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Determination of Electrical Resistance of the Isolated Cortical Collecting Tubule and Its Possible Anatomical Location

SANDY I. HELMAN

Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112

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The electrical properties of renal epithelia have been determined principally by micropuncture studies of nephron segments located at the surface of the kidney. Deeper segments, owing to their inaccessibility to micropuncture cannot be studied directly, and consequently, their contribution to the ultimate formation of urine has been assessed by indirect methods. The introduction of the isolated tubule perfusion technique(1) provided the opportunity for studying individual nephron segments not only under conditions which allowed greater experimental manipulation, but which also permitted direct study of nephron segments such as the cortical collecting tubule.

One of the early goals was to develop techniques to determine the potential difference and the transepithelial resistance in this nephron segment. Studies by Burg, Isaacson and Grantham(2) showed that the cortical collecting tubule was capable of maintaining a negative luminal potential near −25 mV and that the transepithelial resistance was much larger than that of the proximal tubule. These studies, however, were subject to some uncertainty owing to the possibility that significant leaks could have occurred not only at the perfusing end of the tubule, but more importantly at the distal end of the isolated tubule where the tubule was held in a single glass micropipette. In order to overcome this difficulty which could lead to a significant source of error in the measurements of both potential difference and resistance, Sylgard 184 was used to electrically seal the distal end of the tubule into the collecting pipette(3–5). This modification of the original system proved useful not only in studies of the electrical properties of the isolated tubules, but also in studies using isotopic tracers to determine the permeability to nonelectrolytes(6). In order to establish the validity of the techniques, studies were undertaken to show that the isolated tubule behaved like

an electrical cable and further, to show that predicted changes in cable properties could be produced and these changes measured.

In order to perfuse the tubule and to insulate electrically the tubule lumen from the peritubular bathing solution, a large bore pipette was advanced several hundred microns into the tubule lumen. As indicated above, insulation of the distal end of the tubule was achieved with Sylgard 184. The perfusion pipette served two additional functions. The pipette was connected to a source of variable hydrostatic pressure. By varying the pressure, lumen diameter could be changed at will. A silver chloride electrode was also connected to the perfusion pipette. This electrode was used to inject current (I_0) into the tubule lumen and to record the voltage change (V_0) in the lumen in response to the current. A second silver chloride electrode was used to record the change in voltage at the distal end of the tubule lumen (V_L). These changes in voltage in response to the known current pulse are the only measurements besides tubule length (L) needed to determine the values of core resistance (R_c) and transepithelial resistance (R_T).

Owing to the cable-like geometry of the tubule, core resistance and transepithelial resistance are thought of as being distributed along the length of the tubule (Fig. 1). During injection of constant current, luminal voltage changes at every point along the length of the tubule. For a tubule electrically insulated at both ends, it is necessary to measure only the change in voltages V_0 and V_L. As Eq. 1–3 derived from two-dimensional cable theory indicate, the ratio of tubule length to the length constant λ, L/λ, is computed from the ratio V_0/V_L. Accurate measurements of the voltage changes are not limited by the recording equipment since the changes in voltage are normally greater than 5 mV with noise levels typically less than 0.5 mV. The magnitude of the voltage changes can be controlled by choosing the appropriate value of constant current. Typically, currents between 10 and 50 nA have been used. Since I_0, V_0, V_L, L and λ are known, transepithelial resistance and core resistance can be determined.

$$L/\lambda = \cosh^{-1}(V_0/V_L) \tag{1}$$

$$R_T = \frac{V_0\lambda}{I_0}\tanh(L/\lambda) \qquad \text{ohm} \cdot \text{cm} \tag{2}$$

$$R_c = \frac{V_0}{I_0\lambda}\tanh(L/\lambda) \qquad = \frac{4\rho}{\pi D^2} \quad \text{ohm/cm}. \tag{3}$$

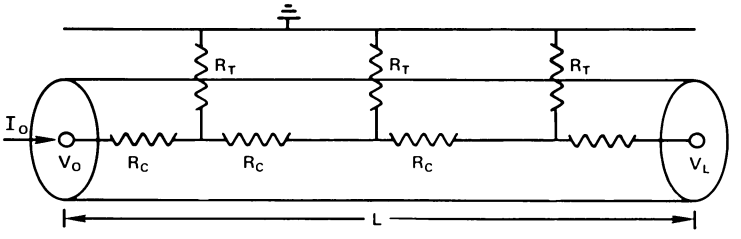

FIG. 1. Two-dimensional electrical cable model of isolated tubule of length, L. V_0 and V_L are the voltage changes at the perfusion and collection ends of the tubule, respectively, in response to the injected current, I_0.

One of the unique advantages in using cable theory with the isolated tubule is that the core resistance is calculated directly from the data of the electrical measurements. If the core can be represented by a cylinder of constant diameter, then the electrical diameter, D, can be computed from the core resistance and the volume resistivity of the perfusion fluid, ρ. It was of interest to find that when the electrical diameter of the core was compared with the optically measured diameter of the lumen, the values were identical(4). Tubules between 0.3 and 2.0 mm in length with lumen diameters between 18 and 30μ were studied. This identity held in every tubule despite the large differences in tubule length and lumen diameter and was consistent with the view that the core resistance was determined by lumen geometry and the volume resistivity of the fluid perfusing the tubule lumen.

This hypothesis was tested further in two ways. First, while maintaining the volume resistivity of the perfusion fluid constant, lumen diameter was changed. In the second test, lumen diameter was kept constant and the volume resistivity of the perfusion fluid was changed. In both groups of studies the measured change in core resistance was compared with the change predicted.

The results of the studies in which the first approach was used have been published previously(4). In brief, lumen diameter was varied by raising or lowering the hydrostatic pressure to the perfusion pipette, and in so doing core resistance was changed. Throughout these changes the optical diameter of the tubule lumen was measured from photographs. At the lower perfusion pressures where the value of core resistance was high, the cells bulged into the lumen and accurate estimates of optical diameter could not be made. At the highest perfusion pressures which corresponded with the lowest core resistances, optical diameter was more uniform and the equivalence between optical and electrical diameter was best observed. It was consistently observed that despite the inability to accurately quantitate optical diameter at the lower perfusion pressures, good agreement between the mean optical and electrical diameter was observed over large ranges of diameter in any particular study.

It was also observed in these studies that transepithelial resistance expressed in units of resistance per unit of tubule length was constant and independent of tubule diameter. This was interpreted to mean that alterations in the geometry of the tubule do not affect the value of transepithelial resistance, and further, that the effective luminal area is not correlated with either electrical or optical diameter. Consequently, the values of transepithelial resistance are expressed in units of resistance per unit of tubule length and thus avoids any assumptions about the membrane area.

In order to obtain further supporting evidence that the core resistance was determined by the geometry of the lumen and the volume resistivity of the perfusion fluid alone, studies were done in which lumen diameter was kept constant and the volume resistivity of the perfusion solution was changed. Two concentric pipettes were inserted into the tubule lumen, each of which contained perfusion fluids of differing volume resistivity. Both fluids contained a reduced concentration of NaCl (56 mm) and were different to the extent that one solution contained

raffinose and the other solution contained an isosmotically equivalent amount of KCl. The volume resistivities of these solutions at room temperature were 124 and 73.9 ohm.cm, respectively, and the ratio of resistivities was 0.6. Both perfusion pipettes were connected to separate sources of hydrostatic pressure. By adjusting the perfusion pressures each of the solutions could be perfused alone. In eight tubules, core resistance ranged between 20.5 and 146.0 Mohm/cm during perfusion of the raffinose–Ringer solution. Perfusion with the KCl–Ringer solution caused core resistance to fall 44.1 $\pm$ 2.9% (SE). The mean ratio of the core resistances determined with KCl–Ringer and raffinose–Ringer solutions was 0.6 $\pm$ .04 (SE), a value identical to the ratio of volume resistivities. Thus, while lumen diameter is kept constant, core resistance varies directly with the volume resistivity of the perfused fluid. This finding, together with the previous findings, supports the idea that core resistance depends only on lumen diameter and the resistive properties of the fluid in the lumen.

Transepithelial resistance ranged between 3.1 and 22.9 $\times$ 10^4 ohm.cm and fell 29.9% $\pm$ 3.3% (SE) when KCl replaced raffinose. This wide range of resistance is not an uncommon finding in the cortical collecting tubule. In other studies transepithelial resistances as low as 1 $\times$ 10^4 and as high as 26 $\times$ 10^4 ohm.cm have been measured. These values are considered to be accurate estimates of the transepithelial resistance since in all studies the identity between optical and electrical diameter was observed.

Before considering further the effects on transepithelial resistance of changing the electrolyte concentration of the solutions bathing the tubule, it seems appropriate to turn to a general consideration of the possible locations of the electrical resistance barriers which may contribute to the measurement of the transepithelial resistance.

When current flows from tubule lumen to the peritubular side of the epithelium, it is commonly assumed that this flow occurs transcellularly, crossing both luminal and peritubular plasma membranes. The resistances of these membranes are labeled R_L for the luminal membrane resistance and R_p for the peritubular membrane resistance (Fig. 2). The electrical resistance through the tight junctions may contribute significantly to the transepithelial resistance. Boulpaep, Windhager and Giebisch have shown that at least in the proximal tubule of *Necturus*

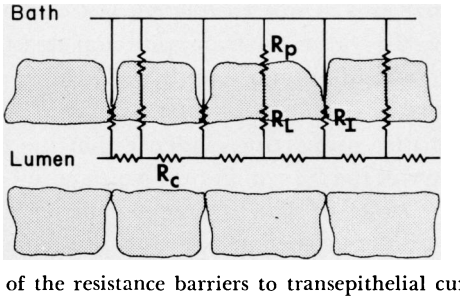

FIG. 2. Possible location of the resistance barriers to transepithelial current flow located at the luminal cell membrane, R_L; the peritubular cell membrane, R_p; and the intercellular route through the tight junctions, R_I.

significant shunting of current occurs around the cells, presumably through the tight junctions(7–9). This intercellular resistance is labeled R_I. Together, this series-parallel combination of resistances would determine the value of the transepithelial resistance. It may be that in the various nephron segments, the relative importance of the transcellular and intercellular pathways may vary considerably and may account for some differences in specific tubular functions.

The observation that the tubule electrical and optical diameters are identical can be interpreted to mean that the principal resistance barrier to current flow through the epithelium must be at or near the luminal membrane. If this barrier were located at the peritubular membrane it would be expected that the electrical diameter would be greater than the optical diameter. Since this is not the case, the data support the view that either the luminal membrane resistance and/or the intercellular resistance are the limiting resistances to current flow through the epithelium.

It was apparent early in the electrophysiological studies of the cortical collecting tubule that changing the concentration of the electrolytes bathing the tubule had marked effects on the value of transepithelial resistance. Some of these data shown in Fig. 3 not only provide further evidence for the applicability of cable theory to the isolated perfused tubule but also raise some questions as to the location of the transepithelial electrical resistance barriers.

In these studies, the effects of replacing raffinose with KCl in both luminal and peritubular solutions were determined. The data in this table is given as transepithelial conductance, C_T, having units of mho/cm. The experimental protocol is shown in Fig. 3. At "a," replacing raffinose in the peritubular bathing solution with 56 mM KCl caused the transepithelial conductance to increase 28.5 $\pm$ 5.8% (SE). At "b," substitution of 56 mM KCl for raffinose in the perfusion fluid caused a larger increase in transepithelial conductance, 57.5 $\pm$ 13.3% (SE). At "c," with the transepithelial conductance still elevated, a further increase in conductance to 83.4 $\pm$ 17.8% (SE) was observed when KCl replaced raffinose in the peritubular solution. Thus, the transepithelial resistance or conductance is determined in part by the concentration of electrolytes in both luminal and peritubular solu-

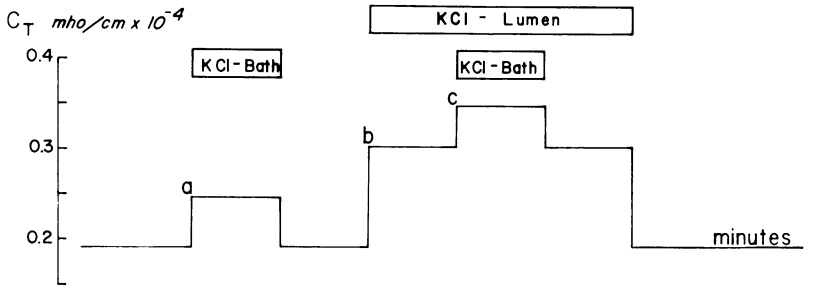

FIG. 3. Tubules were perfused and bathed in solutions in which raffinose isosmotically replaced 56 mM NaCl. At a, b, and c, KCl (56 mM) was substituted for the raffinose and the effects on transepithelial conductance, C_T, determined. Mean conductance values, (N = 8) are given on the ordinate. The order of solution changes is given on the abscissa. C_T was usually determined 4–6 min after solution changes were made.

tions. Although the data is not shown here, it was observed that changes in transepithelial resistance produced by electrolyte changes in the peritubular bathing solution occurred without a change in core resistance, as would be expected if the core and transepithelial resistances were electrically independent variables. The ability to manipulate the transepithelial resistance in the absence of a change in core resistance provides further evidence that the resistances calculated from the electrical measurements and the equations derived from cable theory are indeed adequate.

With regard to the location of the resistance barriers, these data present a paradox. On the one hand, the finding that the electrical diameter exists at or near the luminal membrane leads to the conclusion that the principal resistance barrier is also at or near the luminal membrane. If so, it would be expected that the peritubular membrane resistance would be relatively small in value compared to the resistance of the luminal membrane. Yet, concentration changes in the peritubular bathing solution produced appreciable changes in resistance, presumably at the peritubular membrane. This indicates that the peritubular membrane resistance should have a reasonably high value in order that changes in its resistance would significantly affect the value of transepithelial resistance.

In the transcellular pathway, current flows through luminal and peritubular membrane resistances. The resistance barriers may be located primarily at the luminal membrane, the peritubular membrane, or may be divided between the membranes. In order to determine the relative resistance of the peritubular and luminal membranes, Ling-Gerard electrodes with tip diameters less than 0.5μ were used to puncture into the cell cytoplasm from the peritubular border of the cells. The tips of the pipettes were considered to be within the cells when instantaneous stable changes in potential differences were observed. Since luminal and peritubular membrane resistances are in series, they behave as a voltage divider. Consequently, during passage of current, the microelectrode could be used to determine the relative changes in voltage across the peritubular and luminal membranes. In 64 cells of eight tubules, peritubular membrane resistance was found to be immeasurably small relative to the luminal membrane resistance. Thus it would appear that the principal resistance in the transcellular pathway is located at the luminal membrane.

It is still necessary to account for changes in electrolyte concentration in the bathing solution to produce appreciable changes in transepithelial resistance. The model that seems to fit the data is the one in which the intercellular pathway is considered to be the principal path for current flow through the epithelium. In this model, the resistance barrier through the tight junctions is near the luminal membrane, and since it is bathed by the solutions bordering both the luminal and peritubular membranes, its resistance would depend upon the electrolyte concentrations of both solutions. Thus it may be that the measurement of transepithelial resistance is most likely a measure of the resistance of the intercellular pathway between the cells.

Before concluding this discussion, one more argument in support of this view should be considered. If, for the moment, it is assumed that current flows only

transcellularly through both luminal and peritubular membrane resistances, in series, the value of transepithelial resistance is given by the sum of R_L and R_p (Eq. 4). As noted above, a change in KCl concentration in the bathing solution could have produced a change in peritubular membrane resistance (ΔR_p). Expressed as a percentage change, the percent change in transepithelial resistance is given by Eq. 5. Now consider what happens to the percent change in transepithelial resistance when the luminal membrane resistance is decreased. As R_L falls, the peritubular membrane resistance contributes a larger part to the total resistance. Consequently, a change in R_p will yield a larger percentage change in transepithelial resistance when R_L is reduced. This prediction was tested with the data shown in Fig. 3.

$$R_T = R_L + R_p \tag{4}$$

$$\%\Delta R_T = \frac{\Delta R_p}{R_L + R_p} \times 100. \tag{5}$$

Increasing the KCl concentration in the lumen at "b" caused the transepithelial conductance to increase, presumably by causing "luminal membrane" resistance, R_L, to fall. With KCl still in the lumen, a further change in resistance occurred when KCl in the bath was elevated. The percent change in transepithelial conductance calculated at "c" was compared with that observed at "a." The percent change in transepithelial conductance fell from 28.5 $\pm$ 5.8% (SE) determined at "a" to 14.7 $\pm$ 1.9% (SE) determined at "c." This finding is not consistent with the expected increase predicted by the series resistance transcellular model but does correlate with that predicted from the intercellular resistance model.

In summary, the applicability of core conductor theory to studies of the isolated perfused cortical collecting tubule has been tested under a variety of conditions and found to give predictable results. Transepithelial resistance of the cortical collecting tubule is thought to be a measure of the electrical resistance between rather than through the cells.

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