Osmotically induced changes in electrical resistance of distal tubules of rat kidney

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IT HAS BEEN DEMONSTRATED that colloid-osmotic and hydrostatic pressure of peritubular capillary fluid can influence the net reabsorption of salt and water by proximal tubular epithelium (2-5, 15-17, 21, 25, 31, 41). It is also well documented that changes in osmotic gradients across isolated epithelia such as frog skin, toad bladder, and collecting ducts lead to marked alterations in electrolyte permeability of these tissues (8, 12, 16, 28, 29, 36-38). However, neither physical factors nor osmotic gradients have been evaluated directly in terms of distal tubular electrolyte permeability in vivo. The present study is an attempt to investigate whether changes in peritubular colloid osmotic pressure or transepithelial osmotic gradients affect the transverse electrical conductance of distal tubular epithelium of rat kidneys in vivo. An electrophysiological approach was used in combination with methods of microperfusion of distal convoluted tubules of Sprague-Dawley rats and of rats with congenital diabetes insipidus (Brattleboro strain). There was no demonstrable evidence for changes in electrolyte permeability of distal tubular epithelium in response to changes in peritubular colloid-osmotic or hydrostatic pressure. The results also indicate that transepithelial electrical resistance is related inversely to luminal osmolality and directly to peritubular osmolality. Antidiuretic hormone increases late distal tubular electrolyte permeability not only directly but also indirectly via its effect on tubular fluid osmolality in this part of the nephron.

METHODS

Animal preparation. Studies were performed on male Sprague-Dawley rats (120-250 g body wt) which had free access to a salt pellet diet until 15 h before the experiments and to water until the experiment began. In a smaller number of experiments, male and female Brattleboro rats (23, 39) weighing 120-200 g were used. Rats were anesthetized by intraperitoneal injection of Inactin (Promonta Corp., Hamburg, W Germany), 100 mg/kg body wt, and placed on a thermostatically controlled operating table, adjusted to maintain body temperature at 37°C. A tracheotomy was performed and two indwelling catheters (PE-50, Clay-Adams, Parsippany, N.J.) were inserted into the jugular veins for intravenous infusions and intermittent bolus injections of 0.025 ml of 10% lissamine green. The left kidney was exposed by a flank incision and freed from the adrenal gland and perirenal fat. The capsule was left intact and the kidney was placed in a Lucite holder. To minimize pulsatory movements of the kidney, 1.5% agar in 150 mM NaCl was applied as described by Wright (42). After removing the layer of agar which covered the kidney surface, subsequently used for micropuncture, the kidney was superfused with saline solution at 37°C. Each animal received 1.5 ml of physiological saline to replace surgical losses and was then infused intravenously with 150 mM NaCl at a rate of 0.02 ml/min. Transit time (33) was measured prior to micropuncture and checked every hour throughout the experiment. Animals with a proximal transit time greater than 14 s were discarded. Localization of distal tubular puncture sites was calculated from the ratio of the lissamine green transit time to the puncture site divided by the earliest distal tubular transit time of this kidney (TTR).

Rats with congenital diabetes insipidus were allowed free access to food and water up to the onset of the experiment. They received the same initial intravenous infusion of 1.5 ml of physiological saline, but the subsequent maintenance infusion of 150 mM NaCl was raised to 0.04 ml/min. In this series of experiments, animals with proximal tubular transit times ranging from 14 to 20 s were included. The left ureter was cannulated with a polyethylene catheter (PE-50), and the collected urine was analyzed for osmolality. After completion of the first period during which electrical measurements on distal tubules were performed, the same animals were infused with 88 μU/min vasopressin, (Schwarz/Mann, Division of Becton, Dickenson
and saline infusion during the second period was the same as during the first. Only rats excreting a hypotonic urine prior to antidiuretic hormone (ADH) and responding with an increase in urine osmolality by at least 200 mosmol/kg water after administration of vasopressin were used in this study.

The effect of partial clamping of the renal vein on distal tubular wall resistance was tested in five rats. Control periods preceded venous clamping in all experiments. Partial renal venous occlusion was achieved as previously described (25). Hydrostatic pressure within proximal and distal tubular lumina and in peritubular capillaries was measured by the methods of Wiederhelm (40) as modified by Fcu (11). Partial renal venous clamping was judged adequate when proximal tubular pressure exceeded 20 mmHg, whereas distal tubular hydrostatic pressures ranged from 4 to 11 mmHg, values comparable to those of the control periods. Hydrostatic pressure of distal tubules was measured after the electrical measurements had been obtained on the same tubules.

Measurement of electrical resistance. Estimates of electrical resistance across the wall of distal convoluted tubules on the kidney surface were obtained by application of cable analysis to measurements of voltage attenuations to known distance from a current-injecting microelectrode or, more frequently, to measurements of input resistances using double-barreled microelectrodes.

Single-barreled and double-barreled Ling Gerard microelectrodes were filled with 3 M KCl as described by Tasaki at al. (34). Electrode resistances ranged between 10 and 20 MΩ, and electrodes were selected for tip potentials with less than 5 mV. The microelectrodes were mounted in a micromanipulator which permitted contact with a calomel electrode. Potential differences were measured by means of a Keithley 602 electrometer between the microelectrode and an agar-KCl bridge and calomel electrode submersed in saline solution in the peritubular cavity. The potential differences were all referred to this latter indifferent electrode. Square pulses of 0.6 s duration generated by a Grass S5 stimulator were injected through single-barreled microelectrodes or through one barrel of double-barreled microelectrodes, and the voltage displacement was measured by a second micromanipulator placed at known distances or by the second barrel of a double-barreled micropipette. Currents to the microelectrodes were passed through a 100-MΩ resistor in order to minimize current fluctuations due to changes in microelectrode resistances. Current strength was measured by a Keithley electrometer model 620. Currents (of the order of 2-5 × 10⁻⁴ A) and voltages were displayed on a Brush recorder model 290.

For resistance measurements involving two separate microelectrodes, two impalements of the same distal tubule were made at different distances from the site of current injection with a second single-barreled electrode. Inter-electrode distance was estimated by an ocular filar micrometer. Such length constant (\(\lambda\)) measurements were done by first impaling the tubule at sites most distant from the current injection site to minimize the effect of current leaks on voltage attenuation. With double-barreled electrodes, the effective resistance was evaluated from the current-voltage relationship at the site of the microelectrode impalement. Such resistance measurements were corrected for interbarrel coupling resistance. Specific transverse resistance of distal tubules was calculated from the effective resistance (double-barreled microelectrode measurements) or the tubular geometry according to cable theory (13, 18, 20, 27).

\[
R_s = \frac{\pi^2 \cdot d^4 \cdot R_{eff}^2}{R_i}, \quad \text{or} \quad R_s = 4R_s \lambda^2/d
\]

where

- \(R_s\) = specific wall resistance, Ω cm⁻²
- \(R_i\) = internal resistivity, Ω cm
- \(R_{eff}\) = effective resistance, Ω
- \(d\) = tubular diameter (measured by means of an ocular filar micrometer, cm)
- \(\lambda\) = length constant, cm, is the distance by which the voltage was attenuated 1/e.

Under free-flow conditions, \(R_i\) was taken as 100 Ω cm⁻², as suggested by Malnic and Giebisch (27) and also based on our own conductivity measurements (Radiometer model CDM 2nd) of solutions made up to simulate electrolyte concentrations of early and late distal fluid samples under control conditions (13). Conductivities of these artificial solutions were not different, consistent with the near constancy of the sum of sodium and potassium concentrations along the distal convoluted tubule (13). \(R_i\), the resistivity of distal tubular fluid during partial occlusion of the renal vein, was assumed to equal that of an artificial solution made up according to micropuncture data of Anagnostopoulos et al. (1). This solution had the following compositions: NaCl 94.4 mM, KCl 8.0 mM, NaHCO₃ 5.0 mM, KH₂PO₄ 0.3 mM, CaCl₂ 1.8 mM, MgCl₂ 0.5 mM. The electrical resistivity of this fluid was 68.4 Ω cm⁻¹. In experiments in which tubules were perfused with artificial perfusion fluids, the transepithelial potential differences were corrected for junction potentials by measuring the potential change between indifferent electrodes and recording microelectrode when the latter was placed into the appropriate test solution. Junction potentials varied by no more than −2 to −5 mV. In similar in vitro measurements, coupling resistances of double-barreled microelectrodes were evaluated in the appropriate perfusion fluids. Coupling resistances averaged 10⁴ Ω and never exceeded 5 % of the transepithelial effective resistance observed in vivo.

A number of different approaches were used to ascertain intraluminal localization of the microelectrode tip, as previously described by others (6, 27, 42): 1) the stability of measured potential differences during axial displacement of about one tubular diameter; 2) the effect of ionic substitutions (150 mM choline chloride) achieved by perfusion of the distal tubule, on the electrical potential differences; choline chloride depolarized the distal transepithelial potential difference to values ranging between −5 and +5 mV, an effect which was quickly reversed on reestablishment of normal flow of tubular urine through the distal tubule; or 3) measurements of effective electrical resistance using double-barreled microelectrodes. Resistances higher than 1.0 MΩ were assumed to represent intracellular locations of the microelectrode tip.
FIG. 1. Relationship between transepithelial electrical potential difference and transit time ratios in distal convoluted tubules.

Tubular and peritubular capillary perfusions. Distal convoluted tubules were identified by inspection of the kidney surface after intravenous injection of 10% lissamine green (33). Early segments were selected (average transit time 35 s) for impalement with a micropipette filled with a perfusion fluid. The rate of microperfusion was sufficiently high to minimize changes in ionic composition during passage of the fluid through the distal tubule. These perfusion rates ranged between 60 and 70 nl/min. Peritubular capillaries were perfused as previously described (31).

Distal tubular lumina were perfused with the following solutions:

1) 150 mM choline chloride in water titrated with 0.5 M Trizma base (Sigma Chemical Co.) to pH 7.4 (total solute concentration 309 mosmol/kg water).
2) 120 mosmol/kg Na: 65 meq NaCl (120 mosmol/kg, resistivity 114 Ω·cm).
3) 250 mosmol/kg Na: 134 meq NaCl (250 mosmol/kg, resistivity 60 Ω·cm).
4) 400 mosmol/kg Na: 216 meq NaCl (400 mosmol/kg, resistivity 38 Ω·cm).
5) 600 mosmol/kg Na: 322 meq NaCl (600 mosmol/kg, resistivity 25 Ω·cm).
6) “200 mosmol/kg Raffinose-Na”: 80 mM raffinose plus 65 meq NaCl (201 mosmol/kg, resistivity 111 Ω·cm).
7) “400 mosmol/kg Raffinose-Na”: 280 mM raffinose plus 65 meq NaCl (400 mosmol/kg, resistivity 156 Ω·cm).
8) “500 mosmol/kg Raffinose-Na”: 300 mM raffinose plus 65 meq NaCl (500 mosmol/kg, resistivity 195 Ω·cm).
9) “600 mosmol/kg Raffinose-Na”: 400 mM raffinose plus 65 meq NaCl (600 mosmol/kg, resistivity 200 Ω·cm).

Peritubular capillaries were perfused with the following solutions:

1) 100 mosmol/kg Na: 21 mM NaCl, 25 mM NaHCO₃, 5.1 mM KCl, 1.0 mM CaCl₂, 5 mM glucose.
2) 300 mosmol/kg Na: 123 mM NaCl, 25 mM NaHCO₃, 5 mM KCl, 1 mM CaCl₂, 5 mM glucose.
3) 450 mosmol/kg Raffinose-Na: 150 mM raffinose, 123 mM NaCl, 25 mM NaHCO₃, 5 mM KCl, 1 mM CaCl₂, and 5 mM glucose.
4) 600 mosmol/kg Raffinose-Na: 300 mM raffinose, 123 mM NaCl, 25 mM NaHCO₃, 5 mM KCl, 1 mM CaCl₂, and 5 mM glucose.

These solutions were used with and without the addition of Dextran T-110 (Pharmacia Fine Chemicals) in amounts sufficient to achieve final concentrations of 8 g/100 ml. All solutions were checked for osmolality using an Advanced Instruments osmometer.

Statistics. Results are expressed as mean values ± standard error. Where appropriate comparisons were made by the Student t test, linear regression lines were calculated by the method of least squares.

RESULTS

Control studies. Increasing distal transit time ratios were associated with higher transepithelial potential differences, a finding in agreement with previous results of Wright (42). Figure 1 illustrates the relationship between distal...
ELECTRICAL RESISTANCE OF DISTAL TUBULES OF RAT KIDNEY

transit time ratio, an index of distal tubular length, and the transepithelial potential difference (mv), $-TTR = 0.0172 (mV) + 0.747$ (81 obs, $r = 0.90, P > 0.001$). These results demonstrate that under free-flow conditions the magnitude of the distal tubular transepithelial potential differences can be taken as an index of relative length along the distal convoluted tubule on the kidney surface.

Figure 2 illustrates the relation between transepithelial potential differences and effective electrical resistances across the wall of distal convoluted tubules. In this series of experiments, measurements of potential difference and effective resistance were obtained under free-flow conditions in 110 nondiuretic rats with double-barreled microelectrodes. A nearly linear inverse relationship obtained between transepithelial potential difference and effective resistance, $y = -0.0067x + 0.5877$ ($r = 0.5495, P < 0.001$).

Specific transverse resistances, $R_s$, were calculated from the effective resistances shown in Fig. 2, from optically measured luminal diameters, and the resistivity of artificially prepared distal tubular fluid. Luminal diameters during free flow averaged 19 ± 0.1 μm (310 obs) and resistivity 100 Ω-cm. Figure 3 shows the inverse relationship between transepithelial potential difference and specific transverse resistance in distal tubules. $R_s$ declines from about 180 to some 40 Ω·cm² as the potential difference increases from -10 to -50 mV. The studies of Wright (42), as well as our own data, have shown that the transepithelial potential difference increases as a function of tubular length. The relationship between PD and transverse resistance, $R_s$, illustrated in Fig. 3 therefore demonstrates that in nondiuretic rats in free flow the specific transverse resistance of the epithelium decreases along the length of the distal convoluted tubule.²

In a separate series of 11 experiments, transepithelial resistances were measured during free flow in nondiuretic rats using single-barreled microelectrodes. Voltage attenuation was recorded as a function of interelectrode distance. Measurements were obtained in the midportion of the distal convoluted tubule as judged by the average transit time ratio of $1.5 ± 0.2$ measured at the site of the impalement of the second (more distally located) microelectrode. Length constant measurements averaged 260 μm ± 20.6 SE (11 obs). From optically measured luminal diameters (mean 20 μm ± 0.3 SE; 11 obs) and a resistivity, $R_i$, of 100 Ω·cm, a mean transverse distal tubular wall resistance of 135 Ω·cm² obtains. Thus, length constant-derived estimates of $R_s$ are in good agreement with those based on effective resistance measurements with double-barreled microelectrodes.

Renal venous clamping. To evaluate the effect of alterations in physical factors on distal tubular electrolyte permeability, electrical resistances were measured before and during partial clamping of the renal vein. In 16 measurements on the same distal tubular segments, the effective resistance averaged $0.45 ± 0.026 × 10^5$ Ω during the control periods and $0.42 ± 0.024 × 10^5$ Ω during partial occlusion of the renal vein ($P > 0.1$). Tubular diameters averaged $19 ± 1$ μm in controls and $19 ± 0.3$ μm during clamping. The mean transverse specific resistance, $R_s$, calculated from the effective resistances, $R_{ext}$, the tubular fluid resistivity, $R_i$, and the tubular diameter was $157$ Ω·cm² during control conditions and $186$ Ω·cm² during partial renal venous clamping.

Osmotic gradients. Distal tubules were perfused with a series of sodium chloride solutions varying in osmolality from 120 to 600 mosmol/kg. The average effective resistance during perfusion with 120 mosmol/kg Na (65 meq/liter NaCl) averaged $0.40 × 10^5$ Ω ($± 0.020$ SE; 8 distal tubules in 6 rats). Tubular perfusion with 250, 400, and 600 mosmol/kg NaCl gave average effective resistance values of $0.254 × 10^5$ Ω ($± 0.010$; 9 tubules, 5 rats), $0.157 × 10^5$ Ω ($± 0.018$; 10 tubules, 4 rats), and $0.077 × 10^5$ Ω ($± 0.001$, 10 tubules, 6 rats), respectively. The corresponding values for transverse specific resistance, $R_s$, for 120

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1. The fact that no decline in resistance was found when osmotic changes along the tubules were prevented by rapid tubular perfusion or capillary perfusion argues against a spurious underestimate of specific transepithelial resistance due to branching of the electrical equivalent cable. Furthermore, it can be shown that the hypothetical errors caused by single branching would underestimate the resistance no more than 1.4-fold and 2.6-fold at distances 100 and 10 μm, respectively, from the branching point. This is clearly not enough to explain the decline in resistance along distal tubules of nondiuretic rats. Also, we fail to detect any sharp discontinuity in the relationship between transepithelial resistance and distal tubular length as would be expected if branching would cause serious cable-analytical errors in the segments of distal tubules described in the present paper.

2. It might be expected that the increase in osmolality of late distal fluid in nondiuretic rats should lead to a reduction in the transepithelial potential difference. Actually, such a decrease may occur, but it is apparently overshadowed by the electromotive force of the sodium extrusion mechanism in this part of the nephron (13). Late distal tubular potential differences before and after ADH administration were not different statistically. However, the number of observations in this series is too small to rule out a slight decline in late distal PD after intravenous administration of vasopressin.
mosmol/kg NaCl was 382 ± 38 Ω·cm², and for 600 mosmol/kg NaCl 65 ± 11 Ω·cm².

Another series of distal tubular perfusions was carried out using perfusion fluids in which osmolality was varied by adding different amounts of raffinose to 65 meq NaCl solution. The osmolalities of these solutions ranged from 200 to 600 mosmol/kg. Tubular perfusion with 200, 400, 500, and 600 mosmol/kg "raffinose-Na" gave average effective resistances of 0.2827 × 10⁶ (± 0.0148; 11 tubules, 5 rats), 0.1916 × 10⁶ (± 0.0091; 10 tubules, 5 rats), 0.1790 × 10⁶ (± 0.0139; 10 tubules, 4 rats), and 0.1140 × 10⁶ Ω (± 0.0066; 10 tubules, 4 rats), respectively. In neither series of tubular perfusions did collected end distal fluid differ in osmolality from the injected solution (osmolality ratio: collected/injected = 0.97 ± 0.02, n = 15).

Figure 4 is a summary of the effective resistances measured during perfusion of distal tubules with fluids of different osmolalities. Resistance decreased with increasing luminal osmotic concentration. The relationship for data obtained with pure NaCl perfusion is given by y = -0.0006x + 0.4423 (r = 0.9318, P < 0.001), that for hyperosmotic raffinose-Na solutions by y = -0.0004x + 0.3636 (r = 0.8528, P < 0.001).

In all tubular perfusion studies in which NaCl solutions were used, the early portion of distal surface convolutions was chosen as the site of electrical measurements (TTR < 1.5). Figure 5 shows the effective resistances during perfusion with different osmotic concentrations of NaCl as a function of the transepithelial potential difference prior to microperfusion. Transepithelial resistances decreased markedly as the osmolality of the perfusion fluid increased, but no decline in R_eff was found as a function of increasing initial transepithelial potential difference. The same conclusion is reached when R_eff measured during raffinose perfusion (see Fig. 6), is plotted against initial potential difference measured in the same tubule prior to perfusion. The lack of decline in transepithelial resistance with increasing initial PD, i.e., increasing distance along the tubule, during perfusion is in sharp contrast to the inverse relationship between transepithelial PD and resistance formed during free-flow conditions (see Fig. 2).

Peritubular capillary perfusions were performed to study the effect of alterations in the osmolality of the peritubular fluid environment of distal tubular epithelium while maintaining free-flow conditions within the tubular lumen. Effective resistance (10⁶ Ω) across the epithelium of distal
tubules averaged 0.1258 ± 0.0004 (12 tubules; 4 rats), 0.3933 ± 0.0111 (21 tubules; 10 rats), 0.5475 ± 0.0425 (10 tubules; 4 rats), and 0.7083 ± 0.022 (18 tubules; 7 rats) at peritubular osmolalities of 100, 300, 450, and 600 mosmol/kg, respectively.

Figure 7 illustrates the relationship between the total solute concentration of peritubular perfusion fluid and transepithelial effective resistances. The nearly linear relationship between peritubular osmolality and effective resistance obtained is described by $y = 0.0012x - 0.0037$ ($r = 0.9976; P < 0.001$). Thus, changes in osmolality on the vascular side of distal epithelium exert qualitatively the opposite effect on effective resistance as changes in luminal fluid osmolality.

Figure 8 shows effective resistance measured during peritubular capillary perfusions as a function of the initial transepithelial potential difference at the same tubular site prior to capillary perfusion. The distribution of potential differences demonstrates that early and late distal tubules were punctured in this series of experiments (range of TTR 1.1-1.9). Calculation of regression lines for each of the different osmolalities of capillary fluid failed to reveal significant relationships between PD and effective resistances, in contrast to the results obtained in control conditions.

Figure 9 summarizes the relationships between the osmotic concentration of luminal or peritubular perfusion fluids and the specific transverse membrane resistance, $R_{st}$, across distal tubular epithelium. A nearly linear, inverse relationship was obtained between increasing luminal NaCl concentrations and transverse specific resistance and is given by $y = -0.6652x + 456$ ($r = 0.8650; P < 0.001$). Increasing luminal osmolalities over a range of 200-600 mosmol/kg, by adding raffinose to 65 meq/liter NaCl, also led to a reduction in specific membrane resistance, $y = -0.4543x + 275$ ($r = 0.8335; P < 0.001$). The slope of the NaCl-regression line is significantly steeper than that of the raffinose line ($P < 0.01$). However, a much steeper decline in resistance per unit of increase in luminal raffinose
TABLE 1. Changes in distal transepithelial PD during tubular perfusion

<table>
<thead>
<tr>
<th>Raffinose + 120 mosmol/kg NaCl</th>
<th>∆V (Peritub. Fluid = Ground)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 mosmol/kg</td>
<td>+13.3 ± 0.7 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>500 mosmol/kg</td>
<td>+9.6 ± 1.0 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>400 mosmol/kg</td>
<td>+8.6 ± 0.7 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>120 mosmol/kg NaCl</td>
<td>-7.4 ± 0.5 (10)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TABLE 2. Changes in distal transepithelial PD during peritubular-capillary perfusion

<table>
<thead>
<tr>
<th>Ringer + Raffinose</th>
<th>∆V (Peritub. Fluid = Ground)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 mosmol/kg</td>
<td>-8.0 ± 0.8 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>150 mosmol/kg</td>
<td>-3.0 ± 0.3 (12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>300 mosmol/kg</td>
<td>+0.4 ± 0.2 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>100 mosmol/kg*</td>
<td>+7.8 ± 0.9 (12)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS, not significant. * Dilute Ringer without raffinose. † Paired t-test.

detectable different before and after ADH administration to DI rats. Early and late distal tubular resistances in DI rats were significantly higher than in control rats (P < 0.001 for early and late distal tubules). Administration of vasopressin abolished this difference (P > 0.7 for early and late distal tubules). Late distal tubular fluid is known to remain hypotonic (22), and neither sodium nor potassium concentrations are elevated in diabetes insipidus rats (22), indicating that late distal tubular fluid conductivity is not increased above that of early distal fluid. Hence, the high value of R_eff in late distal tubules must also reflect a high R_e of late tubular epithelium.

DISCUSSION

The aim of this study was to test whether physical factors or transepithelial osmotic gradients influence the electrical conductance of the wall of convoluted distal tubules of rats in vivo. Electrical resistance (or conductance) can be

Tables 1 and 2 summarize the changes in transepithelial PD in response to a sudden change in tubular or peritubular osmolality. Intratubular hyperosmolarity or peritubular hyposmolarity resulted in increased luminal negativity, whereas intratubular hyposmolarity or peritubular hyperosmolarity led to hyperpolarization. These responses were not detectably different in early and late distal tubules.

Peritubular perfusions with and without dextran. To further test whether physical factors influence distal tubular electrolyte permeability, tubular resistance was measured during capillary perfusions with and without addition of 8 g/100 ml high-molecular-weight dextran to perfusion fluids of different osmolalities. Table 3 shows a comparison of the mean effective resistances across distal tubules during capillary perfusion with 600, 300, and 100 mosmol/kg solutions. Addition of dextran failed to show any significant effect on resistance measured during perfusion of capillaries with any of the three solutions.

Diabetes insipidus (DI) rats. Figure 10 summarizes the mean transepithelial effective resistance in early (range of transit time ratios: 1.1-1.4) and late (range of transit time ratios: 1.5-1.8) distal tubules in 14 rats with congenital diabetes insipidus before and after intravenous infusion of ADH. Early distal R_eff averaged 0.54 × 10^6 Ω (± 0.02 SE; n = 13) before and was significantly decreased (P < 0.02) to 0.45 × 10^6 Ω (± 0.03 SE; n = 10) after ADH. Late distal R_eff declined from 0.65 × 10^6 Ω (± 0.08 SE; n = 6) to 0.36 × 10^6 (SE; n = 6) after hormone administration (P < 0.02). Distal tubular lumen diameters averaged 17.0 ± 1.2 (SE) μm (40 obs) and were not different before and after ADH administration to DI rats. Early and late distal tubular resistances in DI rats were significantly higher than in control rats (P < 0.001 for early and late distal tubules). Administration of vasopressin abolished this difference (P > 0.7 for early and late distal tubules).
taken as an index of ionic permeability, since electrical currents are carried through epithelial barriers by ions. As previously described by Wright (42), transepithelial potential differences increased toward the end of the distal tubule. In the present study an inverse relationship was observed between potential and electrical resistance during nonisometric conditions when late distal tubular fluid was isosmotic (7, 14) to systemic plasma. In this context, it should be realized that the so-called “distal tubule” available to micropuncture on the rat kidney surface consists of two morphologically distinct entities. The early portion is composed of epithelium characteristic of the distal convoluted tubule, whereas the ADH-sensitive late distal tubule consists of cells of initial collecting tubules (35).

The question arises whether distal tubular transport characteristics are altered by changes in physical factors as in the proximal tubule. Limiting transepithelial gradients for sodium are not different from controls during partial renal venous occlusion (1). In addition, volume expansion does not reduce distal salt reabsorption below control values (24). On the other hand, nonelectrolyte backleakage from blood to lumen is increased in distal nephrons during partial renal venous clamping (26). In the present study, partial clamping of the renal vein did not lead to any detectable decline in distal tubular wall resistance. Distal tubular hydrostatic pressure was below 8 cmH2O, whereas hydrostatic pressures in the surrounding peritubular capillaries were higher than 25 cmH2O. Others (10) have found a decrease in peritubular colloid-osmotic pressure during partial clamping of the renal vein. The fact that distal tubular electrical resistance was not diminished demonstrates that the reduction in distal tubular reabsorption of fluid (25) is not a consequence of increased passive backleakage of electrolytes from blood to tubular lumen. The present results do not demonstrate but make it likely that active transport of salt by distal tubular epithelium is reduced during partial occlusion of the renal vein. A direct test of the effect of changes in peritubular capillary oncotic pressure also failed to show any detectable alteration in distal tubular electrical resistance. Effective resistances remained nearly unchanged whether or not 8 g/100 ml of high-molecular-weight dextran was present in capillary perfusion fluid at any of the three osmolalities of perfusion fluids used in this series of experiments. It therefore seems safe to conclude that any changes in distal nephron salt and water transport under conditions of reduced peritubular oncotic or increased hydrostatic pressure are not due to augmentation of passive backleakage of electrolytes across the wall of distal convoluted tubules. Increased backleakage of nonelectrolytes in distal nephrons has been observed by Lorentz et al. (26) under conditions of extracellular volume expansion, partial renal venous occlusion, and during partial clamping of the ureter. Increased passive backflux of relatively large nonelectrolytes could be due to the opening of a small number of large-diameter “pores” in an epithelial membrane. However, the microinjection experiments of Lorentz et al. (26) assess permeability of the entire length of the nephron segment between the injection site and the end of the nephron. Thus, the increased nonelectrolyte permeability found may reflect events in collecting tubules and ducts rather than in the distal convoluted tubule. Support for this interpretation comes from the studies of Stein et al. (32) and of Sonnenberg (30) who found sodium reabsorption to be reduced in collecting ducts during expansion of the extracellular fluid volume.

Studies of the effect of osmotic gradients on distal tubular electrical resistance seemed warranted on the basis of two observations. First, the decline in epithelial resistance along distal tubules of nontucretic rats made it logical to postulate a causal relationship between the drop in resistance and the only other well-characterized functional difference between early and late distal tubule, namely the increase in luminal osmolality which occurs in the late distal tubule in the presence of ADH (7, 14). Second, osmotic gradients have previously been shown to alter the electrical resistance of frog skin and toad bladder, epithelia which are functionally quite similar to distal tubular epithelium. In these isolated tissue preparations, increased osmolality of fluid on the outside of frog skins or on the luminal side of toad bladders leads to marked reductions in resistance of the epithelium, whereas elevation of the osmotic pressure has the opposite effect. To assign physiological significance to these findings in terms of mammalian electrolyte and fluid metabolism, an examination of the effect of osmotic gradients was needed. The distal convoluted tubule was studied as one of the nephron segments where physiological changes in transepithelial osmotic gradients are known to occur.

Microperfusion of the lumen of distal tubules with solutions of different osmolalities indeed demonstrated that distal tubular transepithelial electrical resistance of tubular epithelium varied inversely with luminal fluid osmolality (see Fig. 9). In the range of 300–800 mosmol/kg H2O, increases in sodium chloride concentration alone were more effective in reducing transverse specific resistance than osmotically equivalent increments in raffinose concentration. On the other hand, absolute values of specific transverse resistances of the distal tubule corresponding to a given osmolality were always lower when raffinose contributed the major part of the total osmolality than when pure sodium chloride solution was used for the tubular perfusion. This contrasts with the greater effectiveness of NaCl than of nonelectrolytes in reducing epithelial resistances in toad bladders (8). Osmolality of distal tubular perfusion fluid remained unchanged. Hence, differences in transmural net movement of fluid or solutes during perfusion cannot explain the different effect of the two types of perfusion fluid on epithelial resistances. That osmotic gradients acting across the epithelium rather than osmolality of fluid facing luminal or peritubular cell face determine the magnitude of the transepithelial resistance is shown in Fig. 11. A relative increase in luminal as compared to peritubular fluid osmolality leads to a drop in electrical resistance, whether NaCl or a mixture of NaCl and raffinose is used to establish the osmotic gradient. This effect is consistent with the model of DiBona and Civan (8). According to them, solutes diffuse along their concentration gradient into the tight junction interior. The resulting local hyperosmolality leads to water efflux from cells into the tight junction and blister formation. It should be noted that the slopes of the lines for peritubular perfusion experiments and for tubular NaCl perfusions are similar, suggesting that the induced changes in epithelial resistance are
due to effects of the small electrolytes present within the tubular lumen in both instances. The steep curvilinear relationship between raffinose-induced osmotic gradients and transverse resistance (Fig. 10) illustrates again the greater effectiveness of raffinose as compared to salt. It is conceivable that blister formation depends on the reflection coefficient of the lateral wall of the blister to a given solute. Thus, if the reflection coefficient ($\sigma$) for raffinose is greater than $\sigma$ for NaCl, raffinose entering from the tubular lumen would indeed be more effective than NaCl in osmotically attracting water from cells and therefore more effective in lowering the electrical resistance of the tight junction.

Strong support for the notion that changes in tight junction resistance in response to the imposition of osmotic gradients across distal tubular epithelium are the main cause of transepithelial resistance change derives from a consideration of the associated changes in transepithelial potential differences (see Tables 1 and 2). Luminal hyperosmolality or peritubular hyposmolality resulted in decreased transepithelial resistance and simultaneous reduction in transepithelial potential differences. Luminal hyposmolality or peritubular hyperosmolality, on the other hand, led to an increase of transepithelial potential and resistance. The change in transepithelial resistance during luminal perfusions can only be due to alterations in either luminal cell membranes or tight junction resistance. Since diminished resistance of the luminal membrane should increase transepithelial potentials (transepithelial PD = luminal PD + peritubular PD), our results can only be explained by increased electrical shunting through tight junctions under conditions of luminal hyperosmolality and reduced intercellular shunting when tubular contents are hyposmotic compared to peritubular fluid. Similar conclusions have been reached in studies on isolated perfused cortical collecting ducts (28, 29).

Figure 12 summarizes the change in transepithelial potential differences caused by the imposition of osmotic gradients across distal tubular epithelium. A nearly linear relationship obtains between osmotic differences and potential difference. Luminal hyposmolality leads to hyperpolarization and luminal hyperosmolality to depolarization of the distal tubular wall. If the tight junctions are more permeable to cations, then bulk flow out of the lumen due to luminal hyposmolality would lead to hyperpolarization. Likewise, luminal hyperosmolality (bulk flow into the tubular lumen) would have caused transepithelial depolarization. The present results, therefore, demonstrate that distal tubular tight junctions are characterized by greater permselectivity for cations than anions.

Concerning the physiological role of osmotic effects on distal tubular electrical resistance, the following considerations are pertinent. Diabetes insipidus rats failed to show the decline in electrical resistance along the distal convoluted tubule which was found in nondiuretic control rats. Also, in control rats, resistances did not change along the tubule when rapid luminal perfusion prevented significant changes in luminal osmolality or when peritubular capillaries were perfused with fluid devoid of vasopressin. Administration of ADH significantly reduced late distal tubular resistance when compared to early sites and also to late sites prior to ADH. The much smaller decrease in resistance of early distal tubules after ADH administration cannot be related to any known osmotic effects but rather to a direct action of the hormone on cell membrane resistance (19). On the other hand, conversion of water diuresis to antidiuresis is known to result in increased late distal tubular osmolality (7, 14). It therefore is likely that the high resistance of DI
ruts and the low resistance of antidiuretic rats in the late portion of distal tubules is secondary to the prevailing osmotic gradients. This argument is further strengthened by the results obtained in our perfusion studies. Inspection of Fig. 11 indicates that transverse electrical resistance decreases by about 100 $\Omega \cdot \text{cm}^2$ per 100 mosmol increase in luminal osmolality. Assuming late distal tubular fluid to be isosmotic to plasma in antidiuretic rats, while early distal fluid is slightly less than half-isotonic to plasma, the previously shown decline in tubular resistance of about 130 $\Omega \cdot \text{cm}^2$ (see Fig. 3) is nearly accounted for by the osmotic effect.

In summary, changes in peritubular oncotic pressure do not alter the electrical conductance of distal convoluted tubules. The decrease in electrical resistance, observed in antidiuretic rats along late distal tubules, can at least in part be accounted for by the simultaneous increase in luminal osmolality. This osmotic effect acts as a positive feedback for ADH induced osmotic equilibration. Osmotic gradients affect intercellular, rather than transepithelial ion pathways. Hyposmolality of tubular fluid leads to diminished intercellular shunting and thus prevents the dissipation of ion gradients established by active transport. If similar conditions hold for the ascending limb of Henle’s loop, a nephron segment comparable to early distal tubules, the osmotic effect would also constitute part of an intra-epithelial positive feedback in the single effect of countercurrent multiplication.

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