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Electrophysiological Properties of Cellular and Paracellular Conductive Pathways of the Rabbit Cortical Collecting Duct

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Summary. Microelectrode techniques were applied to the rabbit isolated perfused cortical collecting duct to provide an initial quantitation and characterization of the cell membrane and tight junction conductances. Initial studies demonstrated that the fractional resistance (ratio of the resistance of the apical cell membrane to the sum of the resistances of the apical and basolateral membranes) was usually independent of the point along the tubule of microelectrode impalement—implicating little cell-to-cell coupling—supporting the application of quantitative techniques to the cortical collecting duct. It was demonstrated that in the presence of amiloride, either reduction in the luminal pH or the addition of barium to the perfusate selectively reduced the apical membrane potassium conductance. From the changes in G^{te} and fractional resistance upon reducing the luminal pH or addition of barium to the perfusate, the transepithelial, apical membrane, basolateral membrane and tight junction conductances were estimated to be 9.3, 6.7, 8.1 and 6.0 mS cm^{-2}, respectively. Ninety to ninety-five percent of the apical membrane conductance reflected the barium-sensitive potassium conductance in the presence of amiloride with an estimated potassium permeability of 1.1×10^{-4} cm sec^{-1}. Reduction in the perfusate pH to 4.0 caused a 70% decrease in the apical membrane potassium conductance, implying a blocking site with an acidic group having a pK_a near 4.4. It is concluded that both the transcellular and paracellular pathways of the cortical collecting tubule have high ionic conductances, and that the apical membrane conductance primarily reflects a high potassium conductance. Furthermore, both reduction in the perfusate pH and addition of barium to the perfusate selectively block the apical potassium channels, although the site of inhibition likely differs since the two ions display markedly different voltage-dependent blocks of the channel.

Key Words epithelium · cortical collecting duct · ionic permeability · conductance · potassium · hydrogen · barium

Introduction

The rabbit cortical collecting duct (tubule) is known to play an important role in regulating the electrolyte composition of the body fluids. The tissue has been shown to both absorb and secrete a variety of ions, including sodium, potassium, hydrogen and chloride, yet the underlying mechanisms of transport are only partially understood. Much of our present understanding of these mechanisms has been deduced from an analysis of transepithelial ion fluxes, voltages and conductances (e.g. Grantham, Burg & Orloff, 1970; Helman, Grantham & Burg, 1971; Frindt & Burg, 1972; Stoner, Burg & Orloff, 1974; Hanley & Kokko, 1978; Stokes, 1981; O'Neil, 1983) where the tissue has been viewed as a "black box." As a result only limited information has been obtained as to the extent to which ions transverse the epithelium via transcellular or paracellular routes, the importance and magnitude of electrogenic (conductive) and electroneutral transport mechanisms, and the dependency of transport on electrochemical gradients.

Recently, it has been demonstrated that microelectrode techniques can be successfully applied to the cortical collecting duct to directly probe the cellular interior (O'Neil, 1982; Koeppen, Biagi & Giebisch, 1983; Koeppen & Giebisch, *in press;* O'Neil & Sansom, 1984*b*). With these techniques it is now possible to characterize directly the electrogenic transport properties of the cell membranes and of the paracellular pathway (tight junction). The purpose of this study was to use these techniques to characterize the transcellular and paracellular pathways of this tissue and to quantitate the magnitude of the cell membrane and tight junction conductances. Parts of this study have been published in abstract form (O'Neil, 1982; O'Neil & Sansom, 1984*a*).

Materials and Methods

Isolation and Perfusion of the Cortical Collecting Duct

New Zealand white female rabbits were maintained on standard Purina rabbit chow and tap water *ad libitum*. The animals were

sacrificed by cervical dislocation, the left kidney was removed, and segments of cortical collecting duct (tubule) were isolated by free-hand dissection under stereoscopic observation as previously described (Burg et al., 1966; O'Neil & Boulpaep, 1982). A tubule segment was transferred to a Lucite® chamber mounted on the stage of an inverted microscope (Nikon Diaphot or AO Biostar) and was perfused by insertion of a glass pipet into one end of the tubule lumen. The opposite end of the tubule was held in a glass pipet with a small amount of a liquid dielectric, Sylgard 184 (Dow Corning), that served to electrically insulate the end of the tubule from the outer bathing medium.

The tubules were continuously bathed in a medium containing (in mM): 45 NaCl, 40 Na gluconate, 1.2 NaH_2PO_4, 55 choline Cl, 5 KCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 10 PIPES, and 1 g/liter glucose at pH 7.4, and were perfused with an identical solution (control perfusate) but without glucose. These solutions have been modified from the usual solutions (*see* Burg et al., 1966) to provide a "standard" simplified solution in which ion substitution (cations for choline, anions for gluconate on Cl^-) can readily be made (*see* O'Neil & Sansom, 1984*b*; Sansom, Weinman & O'Neil, 1984). In studies using a low pH perfusate to block the apical cell membrane potassium channels, the control solution was exchanged via a manifold arrangement with a solution of the same composition except that 10 mM glycylglycine was substituted for the PIPES buffer, and the pH adjusted to 4.0. The effects of luminal addition of barium were likewise evaluated by substituting 5 mM $BaCl_2$ for 10 mM choline chloride in the control perfusion solution. The perfusion flow rate was controlled via hydrostatic pressure by adjusting the height of a perfusate reservoir. With this arrangement, exchange of the control perfusate with a test solution was rapid, requiring 10 to 20 sec for a complete exchange. The bathing chamber and bathing medium exchange lines were water-jacketted for temperature control at 37°C.

Measurement of Transepithelial Voltage and Conductance

The transepithelial voltage V^{te} was measured via an agar bridge (154 mM NaCl in 3% agar) placed in direct contact with the perfusion solution (O'Neil & Boulpaep, 1982). The agar bridge was connected with a Ag-AgCl wire to a high input impedance dual electrometer (W-P Instruments, model KS-700) whose output was recorded on one channel of a four-channel strip-chart recorder (MFE, model 1400). All voltages were referenced to the bathing medium which was grounded via a second agar bridge and Ag-AgCl wire.

The transepithelial conductance G^{te} was measured using cable analysis as previously described (Helman et al., 1971; O'Neil & Boulpaep, 1982). In brief, constant current pulses of 20 to 200 nA (500-msec duration) were injected into the tubule lumen via the perfusion pipet and the resulting voltage deflections at both ends of the tubule monitored. The voltage deflection at the "perfusion end" of the tubule ΔV_o was measured with the KS-700 electrometer via the agar bridge in contact with the perfusate as noted above. An electrical bridge arrangement was employed whereby the resistance of the perfusion pipet could be nulled before the tubule was connected to the pipet, so that only the voltage deflection due to the input resistance of the tubule was measured. The voltage deflection at the opposite end of the tubule ΔV_L was measured via a second agar bridge inserted down the glass pipet holding the distal end of the tubule. The bridge was connected with a Ag-AgCl wire to a second high-input electrometer (W-P Instruments, model VF-2) whose output was recorded on a second channel of the strip chart recorder. The voltage deflections were recorded within the first 100 msec of the current pulse. Whereupon, the transepithelial conductance G^{te} could be calculated using one-dimensional cable analysis for a tubule of length L (Weidman, 1952; Helman et al., 1971):

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

$$G^{te}(\text{mS} \cdot \text{cm}^{-2}) = \frac{\Delta I_o}{2\pi a \Delta V_o \lambda} \coth(L/\lambda), \tag{2}$$

and

$$R^{te}(\Omega \cdot \text{cm}^2) = 1/G^{te} \tag{3}$$

where R^{te} is the transepithelial resistance, λ is the space constant of the tubule and a is the radius of the tubule lumen measured optically with an eyepiece reticle fitted to the inverted microscope.

It has been shown previously that a major resistance to radial flow of current from the tubule lumen to the bath appears to be located at the level of the luminal border of the tissue (Helman et al., 1971). Consequently, the resistance of the luminal fluid core can be related to the geometric properties of the tissue, so that an "apparent" radius of the luminal fluid core can be defined from electrical considerations alone, a_e, and compared with that measured optically, a. From the cable equations, the core resistance R_c of the tubule lumen can be estimated (Helman et al., 1971; O'Neil & Boulplaep, 1979):

$$R_c(\Omega \cdot \text{cm}^{-1}) = \frac{\Delta V_o}{\Delta I_o \lambda} \tanh(L/\lambda). \tag{4}$$

Combining this with the measured volume resistivity ρ of the perfusate (YSI, model 32 conductivity meter) the apparent radius of the tubule lumen a_e can be estimated:

$$a_e(\text{cm}) = \sqrt{\rho/(\pi R_c)}. \tag{5}$$

If the assumptions of the cable analysis are correct, then the value of a_e should be similar to the optically measured radius a. Hence only tubules were used in which the two estimates of the radii were within 2 μm.

It was assumed in the present analysis that the measured transepithelial conductance was independent of the magnitude of the current injected into the tubule lumen, i.e., the conductance was not voltage-dependent. We have shown in a previous study (O'Neil, 1983) that upon addition of 5 mM barium to the perfusate, the change in G^{te} is voltage-dependent for negative current pulses (lumen-negative voltage deflection) but not for positive current pulses. Hence, in all cases, G^{te} was estimated using positive current pulses.

To evaluate possible voltage-dependent effects of luminal pH on tissue conductance, the "input" current-voltage relation $I_o - V_o$ was determined in some tubules. The relation was generated by injecting pulses of current I_o of varying magnitude (duration 500 msec) into the tubule lumen through the perfusion pipet and monitoring the resulting voltage deflections at the point of current injection V_o via the perfusion pipet (O'Neil, 1983).

Microelectrode Techniques

Microelectrodes used for impaling the epithelial cells were pulled on a Narishige PD-5 microelectrode puller from 1.2 mm OD capillary glass (W-P Instruments). The microelectrodes had pulled tip lengths of 1 to 1.5 cm and electrical resistances of 60 to 200 MΩ when filled with 0.5 M KCl. The filled microelectrodes were inserted into a microelectrode holder (W-P Instruments) fitted with a Ag-AgCl pellet, which in turn was connected to the second channel of the KS-700 electrometer. The microelectrode tip was positioned over the basolateral membrane of a cell using a 3-dimensional hydraulic micromanipulator (Narishige, Model MO-102). The cell was impaled by advancing the microelectrode tip to the cell membrane, followed by gentle "tapping" of the micromanipulator or vibration of the tip by brief application of a positive feedback current (negative capacitance compensation) to the electrode. As discussed in detail previously (O'Neil & Sansom, 1984*b*), most cellular impalements were characterized by an initial negative voltage "spike" which decayed monotonically towards zero. Recordings of this type were discarded. An impalement was accepted if the recorded cell voltage V^b was characterized by an initial negative deflection which was either subsequently maintained at or more negative than the initial deflection, or which was followed by a brief relaxation, but subsequently recovered to a value equal to or more negative than the initial deflection. Since the fractional resistance fR^a was measured in all experiments (*see below*), an additional criterion for acceptance of an impalement was that upon injection of a current pulse I_o into the lumen via the perfusion pipet, a significant change in the basolateral membrane voltage had to be detected so that fR^a could be reliably estimated. This criterion assured that the microelectrode tip crossed a significant resistance barrier, supposedly the basolateral cell membrane. Occasionally it has been observed that upon attempting to impale a cell, a stable negative voltage of 30 to 40 mV was recorded. However, the measured fR^a was 1, i.e., the recorded basolateral membrane voltage was insensitive to injection of I_o. We do not know whether this reflected impalement damage around the microelectrode tip, an artifact, or perhaps impalement of a second cell type which was characterized by a low membrane voltage and a high resistance of the apical cell border relative to the basolateral cell border (*see* O'Neil & Sansom, 1984*b*). Consequently, this type of recording was discarded.

The voltage recorded between the microelectrode and the grounded bath was the electrical potential difference of the basolateral cell membrane V^b. Under zero current or "open circuit" conditions, the electrical potential difference across the apical membrane was given by:

$$V^a = V^{te} - V^b. \tag{6}$$

However, because of the cable properties of the tubule, upon injection of current into the lumen, both axial current flow down the lumen and radial flow across the epithelium to the bath would decay with distance from the point of current injection as a consequence of the radial flow. Consequently, the transepithelial voltage deflections would also decay with distance from the point of current injection. Hence, the transepithelial voltage deflection at the point of the microelectrode impalement at a known distance x from the point of current injection ΔV_x^{te} must be calculated using cable analysis (Weidmann, 1952). Thus,

$$\Delta V_x^{te} = \frac{\Delta V_o \cosh(x/\lambda - L/\lambda)}{\cosh(L/\lambda)}. \tag{7}$$

If the voltage change across the basolateral cell border at point x was simultaneously measured with the microelectrode, the voltage change across the apical border ΔV_x^a could be calculated:

$$\Delta V_x^a = \Delta V_x^{te} - \Delta V_x^b. \tag{8}$$

Estimation of Barrier Conductances

From the above values of ΔV_x^a and ΔV_x^{te} the fraction of ΔV_x^{te} that was dissipated across the apical cell membrane, i.e. the apical membrane fractional resistance fR^a, could be obtained from

$$fR^a = R^a/(R^a + R^b) = G^b/(G^a + G^b) = \Delta V_x^a/\Delta V_x^{te} \tag{9}$$

or

$$1 - fR^a = G^a/(G^a + G^b) = \Delta V_x^b/\Delta V_x^{te} \tag{10}$$

where R^a and R^b are the apical and basolateral cell membrane resistances, respectively, and G^a and G^b are the corresponding membrane conductances, respectively. The analysis is based upon the assumption that upon injection of a current pulse into the tubule lumen, the resulting current flow across the apical border of a single cell is equal to that across the basolateral border (*see* Discussion). Further, by assuming a simple electrical equivalent circuit model in which the transcellular conductive pathway is paralleled by a paracellular conductance consisting primarily of the tight junction G^{tj} then the total transepithelial conductance G^{te} is given by

$$G^{te} = G^a G^b/(G^a + G^b) + G^{tj}. \tag{11}$$

Whereupon Eq. (10) can be substituted into Eq. (11) to get

$$G^{te} = (1 - fR^a)G^b + G^{tj}. \tag{12}$$

It is apparent from this equation that under conditions where G^b and G^{tj} are constant and G^a is allowed to vary, a plot of G^{te} *vs.* $1 - fR^a$ should yield a straight line with a slope of G^b and an intercept of G^{tj} (Lewis et al., 1977). Thus by using microelectrode techniques in combination with a procedure that selectively alters G^a and leaves G^b and G^{tj} unaffected, it is possible to quantitate these three barriers to transepithelial ion transport in the cortical collecting duct. In the present study it was found that this analysis could best be accomplished by inhibition of the apical membrane potassium channel by reduction of the luminal pH.

Statistical Analysis

The data are summarized as mean values ± standard error of the mean (SEM). Differences between two groups were evaluated using the paired or unpaired t-test as appropriate. Relations be-

Table 1. Comparison of the effects of distance to impalement (proximal *vs.* distal) on the estimated fractional resistance fR^a

	Tubule length (μm)	Distance to impalement (μm)	V^{te} (mV)	V^b (mV)	fR^a
A. Proximal impalement	595 ±58	35 ±11	−1.6 ±1.4	−59.3 ±5.2	0.49 ±0.09
B. Distal impalement	595 ±58	476 ±63	−0.8 ±0.7	−55.5 ±3.5	0.45 ±0.08
C. Difference (B − A)	—	441[b] ±69	−0.8 ±1.4	3.8 ±4.9	−0.04 ±0.05

[a] Paired studies, $n = 6$.
[b] $P < 0.01$.

tween various parameters were evaluated using linear regression analysis.

Results

Dependence of Fractional Resistance on Distance to Impalement

The apical cell border of the cortical collecting tubule contains both sodium and potassium conductances (Koeppen et al., 1983; O'Neil & Sansom, 1984*b*), with the potassium conductance being the dominant of the two. In preliminary studies it was determined that the membrane barrier conductances could best be quantitated by selective reduction of the apical cell membrane potassium conductance. Hence, to avoid possible interactions with the sodium conductance, it was blocked in all studies, unless otherwise noted, by the addition of amiloride (50 μM) to the luminal perfusate (Stoner et al., 1974; O'Neil & Helman, 1977; O'Neil & Boulpaep, 1979).

As shown in previous studies, cortical collecting duct cells can be impaled with microelectrodes across the basolateral border to obtain measurements of V^b and fR^a (O'Neil & Sansom, 1984*b*). Since fR^a is needed in the present study to quantitate the barrier conductances, it was necessary to determine whether its value was dependent upon the position along the tubule that the cell was impaled to obtain the measurement. This was evaluated by impaling cells either near the perfusion pipet (proximal end), or at the opposite end (distal end) of the tubule. While obtaining a stable cell impalement at the proximal end was relatively easy, obtaining a stable impalement at the distal end was found to be more difficult. A summary of the results for six tubules in which a stable impalement was obtained at both ends is given in Table 1. In these tubules, the V^b averaged −59.3 mV at the proximal end and did not differ from that observed at the distal end. The fR^a averaged 0.49 at the proximal end and 0.45 at the distal end, values not significantly different from each other (mean difference = 0.04 ± 0.05). This is diagrammatically depicted in Fig. 1 where fR^a is plotted as a function of the distance to the cell impaled given as a fraction of the length constant λ. A significant correlation was not evident. Hence, the value of fR^a would appear to be relatively independent of the distance to the point of cell impalement indicating little cell-to-cell coupling (*see* Discussion). It should be noted, however, that in one tubule the fR^a measured at the distal end was clearly less than that observed at the proximal end (0.47 *vs.* 0.67) which is indicative of apparent cell-to-cell coupling. Hence, while on the average fR^a would appear to be independent of the point of measurement, on an individual tubule basis this may not always be the case.

Interaction of Hydrogen and Barium Ions with the Potassium Channel

It has been shown that the potassium conductance of the apical cell membrane is reduced by the luminal addition of barium (Koeppen et al., 1983; O'Neil, 1983; O'Neil & Sansom, 1984*b*), and by lowering the perfusate pH (O'Neil, 1982; O'Neil & Sansom, 1984*b*). The effects of barium on the potassium conductance were demonstrated to be highly voltage-dependent, particularly for the submillimolar concentration range (O'Neil, 1983). This makes it difficult to quantitate the effects of barium on this conductance other than for a maximum inhibitory concentration of 5 mM which has relatively voltage-independent effects upon passage of lumen-positive current pulses (lumen-positive voltage deflections) (O'Neil, 1983). To evaluate whether the nature of the interaction of H^+ with the potassium channel

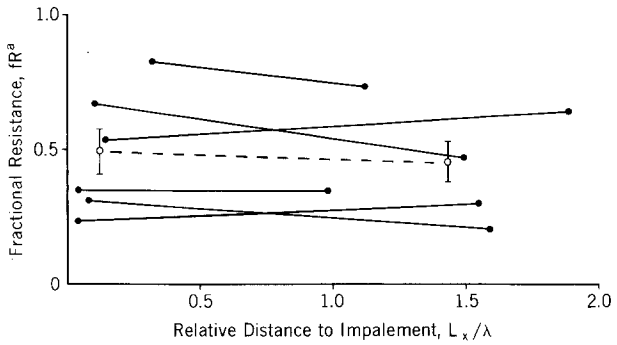

Fig. 1. Relation between fR^a and the distance to the point of impalement of a cell (L_x) given as a fraction of the length constant $\lambda(L_x/\lambda)$. For each tubule, fR^a was measured near the perfusion pipet and near the opposite end of the tubule (solid circles). Measurements from the same tubule are connected with a solid line. The mean value at both ends of the tubule is indicated by the open circles connected with the dashed lined. $n = 6$

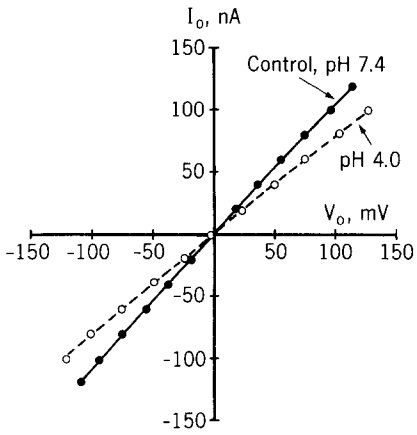

Fig. 2. Representative example of the influence of perfusate pH on the input current voltage ($I_o - V_o$) relation. The $I_o - V_o$ relation was generated in the presence of the control perfusate (pH 7.4, solid line) and the low pH perfusate (pH 4.0, dashed line). The value of V_o is the luminal voltage relative to the grounded bath

was similar, the effect of reducing the perfusate pH on the tubule input current-voltage ($I_o - V_o$) relation was studied (*see* Materials and Methods). As shown in Fig. 2, when tubules were perfused with the control perfusate, pH 7.4, the $I_o - V_o$ relation was relatively linear over the voltage range of −100 to +100 mV, similar to that observed previously (Helman & O'Neil, 1977; O'Neil, 1983). When the same tubule was perfused with the low pH perfusate, pH 4.0, the relation still remained voltage-independent but the slope of the $I_o - V_o$ relation was decreased, consistent with a reduction in the transepithelial conductance.

To assess in greater detail the voltage-dependent effects of luminal pH on the tissue conductance, the input conductance of the tubule G_o was estimated from the slope of the $I_o - V_o$ relation and plotted as a function of V_o. As shown for the summary data in Fig. 3, lowering the luminal pH to 4.0 caused a reduction in G_o by approximately 35% but was apparently voltage-independent—in some tubules, a very slight voltage-dependence was observed. This result is in marked contrast with that observed previously for barium, where the addition of 0.1 mM barium to the perfusate—which is the same concentration of hydrogen ion in the low pH perfusate—has been shown to have little influence on G_o for lumen-negative values of V_o (−50 to −75 mV), but causes a maximum reduction in G_o of near 50% for lumen-positive values of V_o (50 to 100 mV) (O'Neil, 1983).

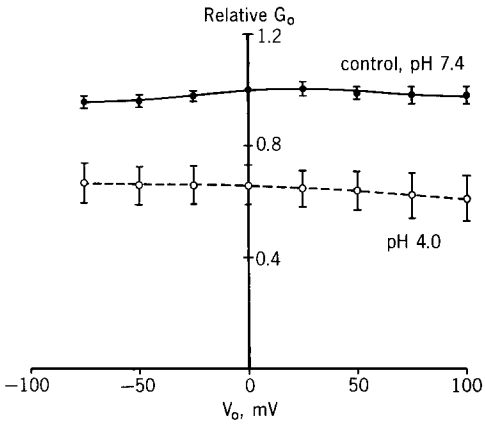

Fig. 3. Summary of the influence of input voltage V_o and perfusate pH on the input conductance G_o. The values of G_o were estimated from the slope of the $I_o - V_o$ relations as given in Fig. 2 ($G_o = \Delta I_o/\Delta V_o$) and normalized to G_o at $V_o = 0$ for the control perfusate (pH 7.4). The influence of V_o on G_o was evaluated for tubules perfused with the control perfusate (pH 7.4, solid line) and the low pH perfusate (pH 4.0, dashed line). $n = 5$

Quantitation of Barrier Conductances

As discussed in Materials and Methods, quantitation of the barrier conductances can be achieved by a step-wise reduction in G^a and plotting the resulting relation between G^{te} and $1 - fR^a$. Since the effects of luminal pH on the tissue conductance are relatively voltage-independent and require 1 to 2 min for a maximum effect on G^{te} and fR^a (O'Neil & Sansom, 1984*b*), this relation can readily be established simply by monitoring G^{te} and fR^a at 15- to 20-sec intervals upon reduction of the luminal pH. Two typical examples employing this method are shown in Fig. 4. As can be seen from the data, upon reduction of the luminal pH to 4.0 (solid circles), the rela-

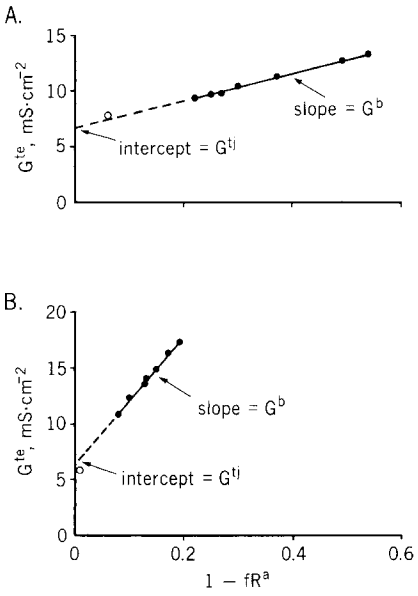

Fig. 4. Two typical examples of the relation between G^{te} and fractional resistance (given as $1 - fR^a$) generated by reducing the apical membrane potassium conductance by lowering the perfusate pH from 7.4 to 4.0 (solid circles). The individual points of the relation were obtained by measuring G^{te} and fR^a at 20-sec intervals during the transient period following reduction of the perfusate pH. Since the relations are highly linear ($r > 0.99$), the slope of the best-fit straight line provides a measure of G^b while the intercept provides a measure of G^{tj}. The single open circle in each panel reflects the effect of addition of 5 mM barium to the perfusate, pH 7.4. *A*. Tubule with an initially low fR^a. *B*. Tubule with an initially high fR^a

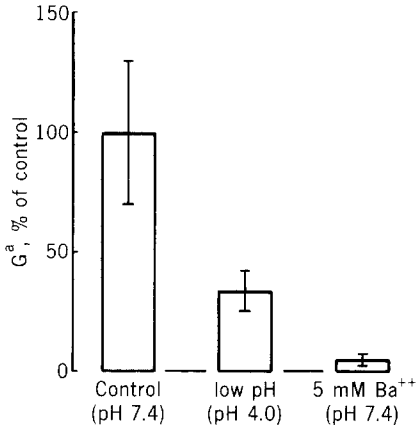

Fig. 5. Influence of perfusate pH and luminal barium on the apical cell membrane conductance G^a

tion between the changes in G^{te} and fR^a (plotted as $1 - fR^a$) are highly linear, supportive of the view that reduction of the luminal pH selectively reduces the conductance of the apical membrane without influencing the other barrier conductances (*see* Discussion). Consequently, it appears that the slope of the relation provides a measure of G^b, and the intercept, a measure of G^{tj}.

The above analysis was extended to use barium as a probe to estimate the barrier conductances, again assuming that barium selectively reduces the apical cell membrane conductance. The effects of addition of 5 mM barium to the perfusate on G^{te} and fR^a are shown in Fig. 4 (open circles) for the same tubules in which low pH was used as a probe. As can be seen after barium, the G^{te} and fR^a were close to the extrapolated line obtained for the low pH perfusate. Taken together, it seems reasonable to conclude that luminal barium and hydrogen selectively reduce the apical membrane conductance (potassium conductance) without significant effects on the basolateral membrane or tight junction conductances. The estimates of the barrier conductances based upon the use of either barium or hydrogen as a probe are summarized for a small group of paired studies ($n = 5$) in Table 2. The estimates of G^{te}, G^b, G^{cell} and G^{tj} were the same regardless of whether low pH or barium was used as a probe to reduce the apical membrane conductance.

The effects of the low pH perfusate and addition of barium on the apical membrane conductance are compared in Fig. 5. The pH 4.0 perfusate decreased G^a by 67% while the addition of 5 mM barium to the perfusate caused nearly a 95% reduction in G^a. If the barium-sensitive conductance reflects the potassium conductance of the apical border as previous evidence indicates (Koeppen et al., 1983; O'Neil & Sansom, 1984*b*), then the reduction in the perfusate pH to 4.0 resulted in a decrease in the apical membrane potassium conductance by approximately 70%.

The effects of 5 mM barium on the transepithelial and cell membrane voltages and conductance are summarized in Table 3 for 21 tubules. The effects of Ba^{++} are similar to those reported previously (Koeppen et al., 1983; O'Neil & Sansom, 1984*b*). Using the data from Table 3, the barrier conductances were estimated to be 6.7, 8.1 and 6.0 mS cm^{-2} for G^a, G^b and G^{tj}, respectively (Table 4). The transcellular conductance G^{cell} estimated as

$$G^{cell} = G^a G^b / (G^a + G^b) \quad (13)$$

averaged 3.3 mS cm^{-2}.

Quantitation of Apical Membrane Potassium and Leak Conductances

The apical membrane of the cortical collecting duct has both an amiloride-sensitive sodium conduc-

Table 2. Comparison of barrier conductances based upon estimates of the effects of either Ba^{++} addition or low luminal pH[a]

	G^{te} (all mS cm^{-2})	G^a	G^b	G^{cell}	G^{tj}
A. Conductance estimates using low pH as probe	11.9 ±1.7	10.1 ±2.6	24.1 ±8.5	5.9 ±1.4	6.0 ±0.8
B. Conductance estimates using Ba^{++} as probe	14.0 ±1.1	12.3 ±4.4	20.6 ±6.9	7.1 ±2.6	6.9 ±0.4
C. Difference (B − A)	2.1 ±1.1	2.2 ±3.4	−3.5 ±1.9	1.2 ±1.4	0.9 ±0.8

[a] Paired studies, $n = 5$.

Table 3. Influence of addition of barium to the perfusate on barrier voltages and conductances[a]

	V^{te} (mV)	V^a (mV)	V^b (mV)	fR^a	G^{te} (mS cm^{-2})	R^{te} (Ω cm^2)
A. Control	−1.0 ±0.4	68.5 ±2.3	−69.5 ±2.3	0.48 ±0.04	9.3 ±1.0	157 ±36
B. + 5 mM Ba^{++}	−1.6 ±0.4	33.4 ±3.3	−35.0 ±3.2	0.89 ±0.02	6.6 ±0.6	205 ±41
C. Difference (B − A)	−0.6[b] ±0.2	−35.2[c] ±2.6	34.5[c] ±2.6	0.41[c] ±0.03	−2.8[b] ±0.8	48[c] ±11

[a] $n = 21$. In all experiments the perfusate contained 50 μM amiloride.
[b] $P < 0.01$.
[c] $P < 0.001$.

tance and barium-sensitive potassium conductance (Koeppen et al., 1983; O'Neil, 1983; O'Neil & Sansom, 1984*b*). Since conductive pathways at the apical border to other major electrolytes such as chloride and bicarbonate have not been observed (O'Neil & Boulpaep, 1982; Sansom et al., 1984), it is likely that the effects of barium at the apical membrane are on the potassium channels only, particularly in the presence of amiloride. Furthermore, since the addition of 5 mM barium to the luminal perfusate has a maximal effect on the tissue conductance (O'Neil, 1983), the barium-sensitive conductance of the apical border likely reflects the magnitude of a barium-sensitive potassium conductance G^a_K. However, in some tubules, a small but significant apical membrane conductance likely remained even in the presence of 5 mM barium, and is defined here as "leak" conductance G^a_L. Defined in this manner, G^a_K averaged 6.1 mS cm^{-2} and G^a_L averaged 0.6 mS cm^{-2} (Table 5).

In the presence of amiloride, the V^{te} was essentially zero so that the potassium distribution across

Table 4. Summary of estiamted barrier conductances[a]

G^{te} (all mS cm^{-2})	G^a	G^b	G^{cell}	G^{tj}
9.3 ±1.0	6.7 ±1.6	8.1 ±2.3	3.3 ±0.9	6.0 ±0.6

[a] $n = 21$.

Table 5. Summary of the estimated "leak" conductance, potassium conductance and potassium permeability of the apical cell membrane[a]

G^a (mS cm^{-2})	G^a_L (mS cm^{-2})	G^a_K (mS cm^{-2})	P^a_K (cm sec^{-1}, $\times 10^4$)
6.7 ±1.6	0.6 ±0.2	6.1 ±1.5	1.1 ±0.3

[a] $n = 21$.

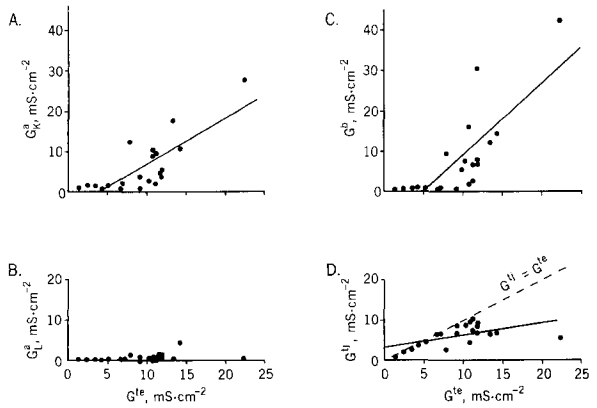

Fig. 6. Relation between barrier conductances and G^{te} for all tubules. *A*. Relation between G^a_K and G^{te}. The solid line is a plot of the least-squares linear regression equation,[1] $G^a_K = (1.15 \pm 0.21)G^{te} - (4.7 \pm 2.2)$. $n = 21$, $r = 0.785$, $P < 0.001$. *B*. Relation between G^a_L and G^{te}. The parameters are not significantly correlated. $n = 21$, $r = 0.352$. *C*. Relation between G^b and G^{te}. The solid line is a plot of the least-squares linear regression equation,[1] $G^b = (1.78 \pm 0.33)G^{te} - (8.5 \pm 3.4)$. $n = 21$, $r = 0.776$, $P < 0.001$. *D*. Relation between G^{tj} and G^{te}. The solid line is a plot of the least-squares linear regression equation, $G^{tj} = (0.29 \pm 0.10)G^{te} + (3.3 \pm 1.1)$. $n = 21$, $r = 0.536$, $P < 0.05$

the cell membranes was likely near equilibrium under these conditions. Whereupon, knowing the value of G^a_K, the potassium permeability of the apical membrane in the absence of barium, P^a_K, could be calculated with a modified form of the Goldman-Hodgkin-Katz equation (Hodgkin & Horowicz, 1959):

$$G^a_K = \frac{-P^a_K \cdot V^a[K^+]_l \cdot (zF^3)}{(RT)^2 \cdot (e^{-zFV^a/RT} - 1)} \quad (14)$$

and

$$P^a_K = \frac{-G^a_K \cdot (RT)^2 \cdot (e^{-zFV^a/RT} - 1)}{V^a \cdot [K^+]_l \cdot (zF^3)} \quad (15)$$

where $[K^+]_l$ is the potassium concentration of the perfusate and z, F, R and T have their usual meanings. Using this approach, the P^a_K was estimated to average 1.1×10^{-4} cm sec^{-1} (Table 5).

Variability of Barrier Conductances

A continual problem in studying the transport properties of the cortical collecting duct has been the extreme variability in the electrophysiological parameters among tubules even when tissues were studied under "similar" conditions (*see* O'Neil & Boulpaep, 1982). This is particularly true of the values for G^{te} which, in the present study alone, varied from near 1.5 to 23 mS cm^{-2}. It has been deduced in previous studies from measurements of G^{te}, that the ionic origin of this variability appeared to be a variable potassium conductance of the apical membrane, and a variable chloride conductance of the basolateral membrane and/or tight junction (O'Neil & Helman, 1977; O'Neil & Boulpaep, 1982; O'Neil, 1983). To directly evaluate this notion in the present study, the magnitudes of the barrier conductances were correlated with the magnitude of G^{te} in each of 21 tubules (Fig. 6). As shown in Fig. 6*A*, the G^a_K was highly variable but directly correlated with G^{te}($r = 0.785$, $P < 0.001$).[1] The apical membrane "leak" conductance G^a_L, on the other hand, was relatively insignificant in most cases, and was not correlated with G^{te} (Fig. 6*B*) and hence, was not likely an important factor relating to the variability of G^{te}.

The basolateral membrane conductance was also found to be highly variable and, like G^a, displayed a strong positive correlation with G^{te} (Fig. 6*C*, $r = 0.776$, $P < 0.001$).[1] The parallel tight junction conductance was less variable than the cell membrane conductances, but still displayed a significant, but weak, correlation with G^{te} (Fig. 6*D*, $r = 0.536$, $P < 0.05$). Since it has been shown in other studies that G^b and G^{tj} are dominated by chloride conductances at these barriers (Sansom et al., 1984), it would appear that the major factors responsible for the notable variations in G^{te} are a variable potassium conductance of the apical membrane, a variable chloride conductance of the basolateral membrane, and to a lesser extent, a variable chloride conductance of the tight junction.

Discussion

Localization and Quantitation of Barrier Conductances

It has been shown recently that microelectrode techniques can be successfully applied to the isolated perfused cortical collecting duct of the rabbit to provide a direct assessment of the electrophysiological properties of the cell membranes (Koeppen et al., 1983; Koeppen & Giebisch, *in press*; O'Neil

[1] The relation between G^{te} and the individual barrier conductances is likely highly complex since all barrier conductances vary simultaneously. The exact relation among parameters is not known. Consequently, linear regression analysis has been used to describe the various relations to determine, as a minimal analysis, whether a correlation exists among the parameters of interest.

& Sansom, 1984*b*). The present study has extended these techniques to provide a means of localizing and quantitating the barrier conductances of this tissue. It was shown that either reduction of the perfusate pH, or addition of barium to the perfusate, could be used as a probe to selectively reduce the potassium conductance of the apical cell membrane (*see below, Assessment of Methodology*). Whereupon, from the induced changes in the transepithelial conductance and fractional resistance upon reduction of the potassium conductance, estimates could be obtained for the initial conductances of the apical membrane, basolateral membrane and tight junction (*also see below, Correlations with Cell Type*).

The barrier conductances of the cortical collecting duct were found to be high. On the average, the apical membrane, basolateral membrane and tight junction conductances were 6.7, 8.1 and 6.0 mS cm^{-2}, respectively. Koeppen and Giebisch (*in press*) have recently estimated the barrier conductances of this tissue, using 2 mM barium as a probe, with similar results. While it is usually considered that a high transepithelial conductance of an epithelium arises as a result of a high paracellular conductance (*see* Boulpaep & Sackin, 1980), the present study demonstrates that for the cortical collecting duct, which is a "moderately tight" epithelium, the high transepithelial conductance arises as a combined result of high paracellular and transcellular conductances in parallel. This does not appear to be exceptional as other studies have recently reported results consistent with high membrane conductances for several tissues (Augustus et al., 1977; Guggino et al., 1982*a*; Greger & Schlatter, 1983; Hebert, Friedman & Andreoli, *in press*). It is interesting that these high membrane conductances may not simply reflect the extent of membrane infolding or the presence of microvilli, but may reflect a high conductance per absolute membrane area. If the present results are corrected for absolute membrane surface area using previous morphometric data (Wade et al., 1979; Welling, Evan & Welling, 1981), the apical membrane conductance would remain near 6.7 mS cm^{-2} since there is little apical membrane infolding. The basolateral membrane conductance would be approximately one-eighth (ratio of apical-to-basolateral membrane area), averaging near 1 mS cm^{-2}. The existence of these high cell membrane conductances was suspected in previous studies, utilizing transepithelial electrophysiological measurements, in which it was demonstrated that the tissue appeared to be characterized by a high potassium conductance of the luminal border and a high chloride conductance of the basolateral border (O'Neil & Boulpaep, 1982; O'Neil, 1983).

The Apical Membrane Conductance

The apical membrane was found to have a high, barium- and hydrogen-sensitive potassium conductance, as discussed previously (Koeppen et al., 1983; Koeppen & Giebisch, *in press*; O'Neil, 1983; O'Neil & Sansom, 1984*b*). Since in the presence of amiloride significant apical membrane conductances to other major electrolytes such as sodium (O'Neil & Boulpaep, 1979; 1982), chloride (Sansom et al., 1984) and bicarbonate (O'Neil & Boulpaep, 1982; Sansom et al., 1984) have not been found, the effects of barium and hydrogen must reflect inhibition of potassium channels alone. This large, barium-sensitive conductance of the apical cell membrane is not unique to the cortical collecting tubule as a similar conductance has been described recently for the apical cell border of several other epithelial tissues (Reuss, Cheung & Grady, 1981; Guggino et al., 1982*b*; Greger & Schlatter, 1983; Hebert et al., *in press*). From a historical perspective, however, it should be noted that an important apical membrane K^+ conductance of the rat distal tubule was described nearly two decades ago by Giebisch and coworkers (*see* Giebisch, Klose & Malnic, 1967). We are just now beginning to demonstrate the presence of, and hence understand the importance of, an apical membrane K^+ pathway in other tissues.

The potassium conductance of the apical cell membrane was found to be highly variable among tubules. It was shown to be directly correlated with the magnitude of G^{te}, and therefore is likely a major determinant of the electrophysiological variability among tubules (*see* O'Neil & Boulpaep, 1982). On the average the apical membrane potassium conductance was 6.1 mS cm^{-2}, corresponding to a potassium permeability of 1.1×10^{-4} cm sec^{-1}, and likely provides the dominant pathway for potassium secretion from the cell into the tubular lumen. Potassium taken up into the cell via the sodium/potassium exchange pump at the basolateral membrane, could therefore exit the cell either by diffusing across the apical border, giving rise to potassium secretion, or alternatively, recycling across the basolateral membrane via diffusion across that barrier (Grantham et al., 1970; O'Neil, 1981; Stokes, 1982; Koeppen et al., 1983; O'Neil & Sansom, 1984*b*). With this series arrangement of permeability barriers, potassium secretion could readily be regulated by controlling the potassium permeability at either or both cell borders. Regulation of these potassium permeabilities may, in part, underlie some of the electrophysiological variability among tubules heretofore mentioned.

The tubular fluid pH may play an important role

in regulating potassium secretion. Reduction of the luminal perfusate pH to 4.0 caused approximately a 70% reduction of the apical membrane potassium conductance (Fig. 4). Since the cortical collecting duct can acidify the tubular fluid to the pH 4 range (Koeppen & Helman, 1982), this could result in a decrease in the potassium conductance which would cause a decrease in potassium secretion. This effect could in part account for the well-known inverse relation in the whole animal between potassium excretion and urinary pH (Toussaint & Vereestraeten, 1962; Malnic et al., 1971; 1972). Indeed, Boudry, Stoner and Burg (1976) have reported a reduction in potassium secretion in the cortical collecting duct upon lowering of the perfusate pH (*see* O'Neil & Sansom, 1984*b*).

The site of inhibition of the potassium channel by luminal hydrogen ion remains speculative. Since the effect of hydrogen ions appears to be voltage-independent, it is unlikely that the active site for hydrogen binding is located deep within the membrane channel, but rather at a site which is near the surface, at the outer or inner boundry of the membrane voltage field. It is interesting that blockage of K^+ channels by H^+ in excitable tissues displays only a weak voltage dependence (Drouin & The, 1969; Hille, 1973; Shrager, 1974; Carbone et al., 1978). This is in marked contrast to that observed for the effects of barium on the potassium channel which have been shown to be highly voltage-dependent in the cortical collecting duct, similar to that observed for excitable tissues (*see* Latorre & Miller, 1983; O'Neil, 1983), implicating a binding site for barium deep within the membrane channel (O'Neil, 1983). If it is assumed that the binding of hydrogen ion to an acidic group causes a proportional decrease in the potassium conductance, it can be shown that given a 70% reduction in the potassium conductance upon reduction of the perfusate pH to 4.0, the pK_a for the acidic group must be near 4.4. This is similar to the pK_a of near 4.6 estimated for several potassium channels of excitable tissues (Drouin & The, 1969; Hille, 1973; Carbone et al., 1978)—this differs from a K^+ channel in crayfish axon which has a pK_a of near 6.3. (Shrager, 1974). These results again demonstrate the similarity of properties among potassium channels from a variety of tissues (*see* O'Neil, 1983; O'Neil & Sansom, 1984*b*).

A small barium- and amiloride-insensitive apical membrane conductance was apparent in the present study. This "leak" conductance was not evident in all tubules, but on the average, accounted for 5 to 10% of the apical membrane conductance. This conductance is not likely anion selective since the luminal addition or removal of either bicarbonate or chloride had no effect on the apical membrane conductance (Sansom et al., 1984). It may, however, reflect an amiloride-insensitive sodium channel or a barium-insensitive potassium channel similar to that described by Lewis and Wills (1981) for the rabbit urinary bladder. This remains to be evaluated in separate studies.

An important apical membrane conductive pathway not evaluated in the present study is the amiloride-sensitive sodium channel. It has been deduced from a qualitative assessment that this conductance is much smaller than the parallel potassium conductance since the effects of luminal addition of amiloride on G^{te} and fractional resistance were much smaller than that observed for barium (Koeppen et al., 1983; O'Neil & Sansom, 1984*b*). In the present study the effects of amiloride on G^{te} and fractional resistance were too small and variable from tubule to tubule to be used to provide a reliable measure of membrane conductances. Nonetheless, considering that the fractional resistance in the absence of amiloride was normally near 0.4 to 0.5, and that the decrease in G^{te} upon addition of amiloride averages near 1 mS cm^{-2} (O'Neil, 1983; O'Neil & Sansom, 1984), as it did in the present studies, the amiloride-sensitive apical membrane conductance would be anticipated to average near 2 mS cm^{-2}, considerably smaller than the apical membrane potassium conductance. This remains to be verified in separate studies.

The Basolateral Membrane Conductance

The permeability properties of the cortical collecting duct basolateral membrane have been partially elucidated. Sansom et al. (1984) have demonstrated that this border has a dominant chloride conductance of approximately 4 to 5 mS cm^{-2}. This is consistent with the results of earlier studies in which it was demonstrated that the G^{te} was highly dependent upon the chloride concentration of the bathing medium (O'Neil & Helman, 1977; O'Neil & Boulpaep, 1982). In addition to the chloride conductance, a significant, but apparently smaller, potassium conductance has also been shown to reside at that border (Koeppen et al., 1983; O'Neil, 1983; Sansom et al., 1984), although its absolute magnitude has not been determined. Evidence of important conductive pathways to other ions such as sodium and bicarbonate could not be demonstrated (O'Neil & Boulpaep, 1982; Sansom et al., 1984), consistent with the view that the basolateral border is characterized by chloride and potassium conductive pathways alone.

The basolateral membrane conductance dis-

plays a high degree of variability which appears to be directly correlated with the variability in G^{te} (Fig. 6). Using microelectrode techniques, the variations in the basolateral membrane conductance have been shown to be directly related to variations in a chloride conductance at that border (Sansom et al., 1984), implicating a chloride conductance as being responsible, in part, for the variability. This view is consistent with the results of earlier studies in which it was deduced from the chloride dependency of G^{te}, that the chloride permeability was higher at the basolateral border of the tubule than at the apical border (O'Neil & Boulpaep, 1982). Since the potassium permeability appears to be lower than the chloride permeability, the potassium permeability is likely to have only a small influence on the variability of the tissue conductance (O'Neil & Boulpaep, 1982; O'Neil, 1983; Sansom et al., 1984).

The Tight Junction Conductance

The magnitude of the tight junction conductance was found to be similar to that of the cell membranes. As such, it represents an important pathway for ion movement. It has been shown that the magnitude of the tight junction conductance among tubules was directly correlated with the chloride-dependent conductance of this pathway and was symmetrically dependent upon the chloride activity in either the bath or perfusate (Sansom et al., 1984). These effects are consistent with a symmetrical single-barrier chloride-conductive pathway.

The permeability of the tight junction to ions other than chloride is likely low under normal physiological conditions. The bicarbonate permeability must be insignificant since, based on the results of bicarbonate substitution experiments, a significant bicarbonate conductance was not evident at either border of the epithelium (O'Neil & Boulpaep, 1982; Sansom et al., 1984). The results of isotopic flux studies of sodium and potassium are likewise consistent with a low paracellular flux of these ions, particularly for sodium (Frindt & Burg, 1972; Stoner et al., 1974; Schwartz & Burg, 1978; Stokes, 1981). This view is supported by the observation that removal of sodium from the bathing medium of perfused tubules has little influence on V^{te} and G^{te} (O'Neil & Helman, 1977; O'Neil & Boulpaep, 1982), and by the ability of this tubule to generate and maintain large electrochemical gradients for both sodium and potassium well above their respective predicted equilibrium values (Grantham et al., 1970). It seems reasonable to conclude that the tight junction of the cortical collecting duct is chloride permselective.

Assessment of Methodology

The analysis of barrier conductances depends upon several assumptions that have been only partially assessed. First, the measurement of fR^a was based on the assumption that upon injection of current into the tubule lumen via the perfusion pipet, the resulting changes in the apical and basolateral membrane voltages were directly proportional to the respective cell membrane resistances (*see* Materials and Methods). This requires that the fraction of injected current entering the impaled cell across the apical cell border was equal to that leaving the cell across the basolateral cell border. Whereupon, the voltage changes—the IR drops—at the two borders would be directly proportional to their respective resistances so that fR^a could be equated to the fraction of the transcellular voltage change dissipated at the apical border (*see* Eq. 9). On the other hand, if the cells were coupled electrically, either via low-resistance intercellular channels or as a result of a low lateral membrane resistance (Clausen, Lewis & Diamond, 1979; Boulpaep & Sackin, 1980) then current entering a cell across the apical border would exit the cell both by spreading to an adjoining coupled cell (or cells) and by directly exiting across the basolateral border to the grounded bath. In terms of equivalent circuits, this implies that the tubule can not be modelled as a simple one-dimensional cable with axial current flow along the tubular luminal core (luminal cable) alone, but must be modelled as a "double" cable with axial current flow both along the luminal core and along the epithelial layer from cell to cell (cell cable). This has been discussed in detail by others (Frömter, Muller & Wick, 1971; Anagnostopoulos, Teulon & Edelman, 1980; Hoshi et al., 1981; Guggino et al., 1982*a*; Greger & Schlatter, 1983). As a consequence, near the point of current injection, the estimated value of fR^a would be greater than the "true" fractional resistance since the current exiting the cell via the basolateral membrane would be less than that entering across the apical border. It also follows that the estimated value of fR^a would decrease from cell to cell with increasing distances along the tubule away from the point of current injection as shown for the rabbit cortical thick ascending limb (Greger & Schlatter, 1983) and the *Triturus* proximal tubule (Hoshi et al., 1981)—this was not observed for the proximal tubule of *Necturus* (Anagnostopoulos et al., 1980; Guggino et al., 1982*a*) and rat (Frömter et al., 1971). Since on the average, the estimated value of fR^a did

not vary between the two ends of the cortical collecting duct in the present study (*see* Fig. 1), cell-to-cell coupling was minimal in most tubules and not likely to influence the measured value of fR^a.

Other indirect evidence is also not supportive of significant cell-to-cell coupling. Kaissling and Kriz (1979) have not been able to demonstrate the presence of gap junctions in this tissue, a finding which is indicative of, but does not prove, the absence of direct cell-to-cell coupling. This view is consistent with recent measurements of single cell input resistances (Sansom & O'Neil, *unpublished observation*). It was observed that upon injection of a current pulse into a cell via the microelectrode, the recorded input resistance of the cell was high, generally over 100 MΩ. This is in the range anticipated for the cell membranes alone in the absence of any low-resistance cell-to-cell pathway.

A second potential problem in estimating fR^a can arise if the resistance of the paracellular pathway cannot be attributed to the tight junction alone, but also includes a significant resistance of the intercellular lateral space. If the lateral space resistance is significant, the paracellular pathway will behave as a distributed resistance including resistance elements of the lateral cell walls and of the lateral space (Clausen et al., 1979; Boulpaep & Sackin, 1980; Nagel, Garcia-Diaz & Essig, 1983). As a consequence, upon injection of a current pulse into the tubule lumen, a fraction of the current flowing through the paracellular pathway will flow into the cell across the lateral membrane, thereby directly influencing the measured change in the basolateral membrane voltage and, hence, the estimated value of fR^a. While this problem has not been directly addressed, it has been demonstrated that treatment of the cortical collecting duct with vasopressin, which induces osmotic water flow and causes a dilatation of the lateral space (Ganote et al., 1968), does not influence the transepithelial resistance (Helman et al., 1971). Since widening of the lateral space would cause a decrease in the lateral space resistance, which should be reflected as a decrease in the transepithelial resistance, it is most likely that this resistance is normally insignificant and hence not a major problem in the present analysis. Some caution must be exercised, however, as this is a rather indirect assessment of the problem which could overlook a small, but important, lateral space resistance.

In view of the heretofore mentioned data, neither cell-to-cell coupling nor a distributed lateral resistance would appear to have a major influence on the estimated value of fR^a in the present study. Hence it would seem reasonable to conclude that the estimated value of fR^a likely provides a good approximation of the "true" fractional resistance of the cortical collecting duct cells.

A particularly important assumption in the present study was that low-luminal pH and the luminal addition of Ba^{++} caused a selective reduction in the apical cell membrane conductance without significant effects on G^b and G^{tj}. As can be seen from Eq. (12), under these conditions a decrease in G^a would result in a linear relation between G^{te} and $1 - fR^a$, with a slope equal to G^b and intercept equal to G^{tj}. Indeed, the time course of the effects of reducing the luminal pH on G^{te} and $1 - fR^a$—shown to be voltage independent—were highly linear ($r > 0.99$) as shown in Fig. 4, thereby supporting this view. It is possible, however, that G^b and G^{tj} were not constant. If this were the case, then the changes in G^b and G^{tj} would had to have been both temporally and quantitatively matched so that the relation between G^{te} and $1 - fR^a$ still remained linear—a highly unlikely possibility. This same relation between G^{te} and $1 - fR^a$ was maintained upon addition of 5 mM Ba^{++} to the perfusate, indicating that reduction in the luminal pH and addition of Ba^{++} were having qualitatively similar effects, supposedly limited to the apical border since Ba^{++} is not likely to enter the cell. Furthermore, the effects of Ba^{++} have been shown to be extremely rapid (O'Neil, 1983). This is particularly demonstrable from the voltage-dependent effects of Ba^{++} at low concentration (0.1 mM). It was shown that upon injection of lumen-negative current pulses (lumen-negative voltage changes) the blocking action of Ba^{++} was completely abolished whereas lumen-positive current pulses caused a maximum blockage similar to that observed with 5 mM Ba^{++}. These effects were rapid, occurring within the 100-msec response time of the electrical system and, therefore, not likely to cause significant secondary changes in G^b and G^{tj}. In view of these considerations, it would appear that the effects of luminal barium and reduction in luminal pH were on the apical cell membrane conductance alone, and therefore should provide appropriate probes for quantitation of barrier conductances as done in the present study.

Correlations with Cell Type

The cortical collecting duct is a simple epithelium consisting of two well-defined cell types—the principal cell and the intercalated cell. The principal cell accounts for approximately two-thirds of all cells and appears to be the cell responsible for sodium and potassium transport in this tissue (Wade et al., 1979; Kaissling & Le Hir, 1982; Le Hir et al., 1982;

O'Neil & Boulpaep, 1982; O'Neil & Hayhurst, 1984). The intercalated cell contains high levels of carbonic anhydrase (Rosen, 1972; Dobyan et al., 1982) and is thought to be involved primarily in acid secretion (Kaissling & Kriz, 1979; Steinmetz & Anderson, 1982; Koeppen & Steinmetz, *in press*). The cortical collecting duct cells impaled with microelectrodes in this and previous studies (Koeppen et al., 1983; O'Neil & Sansom, 1984*b*) have properties expected for the principal cell. The apical border of the cells was characterized by an amiloride-sensitive sodium conductance and a barium-sensitive potassium conductance, while the basolateral border was characterized by a ouabain-inhibitable sodium pump. In a recent series of studies, interference contrast and fluorescence microscopy techniques were combined to positively identify the intercalated and principal cells in the perfused tubule (O'Neil & Hayhurst, 1984). It was demonstrated that upon passage of current pulses (150 nA for 10 to 20 sec) from lumen to bath via the perfusion pipet, principal cells were observed to swell, but not intercalated cells. The current-induced swelling could be blocked by the luminal addition of amiloride and barium. This provides strong support of the view that the two cell types are functionally distinct, and that the principal cell is indeed the cell responsible for sodium and potassium transport in this tissue.

It has been observed in this study, as reported previously (O'Neil & Sansom, 1984*b*), that occasionally upon impalement of a cell, the recorded V^b was low, near -30 to -40 mV, and the fR^a essentially equal to 1. Koeppen has likewise noted this phenomenon (*personal communication*). With the fR^a equal to 1, the cell was discarded since it was not possible to ascertain whether the basolateral membrane had been damaged upon impalement or if a cell membrane resistance barrier had not been crossed with the microelectrode tip, i.e., the recording was an artifact (*see* Materials and Methods). It could be, however, that the recorded voltage was real, reflecting properties of a second cell type such as the intercalated cell, where the resistance of the apical cell membrane was exceedingly high relative to the basolateral cell membrane. It is interesting in this regard, to compare these results with those of recent microelectrode studies of the outer medullary (inner stripe) collecting duct of the rabbit (Koeppen, 1983). The cells of this segment are carbonic anydrase-rich and hence considered to primarily reflect acid-secreting cells (Rosen, 1972; Dobyan et al., 1982) such as the intercalated cells of the cortical collecting duct. These cells were characterized by a low V^b of near -39 mV and a high fR^a of 0.99. By analogy, it may be that the cells characterized by a low V^b in the cortical collecting duct do indeed reflect properties of acid-secreting cells, supposedly the intercalated cell. Since, as noted above, techniques are presently being developed to distinguish between the two cell types of the cortical collecting duct (O'Neil & Hayhurst, 1984), future studies will focus on combining optical and microelectrode techniques to differentiate between cell types.

If it is assumed that the cells which were accepted as being "successfully" impaled in the present study were the principal cells, then the estimated tight junction conductance should reflect the sum of all pathways in parallel with the principal cells. This would include the "true" paracellular conductance (tight junction) and the transcellular conductance of the intercalated cell. While the exact relation between the paracellular conductance and the intercalated cell conductance has not been directly evaluated, as noted above, there is increasing evidence that the intercalated cell apical membrane conductance may be small relative to the paracellular conductance. If so, the intercalated cell conductance may not significantly contribute to the estimated tight junction conductance. First, as noted above, if this cell type is indeed characterized by a value of fR^a near 1, the resistance of the apical cell membrane is likely high, so that the transcellular conductance would be negligible. Secondly, as discussed in a previous study (Sansom et al., 1984), there is little evidence of significant Na^+, K^+, Cl^- or HCO_3^- conductance of the apical cell membrane other than for the principal cell, at least for Na^+ and K^+. Finally, the only ion that has not been dismissed is H^+. However, in the present studies its activity was low and the experiments were performed in the absence of bicarbonate and CO_2 in the solutions, thereby abolishing active acid secretion (Stoner et al., 1974; Koeppen & Helman, 1982). Hence, any hydrogen conductance would necessarily be miniscule. In view of these considerations, it would seem most probable that the intercalated cell transcellular conductance was low relative to the "true" tight junction conductance. The estimates of the tight junction conductance in the present studies, should therefore provide a good first approximation of the "true" tight junction conductance of the rabbit cortical collecting duct. Studies are presently being conducted to directly evaluate this notion.

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