Electrical properties of isolated perfused rabbit renal tubules

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Electrical potential difference (PD) was measured across isolated perfused rabbit proximal renal tubules and collecting tubules in vitro. In the collecting tubule the PD stabilized after 2–3 hr of perfusion at a mean value of -25 mv (lumen negative). Ouabain caused depolarization, interpreted as indicating that the PD may be related to active Na+ transport. Vasopressin caused a transient increase in PD followed by a sustained decrease. Electrical resistance of the collecting tubule wall was evaluated from measurements of the length constant for the decay of PD from a current source within the tubule lumen. This calculated resistance is much greater than that previously found in rat proximal tubule in vivo. In proximal convoluted tubules under conditions optimal for net fluid absorption mean PD was -0.7 mv. This is in agreement with the recent finding that there is no measurable PD in rat proximal convoluted tubules in vivo.

Measurements of electrical PD across several segments of the nephron have been performed in vivo, using micropuncture techniques (19). In the present studies a method was developed for measuring electrical PD across isolated perfused rabbit tubules in vitro. PD was measured in both the cortical collecting tubule and proximal convoluted tubule. The effects of ouabain and vasopressin on the observed potential were studied in the collecting tubule. In addition the electrical resistance of the collecting tubule wall was estimated from measurements of the length constant for decay of PD in the tubule.

METHODS

Individual rabbit cortical collecting tubules or proximal convoluted tubules were dissected and perfused, using methods previously described (2, 9). The outside bathing medium used for dissection and perfusion of the collecting tubules contained NaCl 115 mM, KCl 5 mM, NaHCO3 25 mM, Na acetate 10 mM, Na2HPO4 1.2 mM, MgSO4 1.2 mM, CaCl2 1.0 mM, 5 % v/v calf serum (Microbiological Associates, Bethesda, Md.), and dextrose 5.5 mM. The perfusion solution contained NaCl 150 mM, K2 HPO4 2.5 mM, MgSO4 1.2 mM, and CaCl2 1.0 mM pH adjusted to 7.4 with dilute HCl. A gas mixture containing 95 % O2 and 5 % CO2 was constantly bubbled through the outside bathing solution. The temperature of the bath was maintained at approximately 23 C during perfusion.

Proximal convoluted tubules were studied under conditions found to be optimal for active net fluid absorption (4). The tubules were dissected in chilled rabbit serum, then perfused with an ultrafiltrate of serum while bathed in the same serum at 37 C. 95 % O2 plus 5 % CO2 was used as the gas mixture.

The arrangement for measuring electrical PD is shown in Fig. 1. The proximal tubules were perfused by a pump at 6–10 n liters min⁻¹. For perfusion of collecting tubules, pressure was provided by gravity, 15–20 cm of water generally being required and perfusion rate was not measured. The tip of the perfusion pipet within the tubule lumen was elongated, allowing it to be threaded down the lumen beyond the end of the outer pipet. A Ag-AgCl electrode was placed in the rear of the perfusion pipet and connected to the input of a wideband Standard Bak Electrometer for the experiments with collecting tubules or a model 600A Keithly electrometer for the experiments with proximal tubules. The mean resistance of the inner perfusion pipets was 4 megohms. A second (reference) Ag-AgCl electrode was connected in series through a Heath VR-1 voltage reference source to ground. Both Ag-AgCl electrodes were immersed in identical solutions. The output from the Bak electrometer was fed into a Varian G-10 graphic recorder; the Keithly electrometer was read directly.

Prior to incubation of each tubule, the voltage reference source was adjusted to cancel out any interelectrode electrical asymmetry; this was usually less than 3 mv.
During the tubule perfusion the exploring electrode was periodically disconnected and the electrometer input lead short circuited to ground in order to negate the effects of instrumental drift. Interelectrode drift was less than 2 mv over periods lasting up to 8 hr. Also, during the tubule perfusion an occasional check was made to be sure that a voltage applied from the reference source was recorded in full by the electrometer.

Figure 2 shows the arrangement used to measure length constants. The perfusion pipet was modified to permit introduction of an inner voltage recording pipet. This was freely movable and could be made to project up to 600 μ beyond the orifice of the perfusion pipet. Its outer diameter was small (5 μ) compared to the usual inner diameter of the collecting tubule (20 μ). The current source was a 1.5-v dry-cell battery, the negative pole of which was connected via a variable resistor to the Ag-AgCl electrode in the perfusion pipet. The current passed was measured with the Keithly 600A electrometer. The resistance of the inner pipet was approximately 60 megohms; that of the perfusion pipet, with the inner pipet in situ, approximately 6 megohms. The specific resistivity \( \rho_c \) of the perfusion fluid was 69 ohm cm at 23 C as measured with 1,000 cycles/sec alternating current through a conductivity cell and Wheatstone bridge. Prior to determination of a length constant, the voltage measuring pipet was advanced down the tubule lumen to the limit of its protrusion in order to assure that the spontaneous voltage in the absence of applied current did not vary by more than 2 mv over this tubule length. Then a constant electric current (0.21 to 1.7 \( \times \) 10\(^{-7} \) amp) was introduced into the tubule lumen via the perfusion pipet and the increment of PD above the spontaneous electric PD measured at varying distances down the length of the tubule. (Owing to the resistance of the salt bridge between the bath and the fluid in which the indifferent Ag-AgCl electrode was immersed (Fig. 2) there was a PD recorded when current was passed even without the tubule in place. This constant increment in base-line PD was subtracted from all measurements made with the tubule in place.) The distance between the orifice of the perfusion pipet and that of the voltage measuring pipet at each site was measured with an ocular micrometer. Tubule dimensions (total length and internal diameter) were measured in the same manner.

RESULTS

**Transitubular potential differences of collecting tubules.** When the inner perfusion pipet was first introduced into the tubule lumen, potential differences of +2 to −21 mv (lumen compared to reference electrode) were observed.
As the pipet was advanced down the tubule lumen, the PD became increasingly more negative, usually attaining constant values at distances of 200–300 μ from the orifice of the outer perfusion pipet. When a constant level had been reached the tip of the inner perfusion pipet was left at that position. The outer diameter of this pipet was large enough to fill the lumen of the tubule completely, or nearly so during measurements of PD. If perfusion pressure was increased, the lumen expanded and its diameter exceeded that of the perfusing pipet. Under these circumstances the potential difference decreased. For this reason high perfusion pressures were avoided. Presumably, the glass pipet in the lumen of the tubule acts as an insulator preventing short circuiting of the PD through the broken proximal end of the tubule.

Initially, the time course of the change in potential difference varied among individual tubules (Fig. 3). However, after 1 hr all became progressively more negative and reached a steady value by 80–220 min. Once attained, the steady state persisted for 3 hr or more. This initial time course resembles that previously observed for water permeability (9) and it is possible that the same unknown factors may be affecting both of these early measurements. The mean potential difference in the steady state was −25 ± 3 SEM mV in 11 experiments.

Effect of ouabain. Cardiotonic glycosides inhibit active Na transport in renal tissue (3, 15). The effect of ouabain on PD in the collecting tubule was tested in six experiments. Ouabain (10−5 M) caused the potential difference to fall from a mean control value of −23 to 0 mV in 10–35 min. (The PD fell to one-half the initial value in 5–13 minutes.) In some experiments the potential difference became slightly positive (up to +4 mV) after ouabain. A representative experiment is shown in Fig. 4.

Effect of vasopressin. The effect of Pitressin (.05–25 μunits ml−1) or purified arginine vasopressin (30 μunits ml−1) was tested in 11 experiments on 9 isolated perfused tubules. The antidiuretic hormone caused a decrease in the electrical PD from the mean control value of −24 mV to 0 to −12 mV, 90–120 min after the hormone was added. In more than one-half of the experiments an immediate transient increase in potential difference was observed which lasted 15–30 min after the hormone was added. The time course of the change in potential difference in the 11 experiments following vasopressin is shown in Fig. 5. Potential difference increased to control values again when the hormone was removed (Fig. 6) indicating that the effect is reversible. When the preservative in Pitressin (chlorbutanol) was added alone, it was without effect on PD.

Electrical resistance of collecting tubules. In order to determine the electrical resistance of collecting tubules the length constant for PD was measured 203–285 min after the start of the perfusion using the arrangement illustrated in Fig. 2. If it is assumed that when a current is
passed, the superimposed PD is distributed along a tubule in accordance with simple cable theory (16)

\[ R_m = \lambda^2 R_e \]  

where \( R_m \) is the resistance of the tubule wall per unit tubule length (ohm cm), \( \lambda \) is the length constant (cm), and \( R_e \) is the resistance of the tubule fluid per unit tubule length (ohm cm\(^{-1}\)). Also,

\[ \lambda = -\pi \ln \left( \frac{V}{V_o} \right) \]  

where \( V_o \) is the increment in PD at some point in the tubule lumen distal to the current source, \( x \) is a distance (cm) distal to the point at which \( V_o \) is measured, and \( V \) is the increment in PD at \( x \).

It had been anticipated that \( V/V_o \) would decrease as a logarithmic function of \( x \), providing a measure of \( \lambda \) and thus indirectly of \( R_m \). Actually, it was found that the function was approximately linear rather than logarithmic (Fig. 7) and that extrapolation to \( V/V_o = 0 \) led to an intercept near \( x/L = 1 \), where \( L \) is the length of the tubule (cm). The most likely explanation for this result is that the tubule wall has a very high resistance (\( R_m \), Fig. 8) and that there is a low resistance pathway (\( R_k \), Fig. 8) at the distal end of the tubule where it is held in the collecting pipet. Then, virtually all of the current will flow axially down the tubule lumen going out of the distal end, and \( V/V_o \) will decrease linearly with distance.

Although \( \lambda \) cannot be calculated directly from these data, it is possible to derive a lower limit for \( \lambda \) and thus for \( R_m \). The observed linear fall in \( V/V_o \) may be attributed to a high value for \( \lambda \). For lower values of \( \lambda \) the results should deviate from linearity, approaching a logarithmic function. The amount of deviation to be expected for a given value of \( \lambda \) can be calculated. This deviation might have been detected if \( \lambda \) were as short as the mean length of the tubules studied (14 cm). For \( \lambda \leq 1 \), the deviation from linearity at \( x/L = .3 \) (selected arbitrarily) would be \( > 0.38 V_o \) (see APPENDIX). Inspection of the results (Fig. 7) shows that this much deviation is probably not present. Therefore, \( \lambda \) must be greater than the length of the tubule. From this, an approximate lower limit for the resistance of the membrane can also be calculated. Introducing specific resistances,

\[ r_m = R_m \pi D \]  

and

\[ r_e = R_e \pi D^2/4 \]  

where \( r_m \) is the specific resistance of unit area of tubule wall (ohm cm\(^2\)), \( D \) is the tubule diameter (.002 cm), and \( r_e \) (ohm cm) is the specific resistivity of the tubule fluid (69 ohm cm). Combining equations 1, 3, and 4, and assuming that \( \lambda \geq L = .14 \) cm,

\[ r_m = 4r_e \lambda^2 \geq \frac{(4)(69)(.14)^2}{.002} \geq 2700 \text{ ohm cm}^2 \]

Given that \( \lambda \geq L \) it can also be deduced from the intercept on the abscissa in Fig. 7 that the distal end of the tubule is relatively poorly insulated at its junction.
with the glass pipet. Thus in order for \( V/V_0 \) to decrease linearly to zero at \( x/L = 1 \) (corresponding to the end of the tubule) the resistance at the distal end of the tubule (\( R_k \)) must be comparatively low. For higher values of \( R_k \), \( V/V_0 \) would be greater than zero at \( x/L = 1 \). On the other hand, if \( \lambda \) were very small relative to \( L \), the intercept of the initial slope of the logarithmic curve would be at \( x/L < 1 \). Thus, the observation that \( x/L \) is approximately one at \( V/V_0 = 0 \) in the present experiments is consistent with the assumptions both that \( \lambda \geq L \) and that \( R_k \) is relatively low.

The effective resistance of the tubule, \( R_{eff} \), can be calculated on the basis of Ohm’s law from the relationship between the applied current, \( I_c \), and the PD adjacent to the current source. \( R_{eff} \) was .28 and .29 megohms in the first two experiments done (tubules .105 and .206 cm long, Fig. 7) and the spontaneous PD was -3 and -7 mv. Following these studies the shapes of the pipets were altered in an effort to seal the ends of the tubule more effectively. In the subsequent four experiments the mean \( R_{eff} \) was 1.2 megohms and the mean spontaneous PD -13 mv. This increase in resistance is probably not due to improvement in the seal at the distal end of the tubule, since the intercepts at the abscissa in Fig. 7 for these four experiments were still near \( x/L = 1 \). Therefore the increased \( R_{eff} \) is probably due to improvement in the seal at the perfusing end. There may still be a significant leak of current out of the perfusion end, however, even in the latter experiments. \( R_{eff} \) can be calculated for a tubule perfectly insulated at the proximal end (\( R_0, \) Fig. 8, \( \infty \)) but with a short circuit at the distal end (\( R_k, \) Fig. 8, \( \approx 0 \)) using equation 15 in the appendix. For \( \lambda = .14 \text{ cm and } R_0 = 22 \text{ megohms cm}^{-1} \) the resistance of tubules .078 to .280 cm long (the range of lengths in Fig. 7) should be 2.0 to 2.8 megohms. Since even the highest measured resistances were half of this, the seal at the perfusing end, although improved over earlier experiments, may not be completely satisfactory.

**Electrical potential difference across proximal convoluted tubules.** The rate of fluid absorption in isolated perfused proximal convoluted tubules is apparently normal during the 1st hr of perfusion when they are bathed in rabbit serum and perfused with an ultrafiltrate of the same serum at 37 C (4). Electrical potential difference was measured in seven tubules under these conditions after the perfusing pipet had been advanced as far as possible (usually approximately 200 \( \mu \)) down the tubule lumen (Fig. 9). It was not possible to advance the pipet more than 300 \( \mu \) in any experiment because of the convoluted shape of the tubules. However, if the length constant of the proximal tubule in the rabbit is as short as that in the rat (86 \( \mu \)) (10), placement of the electrode (pipet) more than two length constants away from the “open” ends should permit detection of a PD were it present.

Under these circumstances no measurable PD was recorded in seven tubules during the 1st hr of perfusion (PD = 0.7 ± .6 sem mv), nor was any significant PD detected in more prolonged experiments. (The latter observations are considered of less importance since fluid absorption may decrease after 1 hr in this preparation.)

**DISCUSSION**

Electrical potential measurements have been made across the walls of all segments of the renal tubule accessible to micropuncture in a variety of species. In most segments a PD has been found (lumen negative), which ranges from -1 to -3 mv (the thin limb of Henle’s loop) to -35 to -60 mv (distal convoluted tubule) (19). However, the proximal convoluted tubule may be an exception. On the basis of recent studies, in contrast to results of earlier work, it has been concluded that no transtubular PD is normally present in that segment, at least in the rat (6, 7).

The PD across cortical collecting tubules had not been previously measured since this segment is not readily accessible in vivo. In the present studies a mean steady state PD of -25 mv was found in isolated perfused rabbit collecting tubules in vitro. This is comparable to previous observations in other distal segments, namely -14 mv in the papillary segment of the collecting duct of golden hamsters (18) and -60 mv in the rat distal tubule (19). The prompt depolarization of the collecting tubule epithelium by ouabain is interpreted as indicating that the PD may be related to active Na transport. It is not clear whether this is a diffusion potential resulting from ionic Na and K gradients set up by the active transport (13) or a consequence of Na transport by an electrogenic pump (12).

It had been found previously (9) that when the bath and perfusion fluids were buffered differently (bicarbonate and acetate in the former replaced with chloride in the latter), the collecting tubules remained morphologically intact for a longer period. The resultant difference in chloride concentration (120 vs. 152 mM) could affect the observed potential. Depending on the ionic permeability characteristics of the tubule, this chloride ratio could result in a PD of up to +6 mv. However, the observed PD under control conditions was of opposite polarity, and therefore can not be accounted for by the differences in chloride concentration. Further, in other experiments (unpublished observations) the small positive PD occasionally observed after ouabain was not altered when the...
Cl gradient was eliminated by replacing the bath solution with perfusion solution, indicating that the positive PD after ouabain also was not due to the asymmetry in chloride concentration.

The effect of vasopressin on the PD is of interest since it apparently differs from that previously noted in amphibian membranes. In both frog skin (8) and toad bladder (5) vasopressin caused an increase in PD. The increment in PD was greatest when the initial PD was lowest. In the present studies although an initial increase in PD was frequently observed following addition of vasopressin, the increase was transient and disappeared within 30 min. It was invariably followed by a sustained decrease in PD. (The magnitude of the initial increase, when present, was independent of the previous PD.) The increase in PD induced by vasopressin in amphibian membranes is associated with stimulation of net Na transport (8), and electrical resistance actually decreases (3). It is conceivable that an initial increase in Na transport, followed by a reduction in net transport, could explain the present findings in the cortical collecting tubule. However, this has not been established and requires more detailed examination of changes in sodium flux and electrical resistance.

In the present studies the length constant and effective resistance of collecting tubules were measured in order to calculate electrical resistance of the membrane. The length constant was too long to be measured precisely so only a lower limit for the resistance can be established. This result differs from previous measurements in the proximal tubule of the rabbit. The length constant measured in the proximal tubule was .0086 cm (10) compared to an approximate minimum value of .14 cm for the collecting tubules in the present studies. Clearly the resistance of the collecting tubule in the rabbit is several orders of magnitude greater than that of the rat proximal tubule. The same conclusion follows from comparison of the effective resistance which was 05 megohm for the proximal tubule (10) compared to at least 1.2 megohms for the collecting tubule. The high electrical resistance of the cortical collecting tubule presumably indicates a low ionic permeability. This is consistent with observations that large gradients for ions may normally exist in the distal but not in the proximal portions of the nephron. Since electrical resistance could not be measured precisely in the collecting tubule, it was not possible to determine whether it was affected by vasopressin.

Originally it had been found that the PD across the proximal convoluted tubule was approximately −20 mv (19), and on the basis of these measurements it was concluded that chloride (11) and potassium (1, 14, 17) are transported actively in the proximal tubule. However, it has recently been shown that there is no measurable PD across proximal tubule cells under ordinary conditions. The previously recorded PD values appear to have been intracellular potentials, since the tip of the recording pipet was most likely in the tubular cells rather than in the lumen as intended (6, 7). The absence of a measurable PD has been confirmed in the present studies in which a perfusion pipet was used to measure PD. Under these circumstances it can be ascertained with certainty that the pipet (electrode) is within the tubule lumen. If no PD normally exists in the proximal tubule, previous conclusions concerning active chloride and potassium transport must be reexamined.

It is obviously impossible to prove from in vitro studies that no PD is present in the proximal tubule in vivo. The tubules could have been altered during the dissection, so that the PD was lost entirely or was greatly delayed in its reappearance, as with the collecting tubule. All that can be firmly established is the relationship between PD and the transport processes. In the present studies it is shown that active fluid absorption (presumably secondary to sodium chloride transport) which persists in these tubule fragments does not require or generate an electrical PD.

**APPENDIX**

### 1. Calculation of Deviation From Linearity of Voltage Distribution Within Tubule Lumen

**Definitions.**

\[
V = \text{potential difference across the tubule wall} \quad (v) \\
L = \text{the length of the tubule} \quad (\text{cm}) \\
x = \text{axial distance down the tubule lumen} \quad (\text{cm}) \\
I_c = \text{core or tubule fluid current} \quad (\text{amp}) \\
I_m = \text{membrane or tubule wall current per unit tubule length} \quad (\text{amp cm}^{-1}) \\
R_m = \text{membrane or tubule wall resistance per unit tubule length} \quad (\text{ohm cm}) \\
R_c = \text{core or tubule fluid resistance per unit length} \quad (\text{ohm cm}^{-1}) \\
\lambda = \sqrt{R_m/R_c} = \text{length constant} \quad (\text{cm})
\]

The approach used is similar to that for core conductor models (16). It will be assumed that the resistance of the tubule fluid and wall are uniform and that the electrical potential external to the tubule is also uniform. However, no assumption will be made about the state of the system at \( x = L \). Then, from continuity conditions,

\[
I_m = -\frac{dI_c}{dx} \tag{5}
\]

Also, by Ohm’s law,

\[
I_m = \frac{V}{R_m} \tag{6}
\]

and

\[
I_c = \frac{1}{R_c} \frac{dV}{dx} \tag{7}
\]

Combining equations 5, 6, and 7, we have

\[
\frac{d^2V}{dx^2} = -\frac{R_c}{R_m} V = 0
\]

whose solution is

\[
V = \alpha e^{\lambda x} + \beta e^{-\lambda x} \tag{8}
\]

where \( \alpha \) and \( \beta \) are constants. From the boundary condition that at \( x = 0 \), \( V = V_0 \), we have

\[
V_0 = \alpha + \beta \tag{9}
\]
Letting \(k = (\alpha - \beta)/\lambda\), expanding equation 8 in a Taylor series, and substituting equation 9 yields

\[
V = V_0 + k\lambda + V_0 \frac{x^2}{2\lambda} + k\lambda \frac{x^2}{6\lambda} + \cdots \tag{10}
\]

Thus, \(V\) may be treated as a linear function of \(x\) for small values of \(x\), and may be expressed as

\[
V = V_0 + k\lambda + \delta(x) \tag{11}
\]

where \(\delta(x)\), the deviation from linearity, is given by

\[
\delta(x) = \frac{V_0 + k\lambda}{2} e^{x/\lambda} + \frac{V_0 - k\lambda}{2} e^{-x/\lambda} - (V_0 + k\lambda) \tag{12}
\]

From Fig. 7 we see that \(k\) is less negative than \(-\frac{V_0}{0.6L}\). Thus, for \(x = 0.3L\) and \(\lambda \leq L\),

\[
\delta \geq 0.038V_0 \tag{13}
\]

2. Calculation of Effective Resistance

The effective resistance, \(R_{\text{eff}}\) (ohm), is defined as

\[
R_{\text{eff}} = \frac{V_0}{I_0} \tag{14}
\]

REFERENCES


