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CELL MEMBRANES AND PARACELLULAR RESISTANCES IN ISOLATED RENAL PROXIMAL TUBULES FROM RABBIT AND *AMBYSTOMA*

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SUMMARY

1. Transepithelial specific resistance (R_e) was measured in isolated and perfused rabbit proximal convoluted tubules by cable analysis and intracellular micro-electrode techniques were used to calculate the electrical resistances of the cell membranes and of the paracellular pathway.

2. R_e was 16 ± 2 Ω cm² and the space constant was 130 ± 14 μm, $n = 29$. R_e was significantly increased by a decrease in temperature from 37 to 10 °C, and was practically abolished by nominal removal of Ca^{2+} from the bathing solution (to $2{\cdot}0 \pm 0{\cdot}3$ Ω cm², $P < 0{\cdot}001$, $n = 6$).

3. The apparent ratio of cell membrane resistances (luminal to basolateral) was $3{\cdot}1 \pm 0{\cdot}3$.

4. The control values of apical and basolateral membrane resistances (R_a and R_b) were calculated from the values of (1) R_e, (2) the apparent ratio of cell membrane resistances, and (3) the effects of addition of either Ba^{2+} (1 mM) to the bath solution or glucose (8 mM) to the perfusate on basolateral and apical membrane voltages (assuming that the initial effects of Ba^{2+} and glucose are restricted to the ipsilateral membrane).

5. Control values of R_a (Ω cm² of epithelium) were 249 ± 68 (Ba^{2+} method) and 227 ± 42 (glucose method). Values of R_b were 70 ± 11; and 66 ± 12 respectively. The low paracellular resistance values obtained with the Ba^{2+} and glucose methods, respectively, 17 ± 5 and 15 ± 1 Ω cm², explain the low transepithelial resistance.

6. The use of the Ba^{2+} and glucose methods provides alternatives to cell cable determinations for the calculation of cell membrane resistances.

7. Cell membrane and shunt resistances measured by the same methods in isolated perfused *Ambystoma tigrinum* proximal tubules (in Ω cm² of epithelium) were: R_a, 2650 ± 180 (glucose method) and 2368 ± 350 (Ba^{2+} method). Values of R_b were 665 ± 99 (glucose method) and 701 ± 124 (Ba^{2+} method). The paracellular resistance values were 58 ± 11 (glucose method) and 84 ± 12 (Ba^{2+} method). These results are in good agreement with previously reported values obtained by intracellular cable analysis (Maunsbach & Boulpaep, 1984).

INTRODUCTION

The proximal renal tubule is a salt-transporting epithelium of high ionic conductance and high hydraulic conductivity. The existence of an ion-selective paracellular pathway of high conductance complicates electrophysiological studies in leaky epithelia because unilateral modifications of the ionic composition of the external medium cause intra-epithelial current flow. Hence, the change in ipsilateral membrane voltage elicited by unilateral changes in ionic composition is not equal to the change in equivalent electromotive force (i.e. zero-current voltage). It follows that the determination of cell membrane ionic permeabilities from intracellular microelectrode data requires the measurement of cell membrane and shunt resistances in addition to membrane and transepithelial potentials (Boulpaep, 1971; Schultz, 1972; Reuss & Finn, 1975).

In renal tubules, the problem is complicated further by the cylindrical configuration, which precludes the delivery of a homogeneous current across the tissue. Cable analysis has been used to estimate the transepithelial resistance of proximal and collecting tubules (Burg, Isaacson, Grantham & Orloff, 1968; Helman, 1972). The measurement of cell membrane resistances requires intracellular recordings which are difficult to obtain in mammalian cells because of their small size. Nevertheless, cell membrane potentials have been measured *in vivo* in rat proximal tubules and a method for the calculation of cell membrane resistances based on the changes in membrane voltages induced by glucose has been reported by Frömter (1982). Only recently, measurements of intracellular potential have been reported in isolated and perfused rabbit renal tubules (Biagi, Kubota, Sohtell & Giebisch, 1981; Greger, 1981; Bello-Reuss, 1982; Koeppen, Biagi & Giebisch, 1983; Lapointe, Laprade & Cardinal, 1984). This preparation is advantageous because it allows for easy manipulation of the peritubular bathing medium and permits *in vitro* study of segments otherwise inaccessible. Estimates of the electrical resistances of the cell membranes in rabbit proximal tubule have been obtained by Lapointe *et al.* (1984), who used a similar method to that employed by Frömter (1982).

In the experiments reported here, the method previously described by Helman, Grantham & Burg (1971) was used to determine the transepithelial resistance (R_e) of proximal convoluted tubules of the rabbit. This value is still an issue of some debate, since it has been reported to be as low as 6·9 Ω cm^2 (Lutz, Cardinal & Burg, 1973) and as high as 15·7 Ω cm^2 (Berry, 1983). Two experimental perturbations were used to validate the measurement of R_e: decrease in temperature, and nominal removal of Ca^{2+} from the bathing solution. Experiments were also conducted to evaluate several techniques to measure the electrical resistances of apical and basolateral cell membranes and paracellular pathway, since intracellular cable analysis is not feasible in this and similar preparations. Finally, to validate these techniques experiments were also performed in proximal tubules of *Ambystoma tigrinum*. Values of cell membrane and paracellular resistance for this segment, obtained by intracellular cable analysis, are available for comparison (Sackin & Boulpaep, 1981; Maunsbach & Boulpaep, 1984).

METHODS

Proximal convoluted tubules were dissected free from slices of rabbit kidneys as previously described (Burg, Grantham, Abramow & Orloff, 1966; Bello-Reuss, 1980). In brief, female New Zealand white rabbits were killed by decapitation. The kidney was rapidly removed and the tubules were dissected from slices immersed in a chilled (4 °C) solution containing (in mM): NaCl, 105; KCl, 5; $NaHCO_3$, 25; $MgSO_4$, 1; $CaCl_2$, 1·8; Na_2HPO_4, 2; Na-acetate, 10; glucose, 8·3. Segments were selected from nephrons that originated near the surface of the kidney and were transferred to a lucite chamber of about 0·3 ml capacity where they were held by glass pipettes. One side was cannulated for perfusion and in some experiments the opposite end of the tubule was also cannulated. R_e values obtained from these experiments ($17 \pm 3\ \Omega\ cm^2$) were not different from the ones obtained with no collecting cannula ($18 \pm 2\ \Omega\ cm^2$). Uncured Sylgard 184 (Dow Corning Corporation, Midland, MI, U.S.A.), a resin that has been used previously as an electrical insulator of the collecting side, was not employed in these experiments; the use of acid-cleaned glass and filtered solutions was sufficient to provide electrical insulation because the basement membrane adheres firmly to the glass, as shown by the similarity of values of transepithelial resistance measured with the two techniques (17 ± 3 and $16 \pm 3\ \Omega\ cm^2$, respectively). The bath was continually perfused at a rate of about 15 ml/min from a thermoregulated reservoir. Solutions were kept at 37 °C and gassed with 95% O_2–5% CO_2; the pH was 7·40. The tubules were perfused from a pressurized reservoir at a rate of about 15–20 nl/min. Bath and perfusion solution were identical unless otherwise indicated. The pH of the bath was continually monitored by means of a glass electrode.

Electrical measurements

The transepithelial voltage was measured as the difference in potential between two matched calomel half-cells connected through 3 M-KCl–agar bridges to the bath and the perfusion solution. A high input-impedance electrometer (model 725, WP Instruments, New Haven, CT, U.S.A.) was used. The basolateral membrane voltage was measured with micro-electrodes filled with 3 M-KCl, having tip resistances of 40–60 MΩ, as previously described (Bello-Reuss, 1982). Voltages were recorded (Gould Brush, St. Louis, MO, U.S.A.) and monitored in a storage oscilloscope (Tektronix, Beaverton, OR, U.S.A.).

Positive and negative square current pulses (40–60 nA, 1 s duration) were passed between a Ag–AgCl electrode, located in the collecting pipette, and the bath electrode. The same electrode was used to measure the transepithelial voltage deflexion at the collecting end (V_o). The current source was a 305–1 stimulus isolator driven by a 302-T Anapulse stimulator (WP Instruments, Inc., New Haven, CT, U.S.A.). From the voltage changes at the perfusion and collection ends (V_l and V_o) and the length of the tubule (L), the space constant (λ) was determined. The transepithelial resistance and the luminal core resistance were calculated from the following equations (Helman, Grantham & Burg, 1971; Lutz, Cardinal & Burg, 1973):

$$R_t = \frac{V_o \lambda}{I_o} \tanh(L/\lambda), \tag{1}$$

$$R_c = \frac{V_o}{I_o \lambda} \tanh(L/\lambda), \tag{2}$$

$$L/\lambda = \cosh^{-1}(V_o/V_l), \tag{3}$$

where R_t is the transepithelial resistance (Ω cm), R_c is the core resistance ($\Omega\ cm^{-1}$) and I_o is the current.

Cell membrane resistances (R_a and R_b) and paracellular resistance (R_s) were calculated using the equations which describe the equivalent circuit previously proposed (Bello-Reuss, 1982) and shown in Fig. 1. The cell membranes (apical and basolateral) and the paracellular pathway are represented each by a Thevenin equivalent (equivalent electromotive force, E, in series with an equivalent resistance, R). To determine R_a, R_b and R_s, three independent determinations are needed (Boulpaep, 1979).

(a) The apparent ratio of apical to basolateral membrane resistance (a) which was calculated from:

$$a = R_a/R_b = [\Delta V_x/\Delta V_b]-1, \quad (4)$$

ΔV_b, the change in basolateral membrane voltage produced by the transepithelial current pulse, was experimentally measured; ΔV_x, the change in transepithelial voltage at the site at which the intracellular micro-electrode is positioned (x μm from the current source), was calculated from the cable properties of the tubule:

$$\Delta V_x = \Delta V_0 \frac{\cosh[(L-x)/\lambda]}{\cosh(L/\lambda)}. \quad (5)$$

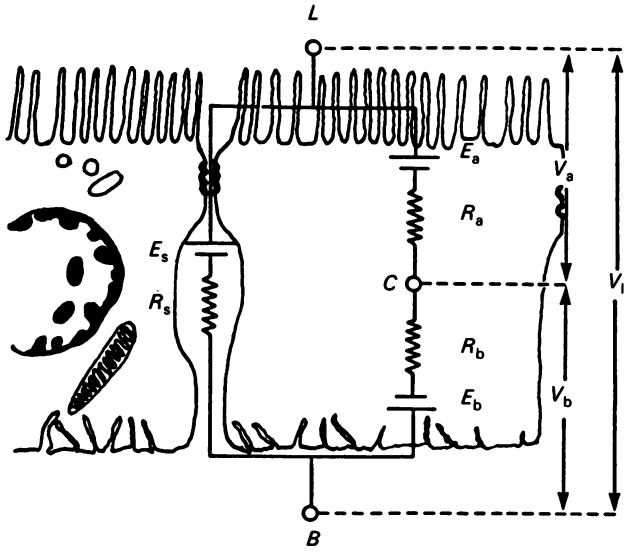

Fig. 1. Equivalent electrical circuit for proximal renal tubule. Apical (a) and basolateral membranes (b) and paracellular (shunt) pathway (s) are represented each by a Thevenin equivalent (equivalent electromotive force, E, in series with an equivalent resistance, R). L, C and B refer to the lumen, the cell interior, and the bath solution, respectively. The electrical potentials V_a (apical membrane), V_b (basolateral membrane) and V_l (transepithelial) are indicated between arrows.

(b) R_e, the transepithelial specific resistance (Ω cm^2), which was determined from luminal cable analysis and from the area of tubule wall, assuming a cylinder of radius r (measured optically) and disregarding microscopic cell membrane infoldings.

(c) The third independent measurement was obtained by two different approaches, either Ba^{2+} was added to the basolateral side, or glucose was added to the luminal side. Assuming that the immediate effect of Ba^{2+} is restricted to the basolateral membrane and that the immediate effect of glucose is restricted to the apical membrane (see Discussion) ratios of resistances (R_a+R_s/R_a and R_b/R_s) can be calculated from the changes in membrane voltages observed immediately after exposure to any of these agents.

In the case of Ba^{2+}, it can be shown that (Frömter & Gebler, 1977)

$$\Delta V'_b/\Delta V'_a = (R_a+R_s)/R_a, \quad (6)$$

where $\Delta V'_b$ and $\Delta V'_a$ are the initial changes in basolateral membrane potential and apical membrane potential, respectively, produced by exposure to Ba^{2+}, and R_a and R_s are the apical and paracellular resistance respectively. This relationship is valid only in case of basolateral substitutions.

In the case of glucose, assuming an initial effect at the apical membrane only, it can be shown that (Frömter, 1982):

$$\Delta V''_b/\Delta V''_l = R_b/R_s, \tag{7}$$

where $\Delta V''_b$ and $\Delta V''_l$ are the initial changes in basolateral membrane and transepithelial potential elicited by glucose addition to the luminal side, and R_b and R_s are the resistances of the basolateral membrane and the paracellular pathway. The calculations of R_a, R_b and R_s can be carried out by solving three simultaneous equations,

(a) $$R_e = [(R_a+R_b)\,R_s]/(R_a+R_b+R_s), \tag{8}$$

(b) $$a = R_a/R_b, \tag{9}$$

(c) either $$\Delta V'_b/\Delta V'_a = (R_a+R_s)/R_a, \tag{10}$$

or $$\Delta V''_b/\Delta V''_l = R_b/R_s.$$

Values of ΔV_b and ΔV_l in the absence of tubule were subtracted from the values obtained in the presence of tubules. Tubule diameters and length were measured with an ocular micrometer. Since some tubules were up to 5 times longer than the space constant, the value of ΔV_l was very small compared to ΔV_0. Appropriate amplification was used to obtain a resolution of 0·01 mV.

Ba^{2+} was added to yield a final concentration of 1 mM in a sulphate-free solution. Prior to the 'glucose' experiments mannitol substituted for glucose in the perfusion solution. When glucose (8·3 mM) was added to the perfusate, mannitol was removed.

Experiments in Ambystoma tigrinum

Proximal tubules were dissected from the pelvic kidney and separated from the neck that joins them to the glomerulus. Tubules were perfused and bathed in a solution containing (mM): NaCl, 94·7; KCl, 2·5; $MgCl_2$, 1; *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid, 13·4; NaH_2PO_4, 0·5; lysine, 0·2; DL-glutamate, 0·05; L-glutamine, 0·5; alanine, 0·5; lactate, 0·05; glucose, 2·2; $CaCl_2$, 1·8; titrated to pH 7·4 with NaOH. Solutions were gassed with O_2.

Electrical measurements and calculations of cell membrane and paracellular resistances were performed as described above for the rabbit segments. Prior to the 'glucose' experiments, mannitol was substituted for glucose in the perfusate. Ba was added to the bath solution to a final concentration of 10 mM. In some experiments, voltage changes caused by a K-for-Na substitution in the bath were used to calculate cell membrane and shunt resistances, using the same assumptions as in the Ba method. In these experiments a 3 M-KCl liquid junction reference electrode (Mere 1, W.P. Instruments, New Haven, CT, U.S.A.) replaced the 3 M-KCl–agar bridge-calomel electrode.

Data from these experiments were digitized with a 12-bit analogue–digital converter and displayed and analysed using an IBM PC computer.

Results are expressed as means ± S.E. of mean. Statistical significance was assessed with Student's test for paired or unpaired comparisons, as appropriate.

RESULTS

In order to calculate the transepithelial resistance from luminal cable analysis, it is necessary to prove that the applied current causes linear voltage changes. Therefore, linearity of the transepithelial current–voltage (*I–V*) relationship was systematically tested, at the beginning of the experiment, in the range of current of −60 to +60 nA. In this range, the response was linear, as illustrated by the typical plot shown in Fig. 2. In most experiments, a current of 40–50 nA was employed. The resulting voltage deflexions were easily measurable at both ends of the tubule.

In twenty-nine tubules (mean length 655 μm, range 200–1225 μm) the space constant was found to be 130 ± 14 μm. The optically measured luminal diameter of the tubules under study was 22 ± 1 μm and the diameter calculated from the core resistance and the resistivity of the luminal fluid was 24 ± 3 μm.

To validate further the transepithelial resistance measurements, two experimental perturbations were employed, both expected to result in changes in predictable directions. First, the temperature of the bath was decreased. This is expected to increase R_e because of: (a) increases in the resistivities of intracellular and extracellular fluids (including the luminal fluid, because of its small volume and flow rate),

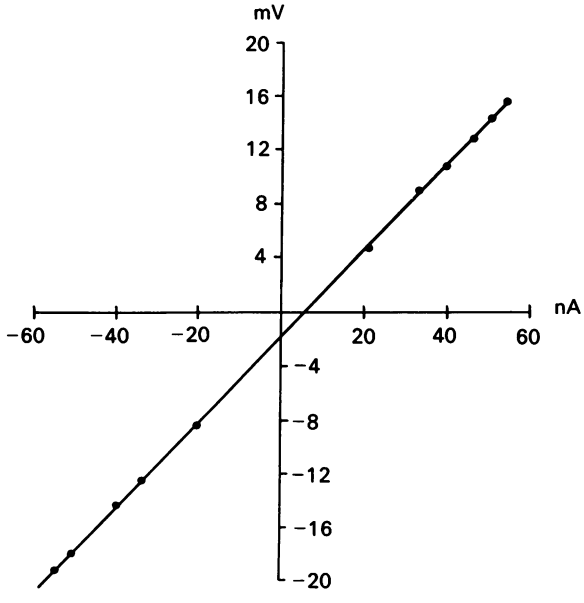

Fig. 2. Transepithelial current–voltage relation in isolated perfused proximal convoluted tubule (rabbit). Linearity was observed between −60 and +60 nA.

and (b) increase in the paracellular resistance, due to inhibition of fluid transport, which should result in narrowing of the lateral intercellular spaces. Secondly, Ca^{2+} was nominally removed from the bath. Extracellular Ca^{2+} is necessary for the maintenance of tight junction integrity in cultured epithelial cells (Cereijido & Meza, 1982). Therefore, removal of extracellular Ca^{2+} would be expected to decrease R_e.

The transepithelial resistance values at 10, 23 and 37 °C are summarized in Fig. 3. Significant increases in transepithelial resistance were observed at the lower temperatures. In six experiments, Ca was nominally removed from the bathing solution and R_e was measured at 2 min intervals during this perturbation. After 20 min of Ca-free solution, or before obvious microscopic alterations were evident, the length constant and the transepithelial resistance had decreased significantly, from 189 ± 35 to 59 ± 9 μm ($P < 0.01$) and 16 ± 1 to 2 ± 0.3 Ω cm^2 ($P < 0.001$), respectively. These changes were irreversible. In two additional experiments, 2 mM-EDTA was added to the Ca-free bath solution and R_e was monitored during the first 2 min following the exchange. As shown in Fig. 4, R_e decreased by more than 50 % in the first 10 s and became indistinguishable from zero, i.e. from the resistance value measured in the absence of tubule, in 30 to 100 s. In Ca-free, EDTA-containing solutions, striking morphological alterations were observed concomitantly with the decrease in transepithelial resistance. The cells separated from the basement mem-

brane and were finally washed out by the luminal perfusate. The electrical resistance of the basement membrane was unmeasurably low.

Results of intracellular impalements

The voltage of the basolateral membrane under control conditions was -54 ± 3 mV. The mean depolarization induced by raising external [K] from 5 to 140 mM (isomolar K-for-Na substitution) was 44 ± 2 mV. Further experimental

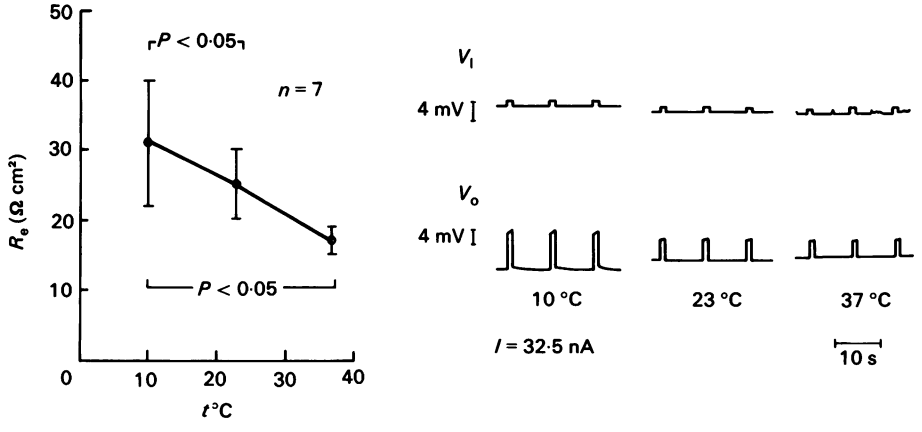

Fig. 3. Rabbit proximal tubule. Specific transepithelial resistances at 10, 23 and 37 °C. Examples, from a typical experiment, of the voltage changes elicited by transepithelial current at 10, 23 and 37 °C are shown on the right-hand side. A significant decrease in R_e was observed as the bath temperature is increased. Statistical significance was assessed with Student's test for pair comparisons.

perturbations were performed only if the depolarization upon K for Na substitution was greater than 35 mV. Cells with a base line basolateral membrane voltage of less than -40 mV were not studied. The control basolateral membrane voltage observed in this series of experiments is in good agreement with the average found by Biagi *et al.* (1981) in the same segment, under similar experimental conditions.

Measurement of 'a', the apparent ratio of apical to basolateral membrane resistances

The apparent ratio of apical to basolateral membrane resistance (a) was calculated from the changes in voltage elicited across the apical and the basolateral membrane (ΔV_a and ΔV_b, respectively) by the transepithelial current pulse (see Fig. 5). ΔV_b was measured by the intracellular micro-electrode. ΔV_a was calculated from $\Delta V_x - \Delta V_b$, where ΔV_x is the current-induced change in transepithelial voltage at distance x from the current source, which corresponds to the site of impalement

$$a = \frac{\Delta V_x - \Delta V_b}{\Delta V_b} = \frac{\Delta V_a}{\Delta V_b}, \tag{11}$$

In principle, ΔV_x can be measured directly, by advancing the intracellular micro-electrode into the lumen of the tubule. However, this frequently results in irreversible cell damage and electric leak at the impalement site. Accordingly, it was

preferred to calculate ΔV_x from the cable properties of the tubule, as explained in the Methods. The calculation of a from eqn. (11) is valid provided that the current flows across the two membranes of the impaled cell (apical and basolateral) are identical. However, it is possible that the tubule cells are electrically coupled, as observed in amphibian proximal tubules (Windhager, Boulpaep & Giebisch, 1967;

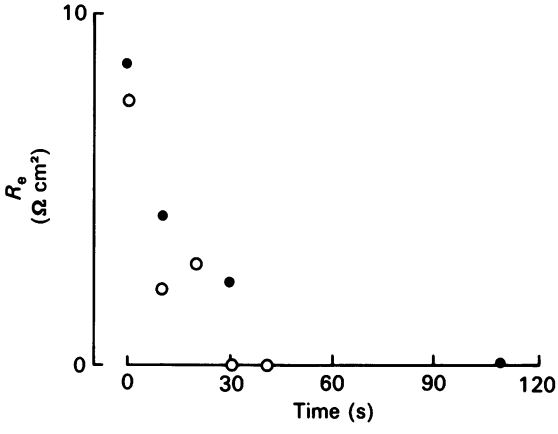

Fig. 4. Rabbit proximal tubule. Effect of a Ca^{2+}-free, 2 mM-EDTA basolateral solution on the transepithelial resistance, R_e. Different symbols denote two different tubules. A rapid decrease in R_e was observed after Ca^{2+} removal, which was indistinguishable from zero at 30 s (open circles) and at 110 s (filled circles), respectively.

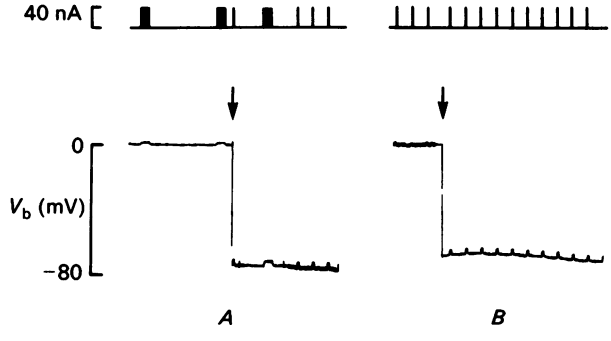

Fig. 5. Cell impalements in rabbit proximal tubule. Note the increase in the size of the deflexions induced by transepithelial current pulses upon impalement.

Guggino, Windhager, Boulpaep & Giebisch, 1982). If such were the case, the basolateral membrane current would be higher than the apical one, because current would either enter the impaled cell both from the lumen and from the neighbouring cells closer to the current source, resulting in an underestimate of the value of R_a/R_b. Inasmuch as the space constant for current flow from cell to cell is not known, it is impossible to estimate the magnitude of this error.

In nineteen experiments, the mean value of a under control conditions was $3{\cdot}1 \pm 0{\cdot}3$.

For calculations of cell membrane resistances (see below) it was assumed that $a = R_a/R_b$.

Selective changes in the electrical properties of luminal or basolateral membrane

To estimate the absolute values of R_a, R_b and R_s it is necessary to have a third independent measurement in addition to R_e and a. This was done in two different

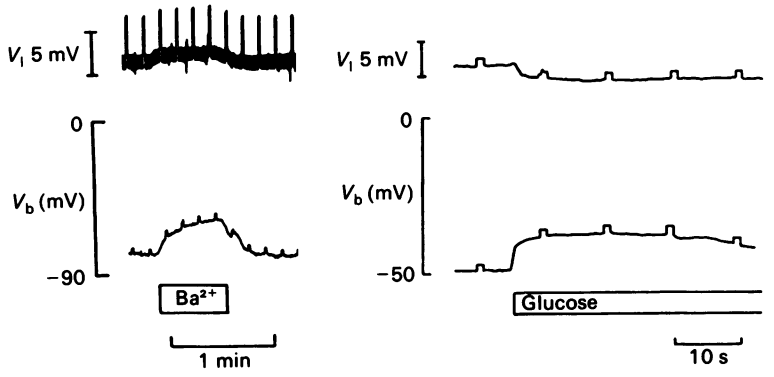

Fig. 6. Effects of Ba^{2+} and glucose on membrane voltages in rabbit proximal tubule. Left panel shows the effect of Ba^{2+} addition to the basolateral side on the transepithelial potential (V_l) and the basolateral membrane potential (V_b). Rapid depolarization of both transepithelial and basolateral membrane voltages is seen. These effects are reversible upon removal of Ba^{2+}. The right side panel shows the effect of glucose addition to the luminal side on the transepithelial voltages (V_l) and the basolateral membrane voltage (V_b). Note the rapid depolarization of the basolateral membrane and the concomitant transepithelial hyperpolarization.

ways: (1) by blocking basolateral membrane K permeability with Ba^{2+} (Nagel, 1979; Biagi *et al.* 1981; Bello-Reuss, 1982), which should result in a decrease in basolateral membrane equivalent electromotive force (Biagi *et al.* 1981; Bello-Reuss, 1982) and in an increase in basolateral membrane resistance; and (2) by adding glucose to the luminal perfusate, since glucose is co-transported with Na at the apical membrane (Kinne, 1976; Sacktor, 1977), glucose addition should result in decreases in both apical membrane equivalent electromotive force and resistance (Frömter, 1982).

Each of these two experimental manoeuvres results in changes in the voltages across luminal and basolateral membranes and across the whole epithelium. As explained in the Methods, the initial changes in two of the voltages are related in simple ways to the electrical resistances of the cell membranes (eqns. (7) and (8)).

Fig. 6 illustrates the effect of addition of Ba^{2+} to the peritubular side. As illustrated in the Figure, and as observed in all experiments of this series ($n = 8$), Ba^{2+} elicited rapid and reversible changes in transepithelial and basolateral membrane voltages. The initial depolarization, lasting up to 2 s was followed by slower depolarization. The Ba^{2+}-induced change in basolateral membrane voltage ($\Delta V_b'$) was measured as the difference between the base-line voltage and the voltage at the inflexion point between the fast and the slow depolarization, i.e. 0·5–2 s after the onset of the Ba^{2+}-elicited depolarization. The change in V_l ($\Delta V_l'$), recorded simultaneously, was

measured from identical time points. Changes in V_b and V_l upon exposure to Ba^{2+}, measured as described, are summarized in Table 1. Calculated resistances of the cell membranes and the paracellular pathway from these measurements and from the values of R_e and R_a/R_b in the same experiments are summarized in Table 2.

TABLE 1. Initial changes in basolateral membrane voltage (ΔV_b) and transepithelial voltage (ΔV_l) induced by the addition of either Ba^{2+} to the bath solution or glucose to the perfusate in rabbit proximal tubules

	$\Delta V'_b$	$\Delta V'_l$
Ba^{2+} ($n = 8$)	15·9±2·0	1·1±0·3
	$\Delta V''_b$	$\Delta V''_l$
Glucose ($n = 7$)	9·8±2·1	2·1±0·5

Values are means±S.E. of mean, n = number of experiments.

TABLE 2. Transepithelial specific resistance, cell membrane and shunt resistances in rabbit proximal tubules

Method	R_e	a	R_a	R_b	R_s
Ba^{2+} ($n = 8$)	16±5	3·3±0·4	249±68	70±11	17±5
Glucose ($n = 7$)	14±2	3·2±0·2	227±42	66±12	15±1

Resistances in Ω cm² of tissue (basement membrane area). R_e = transepithelial specific resistance; a = apparent ratio of cell membrane resistances; R_a = apical membrane resistance; R_b = basolateral membrane resistance; R_s = paracellular resistance.

TABLE 3. Cell membrane and paracellular resistances in proximal tubules of *Ambystoma tigrinum*

		R_a	R_b	R_s
Glucose	($n = 9$)	2650±180	665±99	58±11
Ba^{2+}	($n = 13$)	2368±350	701±124	84±12
K^+	($n = 6$)	2375±472	796±105	90±14
Cable analysis*		2305	591±58	53

* Cable analysis values from Maunsbach & Boulpaep (1984); R_s calculated from the reported values of R_a, R_b and transepithelial resistance.

As shown in right side of Fig. 6, luminal addition of glucose resulted in rapid depolarization of the basolateral membrane and concomitant transepithelial hyperpolarization. The initial change lasted 300±30 ms and was followed by a slower depolarization of both cell membrane and transepithelial voltage that reached apparent steady states in 5–10 s. The significance of the initial changes in potentials has been thoroughly analysed by Frömter (1982) who concluded that the initial depolarization is the expression of intraepithelial current flow secondary to the Na^+-glucose co-transport at the luminal membrane. Current flow through the resistances represented by the basolateral membrane and the paracellular pathway cause the voltage drops $\Delta V''_b$ and $\Delta V''_l$, respectively. The initial voltage changes across the basolateral membrane and the paracellular pathway were used to estimate R_b/R_s (eqn. (7)). The values of the initial depolarization of V_b and the

initial hyperpolarization of V_l are shown in Table 1. The basolateral membrane voltage change was 4·7 times larger than the transepithelial voltage change, indicating that change, $R_b/R_s = 4{\cdot}7$. From these data and the values of R_e and R_a/R_b, all three resistances were calculated. The results are summarized in Table 2.

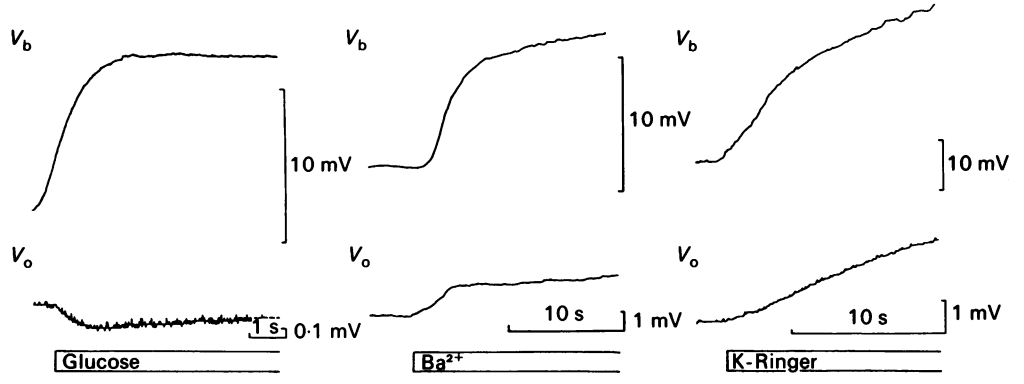

Fig. 7. Effects of glucose, Ba^{2+} and high K^+ on membrane voltages in proximal tubule of *Ambystoma tigrinum*. The left-hand panel shows the effect of addition of glucose to the luminal solution on V_b and V_o. Depolarization of V_b and hyperpolarization of V_o was observed. The centre panel shows the effect of addition of Ba^{2+} to the bath on V_b and V_o. Note depolarization of both voltages. The right-hand panel illustrates the effects on V_b and V_o of a substitution of K for Na in the bath. Note the rapid depolarization of V_b and the slower depolarization of V_o.

Studies in Ambystoma proximal tubule

The proximal tubule of the kidney of neotenic specimens of *A. tigrinum* has been thoroughly characterized in terms of electrophysiological and transport properties by Sackin & Boulpaep (1981) and Maunsbach & Boulpaep (1984). Inasmuch as cell membrane and paracellular resistances were determined by luminal and intra-epithelial cable analysis (Sackin & Boulpaep, 1981), this preparation affords a unique opportunity for comparison of the results obtained with cable analysis and with the methods employed here. Therefore, resistance values were estimated in segments of *Ambystoma* proximal tubules perfused as described in the Methods. In addition to the glucose and Ba^{2+} techniques, K-for-Na substitutions were employed, assuming the paracellular pathway to be anion selective (Boulpaep, 1979), with the results summarized in Table 3. Fig. 7 depicts the basolateral membrane potential and the transepithelial potentials during the aforementioned changes. The results obtained with the three methods reported here were very similar to those obtained frc n cable analysis. (Sackin & Boulpaep, 1981; Maunsbach & Boulpaep, 1984), as alsc shown in Table 3.

DISCUSSION

The validity of the methods employed to determine cell membrane and paracellular resistance values depends on the equivalent circuit chosen to represent the tubule epithelium, the specificity of the actions of Ba^{2+} and glucose, and several technical

problems. Therefore, interpretation of the results requires consideration of these issues. I will discuss first the measurement of transepithelial resistance and then the estimation of cell membrane resistances.

Transepithelial resistance

Several arguments can be given for the validity of the R_e measurements. First, there was good correlation between the temperature-induced changes of resistivity of dilute saline solutions and the measured change in core resistance. Secondly, there was excellent agreement between the optically measured tubule diameter, and the diameter calculated from the electrical data. Thirdly, the transepithelial resistance fell in Ca^{2+}-free solution, and rose at low temperature. Both effects are the predicted ones from the known transport properties of this epithelium (see above).

Thus the present study confirms the existence of a low transepithelial resistance in this segment. The values are in good agreement with the ones reported by Berry (1983) and are about twice the values reported by Lutz *et al.* (1973). Recently an intermediate value ($R_e \cong 8{\cdot}2\ \Omega\ cm^2$) has been communicated (Lapointe *et al.* 1984). Berry (1983) and Lapointe *et al.* (1984) ascribe these discrepancies to improvements in the electrical insulation of the tubule ends, which would result in the higher values. In addition, Berry (1983) found higher transepithelial resistances in tubules originated near the surface of the kidney ($n = 5$) as compared with juxtamedullary ones ($n = 3$). Although the small number of experiments does not allow one to draw a definitive conclusion, Lapointe *et al.* (1984) stress that they selected for study, tubules from the middle part of the cortex and thus differences intrinsic to nephron population origin were considered a probable explanation for the difference between their values and the ones reported by Berry (1983). It is indeed possible that this is the case, since the tubules employed in the present experiments originated near the surface of the kidney.

Electrical resistances of the cell membrane and the paracellular pathway

The experiments in which cell membrane and paracellular resistances were calculated showed that the low paracellular resistance is responsible for the low transepithelial resistance, in agreement with findings for renal and other leaky epithelia (Boulpaep, 1972; Frömter, 1972; Reuss & Finn, 1975; Boulpaep, 1979; Sackin & Boulpaep, 1981). As stated before, the calculations were based on the assumption that the initial effects of Ba^{2+} are restricted to E_b and R_b. A similar assumption (i.e. only E_a and R_a are initially changed) was made for the action of glucose on the apical membrane. Ba^{2+} produces an initial rapid depolarization followed by a slower depolarization. The initial change is assumed to correspond to the change of the resistance and electromotive force of the basolateral membrane only.

In the rat proximal tubule, resistance measurements based on the voltage efffects of luminal addition of glucose (Frömter, 1982) yield results remarkably close to the ones reported here. Both sets of values are larger than the ones communicated for the perfused rabbit proximal convoluted tubule by Lapointe *et al.* (1984). The latter measurements were made 2–5 s after the exchange of luminal solution, i.e. at a time at which secondary changes are conceivable. Indeed, calculation in the present

experiments based on values at times longer than 300 ms of glucose exposure yield progressively lower cell membrane resistance values (at 1 s, $R_a = 151 \pm 63$ and $R_b = 52 \pm 11\ \Omega\ cm^2$).

The fact that similar results were obtained with the glucose and Ba^{2+} methods supports the validity of the assumptions involved in both sets of experiments. A stronger argument is provided by the excellent agreement between the calculated resistance in *A. tigrinum* and the values reported previously in the literature, which were obtained by double-cable analysis (Sackin & Boulpaep, 1981; Maunsbach & Boulpaep, 1984).

In conclusion, in the experiments reported here the measurement of transepithelial resistance corroborates previous findings in isolated and perfused rabbit proximal convoluted tubules. The transepithelial resistance is low. Measurements of cell membrane and shunt resistance indicate that the main conductive pathway is probably of intercellular location. The cell membrane resistance ratio (apical/basolateral) was found to be about 3. The cell membrane resistances, estimated by the Ba^{2+} and glucose methods in rabbit tubules, were in close agreement. The agreement was also valid for the comparison, in *Ambystoma* tubules between values estimated from the Ba^{2+} and glucose techniques and previously reported values, obtained by cable analysis. I conclude that the methods described here are valid for the estimation of cell membrane resistances in tissues with small cells where more direct approaches are not feasible.

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