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Research Article

Transmembrane potential difference (pd) was studied in isolated perfused segments of rabbit proximal convoluted tubules. At perfusion flow rates above 10 nl/min the pd was -5.80 ±0.3 mv (lumen negative) when perfusing with isosmolal ultrafiltrate of same rabbit serum as the bath. That this pd is generated by transport activity of the tubule is supported by three separate observations: (*a*) pd reversibly decreased with cooling from 37°C to 25°C; (*b*) pd decreased when 10^{-5} M ouabain was added to the bath and reversed to control levels when ouabain was removed; and (*c*) heating to 47°C irreversibly decreased pd to zero. The magnitude of the pd was related to perfusion flow rate at slower rates than 10 nl/min. A decrease in flow rate was associated with a decrease in pd. The tubular geometry and transmembrane hydrostatic pressure were ruled out as the mediating factors governing the magnitude of observed pd.

Flow Dependence of Transtubular Potential Difference in Isolated Perfused Segments of Rabbit Proximal Convoluted Tubule

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ABSTRACT Transmembrane potential difference (pd) was studied in isolated perfused segments of rabbit proximal convoluted tubules. At perfusion flow rates above 10 nl/min the pd was -5.80 ± 0.3 mv (lumen negative) when perfusing with isosmolal ultrafiltrate of same rabbit serum as the bath. That this pd is generated by transport activity of the tubule is supported by three separate observations: (*a*) pd reversibly decreased with cooling from 37°C to 25°C; (*b*) pd decreased when 10^{-5} M ouabain was added to the bath and reversed to control levels when ouabain was removed; and (*c*) heating to 47°C irreversibly decreased pd to zero. The magnitude of the pd was related to perfusion flow rate at slower rates than 10 nl/min. A decrease in flow rate was associated with a decrease in pd. The tubular geometry and transmembrane hydrostatic pressure were ruled out as the mediating factors governing the magnitude of observed pd.

INTRODUCTION

There has been considerable controversy concerning the existence and magnitude of the transepithelial potential difference (pd)[1] in the proximal convoluted tubule of the mammalian kidney. Following the initial reports of Solomon (1) many investigators found a significant proximal transtubular potential difference of 20 mv, with the lumen negatively charged with respect to peritubular fluid. Frömter and Hegel (2), however, challenged these values, attributing them to artifacts arising from the placement of the electrode tips partially inside the tubular epithelial cells rather than in the tubule lumen. When steps were taken to localize objectively the electrode tips in the lumen, these authors were unable to find any measurable pd across the tubular epithelium. Burg, Isaacson, Grantham, and Orloff (3) using isolated perfused segments of rabbit proximal convoluted tubule initially were unable to find any transmembrane pd; however, they subsequently (4) found the lumen to be about -3.8 mv with respect to the bath when Sylgard (Dow Corning Corp., Midland, Mich) was used to obtain a more effective electrical seal between the ends of the tubule segments and the pipettes suspending the tubule in the bath.

The present studies were originally undertaken to examine the effect of the composition of the perfusion fluid on the transtubular pd in isolated perfused segments of rabbit proximal convoluted tubules. During the course of these studies, however, it was inadvertently found that perfusion pressure had a dramatic effect on the pd. The present communication presents these findings and some of our studies designed to elucidate the mechanism whereby perfusion pressure influences the transtubular pd.

METHODS

Isolated segments of proximal convoluted tubules obtained from female New Zealand rabbits were perfused by the general techniques previously described (4). Sylgard 184 (Dow Corning Corp., Midland, Mich.) was used at both the collection and perfusion end to obtain good tissue/glass electrical seal.

The tubules were perfused with isosmolal ultrafiltrate of the same rabbit serum as the bath. The perfusion rate was controlled in two ways: (*a*) by varying the height of the perfusion chamber which was filled with the isosmolal ultrafiltrate, and (*b*) by constricting the collecting end of the tubule with pressure applied by a polished glass pipette. Equivalent bridges of 300 mOsm liter^{-1} Ringer's in 4% agar (PE tubing size 240) were connected to end of the perfusion pipette and the bath. The other end of the bridges were submerged in saturated KCl solution which contained Beckman (Beckman Instruments, Inc., Fullerton, Calif.) calomel half-cells. The circuit was completed by placing a

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[1] *Abbreviations used in this paper:* pd, potential difference.

voltage reference source and a battery operated Keithley model 602 (Keithley Instruments, Inc., Cleveland, Ohio) electrometer in the circuit. The stability of this system was excellent with base line voltage drift of less than ±0.3 mv for the duration of an experiment (2–6 hr).

The effect of ouabain was studied by adding 10^{-5} M concentration to the bath. The reversibility of ouabain was evaluated after exchanging the bath five times with ouabain-free serum.

RESULTS

Shortly after the initial hookup, the tubules had a transmembrane potential of 1.5–2.5 mv with the lumen negative, while at room temperature. After the bath temperature was elevated to 37°C the transmembrane pd quickly became more negative (approximately 80% of the maximum potential) and stabilized at its maximum reading after approximately 60 min of perfusion (Fig. 1). When the tubules were perfused at 20 cm H_2O pressure, the lumen was consistently negative to the bath by 5.8 ±0.3 (SE) mv (n = 12). Though the transmembrane pd varied from − 3.5 to − 8.1 mv, each tubule maintained an extremely constant pd after the initial stabilization period. The maximum variation in pd over a 1 hr period was ±0.5 mv. In several experiments, the transmembrane pd was noted to be stable up to 6 hr.

To evaluate whether the observed transmembrane pd was related to metabolic and transport activities of the tubule several procedures were done. After a stable potential was reached, the heating elements were cut off and the temperature allowed to return to room temperature. Associated with this was a drop in transmembrane pd to approximately − 2 mv. If the temperature at this point was returned to 37°C, the previously noted control pd returned within a few minutes. Thus the effect of cooling on transmembrane pd was reversible in each case (n = 6). If, however, the tubule was heated to 47°C, the transmembrane pd dropped to zero, and this change was irreversible when the bath temperature was returned to 37°C, presumably due to permanent injury of the tubule. When 10^{-5} M ouabain was added to the bath, there was a transient (30 sec) period of hyperpolarization of approximately − 0.3 mv, followed immediately by rapid depolarization to − 0.5 to − 1.8 mv, with a mean of − 1.2 mv (n = 6). That the effect of ouabain is reversible and not due to nonspecific killing of the tubule is shown in Fig. 1 where the ouabain effect was washed off by changing the bath five times with ouabain-free serum. Three such experiments were conducted, and in each case the findings were similar as represented in the single experiment in Fig. 1.

The apparent effect of perfusion pressure on transmembrane pd was examined in six consecutive experiments. The results of these are represented in Fig. 2. As was noted in each case the pd remained constant as the perfusion pressure was dropped from 40 cm H_2O

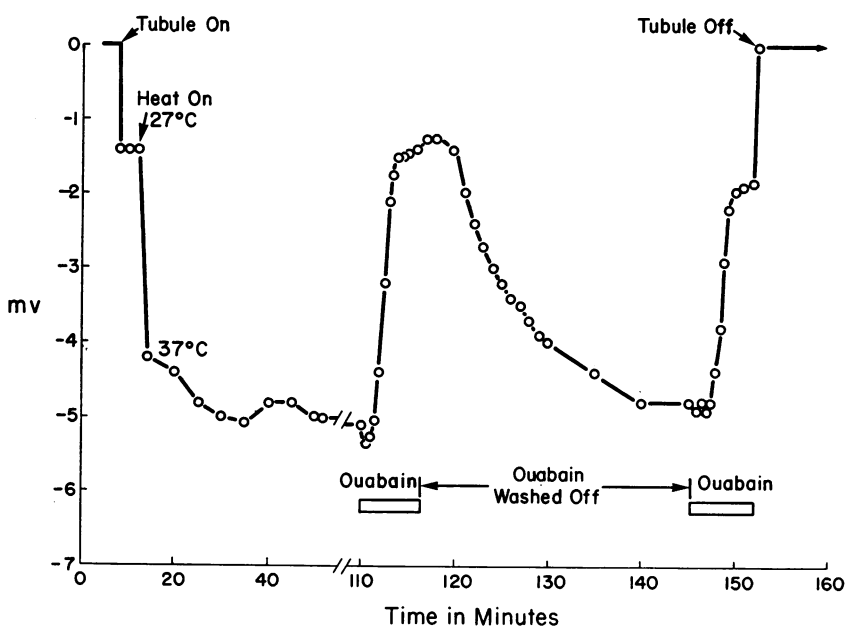


FIGURE 1 Transmembrane potential difference of a single proximal tubule illustrating response to temperature change and effect of 10^{-5} M ouabain added to the bath. The reversibility of ouabain effect is noted by return of potential difference to control levels after ouabain has been removed from the bath.

to 15 cm H_2O.[2] In the range between 8 and 12 cm H_2O the transmembrane pd became quite sensitive to the perfusion pressure. This pattern was consistent and was observed in each tubule.

Our initial impression was that the relationship of perfusion pressure to transmembrane pd was mediated through changes in tubular diameter. As shown in top and bottom left-hand panel of Fig. 3, lowering perfusion pressure from 25 cm to 7 cm resulted in a decrease in inside diameter from 22.8 μ to 4–6 μ with an associated fall in pd from -4.9 mv to -0.6 mv. That the relationship between perfusion pressure and pd is not mediated through either geometry or transepithelial hydrostatic pressure gradients is shown on top and bottom right-hand panels of Fig. 3. When the tubule was perfused at 25 cm H_2O the control pd was -4.9 mv and decreased to -0.8 mv when the perfusion rate was decreased by constriction of the outflow end of the tubule. This fall in pd occurred in association with an increase in the transepithelial hydrostatic pressure gradient and dilation of the tubule from 22.8 μ to inside diameter of 25.9 μ. Similarly, if the collapsed tubule perfused at low pressure is dilated by increasing the resistance at the collecting end, as noted in the lower panels of Fig. 3, there is no increase in transmembrane potential difference. The changes in transmembrane pd in response to partial constriction of the distal end are immediate and reversible when the constriction is withdrawn. Similar changes were noted in each tubule so studied ($n = 6$). In three tubules perfused at 15 cm H_2O the reduction of flow rate by constriction of the outflow end of the tubule resulted in a prompt fall in pd. When the perfusion rate was returned to approximately control levels by increasing the perfusion pressure to 35–40 cm H_2O while maintaining the partial constriction, the pd returned promptly to control levels.

These studies indicate that the apparent relationship between perfusion pressure and pd is mediated, not by transepithelial hydrostatic pressure gradients or tubular diameter, but rather by the perfusion flow rate. The quantitative relationship between pd and flow rate is shown in a representative experiment in Fig. 4. As the perfusion pressure is lowered from 30 to 12 cm H_2O the perfusion rate decreases from 130 nl/min to 10 nl/min without any change in pd; however, as the perfusion rate is reduced below 10 nl/min progressively to less than 1 nl/min the pd falls precipitously to less than -2 mv. Though the pd becomes sensitive to linear flow rates at less than 10 nl/min, the sharp decreases in pd occur at much lower perfusion rates. The mean perfusion rate at the estimated points where the pd is most sensitive to perfusion rate, approximately one half of the observed maximum stable pd, see Figs. 2 and 3, is 2.0 $\pm$0.1 nl/min ($n = 6$).

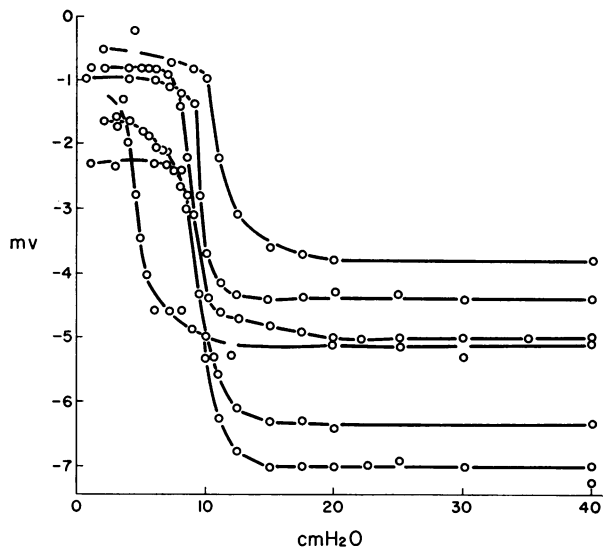


FIGURE 2 The relationship between transmembrane potential difference and hydrostatic perfusion pressure in six consecutive experiments. See footnote 2.

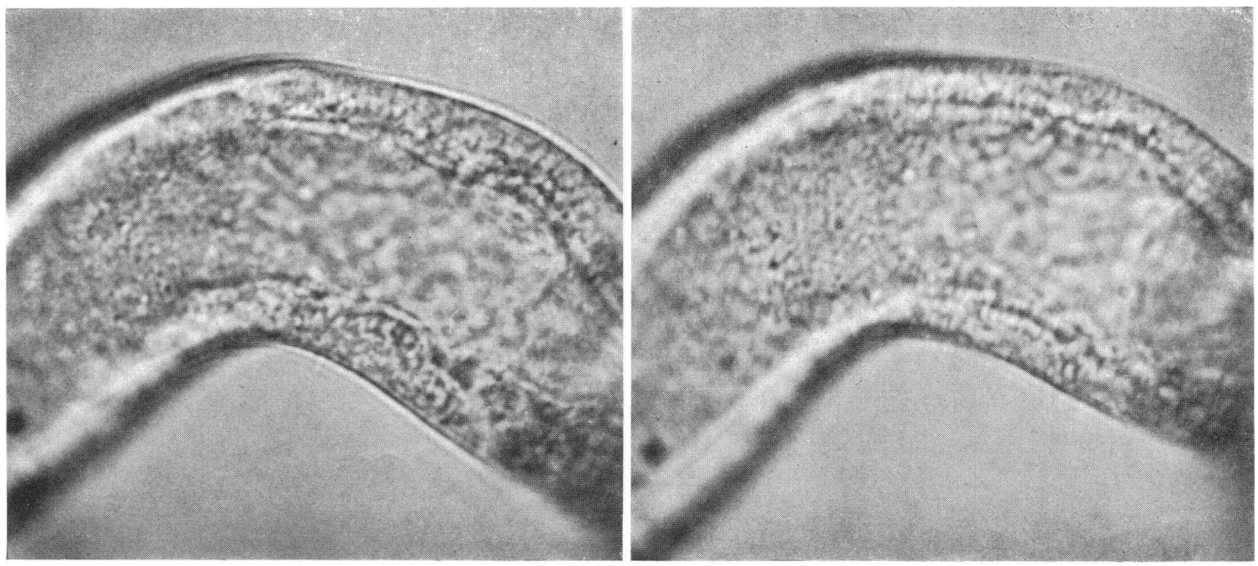
DISCUSSION

The present studies showing that the proximal tubule of the rabbit perfused at relatively high flow rates has a transtubular potential difference of -5.8 mv are essentially in agreement with those of Burg and Orloff (4). The data further suggest that the pd is related to the transport activity of the tubule rather than arising from streaming potentials or diffusion artifacts. First, the pd is temperature dependent, decreasing reversibly as the tubule is cooled to room temperature and then rewarmed to 37°C. Heating the tubule to 47°C causes an irreversible loss of the pd, presumably due to permanent thermal injury of the tubule. Second, 10^{-5} M ouabain added to the bath causes rapid decrease of the pd to approximately -1 mv; the pd returns to the control value when ouabain is removed from the bath.

Considering the evidence that the transtubular pd is related to transport activity of the tubule, it was surprising to find that varying perfusion pressure had such a dramatic effect on the value of the pd. As shown in Fig. 2, lowering perfusion pressure below 10–12 cm H_2O resulted in a precipitous fall in the pd to -1 to -2 mv.

Lowering perfusion pressure is associated with at least three changes in the tubule which might mediate the fall in transtubular potential difference; (*a*) a de-

[2] The perfusion pressure is not to be equated with intratubular hydrostatic pressure for two reasons: (*a*) the major resistance to hydrostatic flow is the tip of the perfusion pipette (most are 10–12 μ I.D. for approximately 500 μ giving pipette electrical resistance from 3.8 to 5.5 MΩ) and (*b*) the distal end of the tubule is in an open-ended pipette.

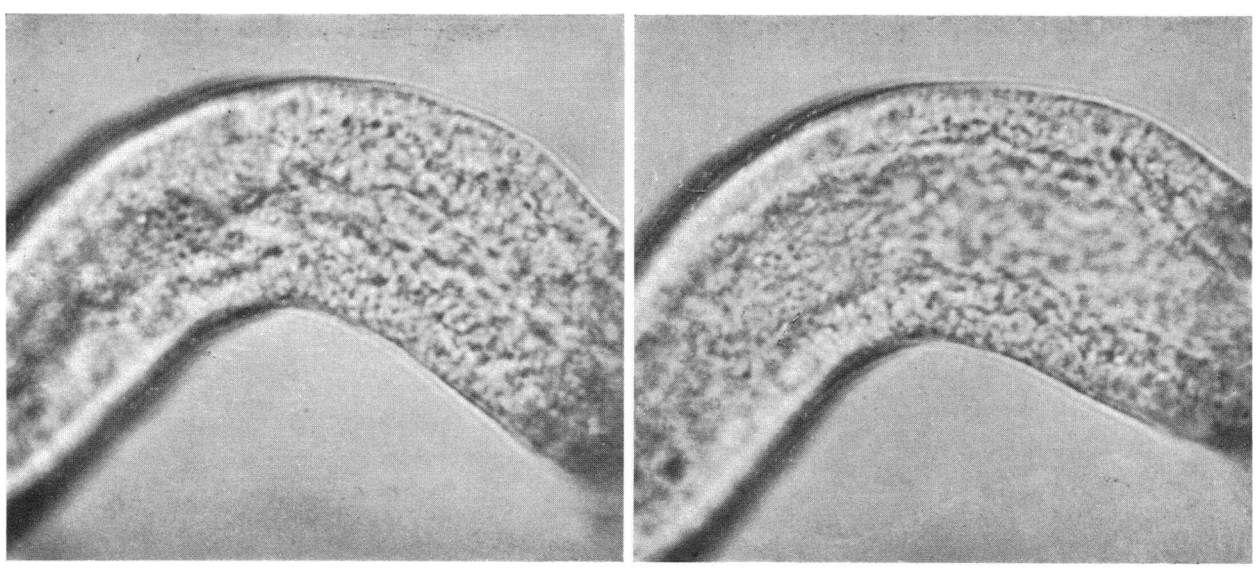


Control at 25 cm H_2O p.p.

O.D. 45.5 μ
I. D. 22.8 μ
mv −4.9

Constricted at 25 cm H_2O p.p.

O.D. 45.5 μ
I. D. 25.9 μ
mv −0.8



Control at 7 cm H_2O p.p.

O.D. 35.7 μ
I.D. 4–6 μ
mv −0.6

Constricted at 7 cm H_2O p.p.

O.D. 40.4 μ
I.D. 14.5 μ
mv −0.5

FIGURE 3 Photographs of a single tubule to illustrate the dissociation of intratubular hydrostatic pressure and tubular geometry from the observed transmembrane potential difference. See text for full explanation. × 400. (p.p., perfusion pressure.)

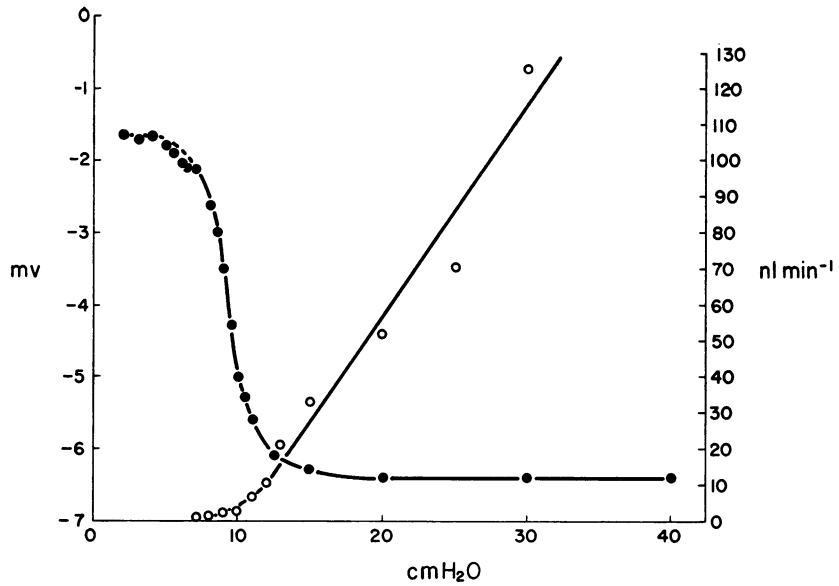


FIGURE 4 Representative experiment of a single perfused proximal tubule illustrating the relationship between transmembrane potential, closed circles, hydrostatic perfusion pressure, abscissa, and, perfusion rate, open circles.

crease in the hydrostatic pressure gradient across the tubule epithelium; (*b*) a decrease in tubular radius; and (*c*) a decrease in the flow rate of tubular fluid.

Initially we considered changes in tubular radius as the most likely mechanism mediating the changes in pd. Subsequent experiments, however, clearly excluded both transtubular hydrostatic pressure gradients and tubular radius as the factors responsible for the fall in pd. In the experiments in which the distal end of the perfused tubule was partially obstructed by external pressure, the transtubular hydrostatic pressure gradient presumably rose (although this was not measured directly) and tubular radius increased. Despite these changes, pd fell. In other experiments, in which the perfusion pressure was first reduced, the tubule was observed to collapse and the pd to fall; outflow constriction of these tubules caused the tubules to dilate towards control radii without any corrective effects on the pd. Experiments such as these clearly dissociated changes in both transtubular hydrostatic pressure gradients and tubular radii from the changes in pd. Thus we were left with the conclusion that the most obvious factor responsible for the change in pd is the change in tubular fluid flow rate.

It is important to note that variations of flow rate in range that influences pd (less than 10 nl/min) coincides with the physiological rates of tubular flow through individual nephrons of the intact rabbit kidney. Burg and Orloff (5) have estimated indirectly that the glomerular filtration rate per nephron in the in vivo rabbit is approximately 14 nl/min, and at end of proximal tubule the flow rate would be about 7 nl/min. By more direct observations, we have found in a few in vivo micropuncture experiments in rabbits (unpublished observations) that the collected tubular fluid rate was of the same range as estimated by Burg and Orloff(5).

A change in tubular flow rate might influence transtubular pd in one of several different ways. One possibility is that variation in tubular flow rate might influence the entrance into the cell of some transported substance such as sodium. This might be mediated through effects on the microvilli-unstirred layer complex which is present on the luminal surface of proximal convoluted tubules. A second possibility is that the composition of the tubular fluid is altered as it passes along the tubule in a manner that might either limit active transport processes or else generate diffusion potentials that would obscure the basic transport pd. Preliminary experiments utilizing artificial perfusion solutions have shown that removal of glucose, bicarbonate, and calcium markedly reduces pd. These results suggest, but do not prove, that at extremely low perfusion rates depletion of these and other critical constituents in the luminal fluid is responsible for the flow dependent fall in the observed pd.

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