. Their use of a single pipet with a bridge circuit to both pass current and measure voltage was satisfactory because the electrical resistance of the collecting tubule was of the same order of magnitude as that of the perfusion pipet. In contrast, the electrical resistance of the proximal tubule is an order of magnitude lower than that of the pipet, making it difficult to accurately measure resistance with a bridge circuit.

In the present study, two techniques were developed to measure electrical resistance and PD in the proximal nephron. Cable analysis was employed and its validity as applied to the proximal tubule was confirmed. The effect of ouabain on transepithelial resistance was studied using one of the techniques described.

METHODS

Proximal convoluted and straight tubules were dissected and perfused with ultrafiltrate of rabbit serum under conditions described previously in detail (4).

Electrical resistance and potential difference were measured using two different pipet arrangements. Figure 1 illustrates the first pipet arrangement used. The tubule was attached to the pipets and electrically insulated from the bath at both ends using liquid Sylgard as reported previously (4). Two concentrically arranged pipets were positioned in the tubule lumen, one to pass current and the other to record voltage. The tips of the pipets were maintained constant in position relative to each other in each experiment with the inner pipet protruding approximately 10 $\mu$ beyond the outer. Ultrafiltrate of rabbit serum was perfused into the tubule lumen by gravity through the middle pipet shown on the left. Perfusion rate was not measured. To inject current into the tubule lumen, electrical contact with the fluid in the perfusion pipet was made with a Ag-AgCl wire. This was connected through a voltage divider to a 90-v battery. Current was measured with a Keithley 600-A electrometer in parallel to a $10^4$ $\Omega$ resistor in the circuit. The floating circuit was completed through a second Ag-AgCl wire in contact with the bath through an agar bridge containing 0.16 $n$ NaCl. Spontaneous and electrotonic potentials were recorded through the lumen of the innermost pipet which was filled with 0.16 $n$ NaCl. A saline-agar bridge provided electrical contact between the fluid in this pipet and a calomel half-cell. This was connected to the input of a BAK standard wide-band electrometer (Electronics for Life Sciences, Rockville, Md.), and the signal was displayed on a Tektronix 564 B storage oscilloscope. The resistance through the lumen of this pipet was 30 M$. The electrotonic voltage at the collecting end of the tubule was measured through a saline-agar bridge between the fluid in the col-
The tubule was aspirated into a holding pipet and the lumen was cannulated with the perfusion pipet, taking care to avoid damaging the tubule cells. Liquid Sylgard, contained in an outer concentrically placed pipet, was advanced over the cannulated portion of the tubule to electrically insulate the lumen from the bath. After aspirating the other end of the tubule into a collecting pipet, a second Sylgard-containing pipet was advanced over the end of the collecting pipet to insulate this end of the tubule (shown on the right side of Fig. 2). Tubules were perfused either by gravity or with a Sage syringe pump. Flow rates were not measured. A wire, attached to the platinum blaclc coating of the perfusion pipet, was connected through a voltage divider to a 90 v battery. Current was measured with a Keithley electrometer in parallel to a 10Ω resistor in the current.

The coupling resistance of each of the pipet systems was determined by passing a direct current to ground through the bath with no tubule in place and measuring the deflection through the voltage-recording pipet. With the pipet system shown in Fig. 1, the mean coupling resistance was 50 kΩ, which was 55% of the mean input resistance (Ri) of the tubules. In contrast, the mean coupling resistance of the platinum-coated pipet was low, being 5 kΩ, or 5% of the mean Ri. The coupling resistance, which was stable in both pipet systems, was subtracted from the input resistance of the tubule. The voltage induced in the collecting pipet by current passed across the resistances in series of the bath and the saline bridge to ground was subtracted from the Vi observed with a tubule in place (see Fig. 3).

After being attached to the pipets, all tubules were examined microscopically for damage. If the tubule was morphologically intact, the serum bathing the tubule was replaced with fresh serum previously heated to 37 C and equilibrated with 95% O2 and 5% CO2. The spontaneous potential difference was then recorded. To measure electrical resistance, a 1- to 2-sec pulse of 2–3 × 10^-7 amps of direct current was injected into the tubule lumen. After the initial voltage artifact, the voltage deflection, which was constant with time, was recorded to calculate the input resistance of the tubule. The tubule was then photographed with a Polaroid camera at X125 final magnification for determination of tubule length and at X300 final magnification to measure tubule diameter.

Ouabain (Calbiochem) was added to the bathing serum as specified in the text.

The results are reported as the mean ± standard error (number of tubules studied).

RESULTS

Cable theory. The electrical equivalent of a renal tubule is shown in Fig. 3. Cable analysis was used to calculate Rp (Ω·cm), the transepithelial resistance; Ri (Ω·cm^-1), the cure resistance; and λ (cm), the length constant, which has the conventional meaning. The general solutions for this electrical cable and the equations used in this analysis have been published previously (12) and are stated in the legend to Fig. 3.

A necessary condition for applying cable analysis to the renal tubule is that the tubule has the characteristics of an ohmic resistor. The voltage/current relationship of proximal convoluted and straight tubules was assessed with each of the pipet systems. Hyperpolarizing and depolarizing currents up to 4 × 10^-7 amps were used.
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FIG. 3. Electrical equivalent of a renal tubule (used by permission of authors, ref. 12). $R_T$ is transepithelial resistance. $R_c$ is core resistance. Current $I$, injected into lumen results in changes $V_o$ and $V_L$ of luminal PD values at ends of tubule. $L$ is length of tubule between Sylgard seals. Input resistance, $R_i$, is ratio $V_o/I_o$. Equations used in analysis are: $R_T = V_o\lambda \tanh (L/\lambda)/I_o$; $R_c = V_o \tanh (L/\lambda)/L_o$; $L/\lambda = \cosh^{-1}(V_o/V_L)$.

With no tubule in place, passage of pulses of current through the serum in the bath resulted in a linear current/voltage relationship. These electroneutral voltages were subtracted from those measured across the tubule wall. With both pipet systems, a linear voltage/current relationship was found in both types of proximal tubule over the range of current tested. A similar correlation has been described previously in proximal tubules (11, 22). Thus, the proximal tubule can be characterized as an ohmic resistor, which allows cable analysis to be applied to measurements of its resistance.

Since the cable analysis employed requires that the transepithelial resistance and the length constant be independent of tubule length, $R_T$ and $\lambda$ of various tubule lengths were determined in a given tubule using the pipet arrangement shown in Fig. 2. Tubule length was decreased by advancing the Sylgard-containing pipet over the tubule from the collecting end, thus insulating a large portion of the tubule from the bath. The results of this experiment in six proximal convoluted tubules are shown in Fig. 4. With the exception of one point, the values for $R_T$ and $\lambda$ remained within 10% of the mean for a given tubule, whereas length was decreased over the range of 345–110 $\mu$, thus confirming the applicability of cable analysis to the proximal nephron.

Comparison of electrical and optical diameters. In considering the renal tubule as an electrical cable (Fig. 3), we assume it is most likely that the perfusion fluid in the lumen constitutes the core of the cable. Then, the resistivity of the core ($\rho$) is that of the perfusion solution, and the diameter of the core (D) is calculated to be $D = [4\rho/\pi R_c]^{1/2}$. The resistivity of ultrafiltrate of rabbit serum at 37 C is 55 $\Omega$cm, measured directly with a conductivity cell and Wheatstone bridge. The diameter of the core calculated from the electrical measurements in each experiment was compared to the diameter of the lumen, measured photographically as the distance between the tips of the brush border (lumen diameter). The results and a paired analysis of the difference between electrical core diameter and lumen diameter are seen in Table 1. The mean electrical core diameter was 6–8 $\mu$ greater than the mean lumen diameter. A similar relationship between the electrical and lumen diameter has been reported previously by Hegel, Frömter, and Wick (11). They calculated an electrical diameter of 26–30 $\mu$ and assumed a lumen diameter of 20 $\mu$. They concluded that the electrical diameter was located between the luminal and peritubular membranes of the tubule epithelium. In their analysis of the difference between the electrical and lumen diameters, they neglected the height of the brush border, which is about 3 $\mu$, measured from electron micrographs of perfused proximal convoluted tubules (2). Thus, the difference between the electrical diameter and the lumen diameter places the resistive barrier to ion movement approximately at the level of the tight junctions. The close correspondence of the two diameters justifies the assumption that the luminal fluid constitutes the core of the cable and adds confidence to the use of cable analysis.

Comparison of methods. The results yielded by the two techniques in experiments with both proximal convoluted and straight tubules are compared in Table 2. For a given nephron segment, the values determined with the two techniques were not statistically different. Thus, the results from both methods were combined to compare proximal convoluted and straight segments.

Proximal convoluted and proximal straight tubules. The mean values from 41 proximal convoluted and 22 proximal straight tubules are presented in Table 3. Although the differences are small, the proximal convoluted tubule has a higher PD and lower transepithelial resistance than the proximal straight segment. For a membrane such as the proximal tubule in which the voltage/current relationship

<table>
<thead>
<tr>
<th>Proximal convoluted tubule</th>
<th>Electrical Core Diameter</th>
<th>Lumen Diameter</th>
<th>$P &lt; 0.001$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\times 10^{-4}$ cm</td>
<td>29.6 ± 0.9</td>
<td>23.2 ± 0.9</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>$n = 30$</td>
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</table>

<table>
<thead>
<tr>
<th>Proximal straight tubule</th>
<th>Electrical Core Diameter</th>
<th>Lumen Diameter</th>
<th>$P &lt; 0.01$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\times 10^{-4}$ cm</td>
<td>29.6 ± 2.4</td>
<td>21.9 ± 1.8</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>$n = 8$</td>
<td></td>
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TABLE 2. Comparison of PD and resistance determined by the two techniques

<table>
<thead>
<tr>
<th></th>
<th>Prox Conv Tubule</th>
<th>Prox Straight Tubule</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Two glass pipets</td>
<td>Platinum-coated pipet</td>
</tr>
<tr>
<td>PD, mv</td>
<td>4.0 ± 0.4</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>R1 = V/I X 10^5, ohms</td>
<td>0.69 ± 0.06</td>
<td>0.90 ± 0.05</td>
</tr>
<tr>
<td>R1 , ohm cm</td>
<td>996 ± 83</td>
<td>950 ± 44</td>
</tr>
<tr>
<td>λ X 10^-1, cm</td>
<td>116 ± 6</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>29</td>
</tr>
</tbody>
</table>

Values are means ± se. For abbreviations, see text and Fig. 3.

is linear, short-circuit current equals the open-circuit PD divided by the transmembrane resistance. The calculated short-circuit current is greater in proximal convoluted tubules than in proximal straight tubules, as is the rate of fluid absorption (Table 3).

**Ouabain effect.** In three proximal convoluted tubules perfused through the platinum-coated pipet, ouabain (10⁻⁵ m) was added to the bath. In these studies, a Sage microperfusion pump was used to assure constant perfusion rate. Figure 5 shows the results of these studies. The PD rapidly fell to zero upon addition of ouabain and returned to control values within 10–15 min after the drug was removed. The transepithelial resistance increased by 200–300 Qcm² upon addition of the drug and returned to within 12% of the control value upon removal of ouabain. The core resistance, lumen diameter, and cell morphology did not change during the 10-min exposure to ouabain.

**DISCUSSION**

Electrical resistance has been measured previously in the isolated perfused rabbit cortical collecting tubule (3, 12). In the first attempt, current was introduced into the tubule lumen through a perfusion pipet and the electrotonic voltages were recorded with a second concentrically placed pipet that was advanced by increments into the tubule lumen. It was possible to use this technique because the collecting tubule is a straight tubular structure. In the later study, a single glass perfusion pipet with a bridge circuit was used both to pass current and record voltage. This method was satisfactory because the input resistance of the tubule was of the same order of magnitude as the pipet resistance.

In the present study, methods were devised to measure accurately the low electrical resistance of the proximal tubule. The two methods used yielded the same value for electrical resistance, thus lending confidence in the result. The essential feature, common to both techniques, was the use of separate circuits to pass current and record voltage. The platinum-coated pipet offers two advantages over the double-pipet arrangement. First, it eliminates the effort of assembling concentric perfusion pipets. Second, it has a very low coupling resistance, which might increase the accuracy of tubule resistance measurements in experiments designed to effect small changes in resistance, such as those reported above with ouabain.

The presence of a transtubular PD in the proximal convoluted tubule when perfused with ultrafiltrate of serum has been reported previously (4). The present study confirms the presence of a spontaneous PD of -4 mv, lumen negative. This PD, which was stable for periods up to 5 h, was abolished by ouabain and returned to control values upon removal of ouabain. This, together with the temperature dependence of the PD (16), suggests that it is generated by an active transport process.

The proximal tubule has a low transepithelial resistance, reflecting the high permeability to electrolytes that characterizes this portion of the nephron. The specific membrane resistance (Rm) of the rabbit proximal convoluted tubule, 6.96 Ωcm², is similar to that measured in the rat (11) and dog (1) by micropuncture. Direct measurements of short-circuit current in renal tubules are complicated by their small size and cylindrical shape. Several methods have been used to circumvent these difficulties. Using the technique of Karger, Eigler, and Hampel (13), Eigler (7) isolated a short segment of *Necturus* proximal tubule between oil drops in order to uniformly short circuit the epithelium. The mean short-circuit current, 4.8 X 10⁻⁶ amp cm⁻², was approximately equal to the net Na⁺ transport rate measured by others (18) under open-circuit conditions. Giebisch,
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Klose, Malnic, Sullivan, and Windhager (9) modified this technique for rat proximal tubules. They found a short-circuit current of $73 \times 10^{-5}$ amp cm$^{-2}$, which was in good agreement with the net Na$^+$ transport measured without short circuiting. More recently, Spring and Paganelli (19) short-circuited Necturus proximal tubules with an axial electrode in the tubule lumen and measured fluid reabsorption (net Na transport) under these conditions. They found close agreement between short-circuit current and net Na flux. Our result is similar. The mean short-circuit current of the proximal convoluted tubule, calculated from the PD and $R_T$ (Table 3), was $39 \times 10^{-7}$ amps cm$^{-2}$ (55 $\times$ $10^{-5}$ amp cm$^{-2}$ for the lumen diameter of $23 \times 10^{-4}$ cm) compared to a net Na$^+$ flux of $28 \times 10^{-7}$ amps cm$^{-2}$ calculated from previous results (15). The theoretically correct comparison would be between the short-circuit current and the net Na$^+$ transport measured in the short-circuited state. However, if these were also approximately equal in mammalian proximal tubules, as they are in anuran membranes (20) and Necturus proximal tubules (19), then Na transport must be active and must be responsible for the generation of the PD. Other electrically neutral ion pumps would not be excluded, however. We are unable to short circuit a sufficient length of the tubule to measure Na transport in the short-circuited state. However, in view of the reasonably close agreement between the short-circuit current and the net Na$^+$ transport measured in the open-circuited state, we suggest, as have others (7, 9, 19), that Na$^+$ is transported actively in the proximal tubule and that Cl$^-$ is reabsorbed, however. We are unable to short circuit a sufficient length of the tubule to measure Na transport in the short-circuited state. However, in view of the reasonably close agreement between the short-circuit current and the net Na$^+$ transport measured in the open-circuited state, we suggest, as have others (7, 9, 19), that Na$^+$ is transported actively in the proximal tubule and that Cl$^-$ is reabsorbed, passively along the resultant electrical gradient. We do not conclude, however, that the present experiments completely exclude an active Cl$^-$ secretion as suggested by Kashgarian et al. (14) and Malnic and Mello Aires (17), particularly since it could be an electrically neutral process (HCl secretion).

As has been reported previously (4), ouabain abolished the transtubular PD in proximal tubules. Presumably, the drug acts by inhibiting active Na$^+$ transport. It is of interest that ouabain also increased the electrical resistance of the proximal tubule. A similar effect of ouabain on the electrical resistance of toad bladder has been reported by Civan (6). In both anuran membranes (20) and proximal renal tubules (21), the transepithelial electrical resistance may be analyzed in terms of the electrical resistances of a pump and a shunt pathway in parallel. The resistance of either or both pathways may be altered by ouabain, as follows: 1) Ouabain may increase the resistance to Na$^+$ movement through the active transport pathway, as a consequence of inhibiting the pump. 2) The drug may alter the passive permeability to Na$^+$ of some membrane in either the pump or shunt pathway. 3) Ouabain may increase the resistance of the intercellular channels by decreasing their width, either as a result of cellular swelling or as a result of diminution of the volume of fluid transported through them. 4) The concentration of ions in the shunt pathway may be decreased when transport of NaCl into the intercellular channels is inhibited by ouabain. It is not possible on the basis of the present studies to distinguish among these possibilities.

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