Evidence for neutral transcellular NaCl transport and neutral basolateral chloride exit in the rabbit proximal convoluted tubule.

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Research Article

The electrical nature of active NaCl transport and the significance of a basolateral membrane chloride conductance were examined in isolated perfused rabbit proximal convoluted tubules (PCT). PCT were perfused with a high chloride solution that simulated late proximal tubular fluid and were bathed in an albumin solution that simulated rabbit serum in the control and recovery periods. The electrical nature of NaCl transport was examined by bathing the tubules in a high chloride albumin solution where there were no anion gradients. Volume reabsorption (Jv) during the control and recovery period was 0.56 and 0.51 nl/mm X min, respectively, and 0.45 nl/mm X min when the tubules were bathed in a high chloride bath. The transepithelial potential difference (PD) during the control and recovery periods averaged 2.3 mV, but decreased to 0.0 mV in the absence of anion gradients, which indicated that NaCl transport is electroneutral. Further evidence that NaCl transport is electroneutral was obtained by examining the effect of addition of 0.01 mM ouabain in PCT perfused and bathed with high chloride solutions. The Jv was 0.54 nl/mm X min in the control period and not statistically different from zero after inhibition of active transport. The PD was not different from zero in both periods. Two groups of studies examined the role of basolateral membrane Cl- conductance in NaCl transport. […]

Evidence for Neutral Transcellular NaCl Transport and Neutral Basolateral Chloride Exit in the Rabbit Proximal Convoluted Tubule

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Abstract. The electrical nature of active NaCl transport and the significance of a basolateral membrane chloride conductance were examined in isolated perfused rabbit proximal convoluted tubules (PCT). PCT were perfused with a high chloride solution that simulated late proximal tubular fluid and were bathed in an albumin solution that simulated rabbit serum in the control and recovery periods. The electrical nature of NaCl transport was examined by bathing the tubules in a high chloride albumin solution where there were no anion gradients. Volume reabsorption (J_v) during the control and recovery period was 0.56 and 0.51 nl/mm · min, respectively, and 0.45 nl/mm · min when the tubules were bathed in a high chloride bath. The transepithelial potential difference (PD) during the control and recovery periods averaged 2.3 mV, but decreased to 0.0 mV in the absence of anion gradients, which indicated that NaCl transport is electroneutral. Further evidence that NaCl transport is electroneutral was obtained by examining the effect of addition of 0.01 mM ouabain in PCT perfused and bathed with high chloride solutions. The J_v was 0.54 nl/mm · min in the control period and not statistically different from zero after inhibition of active transport. The PD was not different from zero in both periods.

Two groups of studies examined the role of basolateral membrane Cl^- conductance in NaCl transport. First, depolarizing the basolateral membrane with 2 mM bath Ba^{++} did not significantly affect J_v or PD. Second, the effect of the presumptive Cl^- conductance inhibitor anthracene-9-CO_2H was examined. Anthracene-9-CO_2H did not significantly affect J_v or PD. In conclusion, these data show that NaCl transport in the PCT is electroneutral and transcellular and provide evidence against a significant role for basolateral membrane chloride conductance in the rabbit PCT.

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Introduction

The mechanism of active NaCl transport in the proximal convoluted tubule (PCT)[1] is poorly understood. From a high chloride solution simulating late proximal tubular fluid, an essentially pure NaCl solution is reabsorbed. In this setting one-third of NaCl reabsorption is passive and paracellular and two-thirds are active (1–4). Active NaCl transport could be due to electrogenic active Na^+ transport with passive Cl^- movement through the paracellular pathway driven by the lumen negative transepithelial potential difference, or it could be due to electroneutral transport where both Na^+ and Cl^- movement are transcellular. Transcellular NaCl transport would require a mechanism for intracellular Cl^- to exit across the basolateral membrane. In several leaky epithelia (5–9) including the PCT (10, 11), intracellular Cl^- is at or above electrochemical equilibrium. Intracellular Cl^- above electrochemical equilibrium in combination with a basolateral membrane chloride conductance could explain basolateral Cl^- exit.

The purposes of the present in vitro microperfusion study were to investigate whether active NaCl transport is electrogenic or electroneutral, and to determine the possible contribution of basolateral membrane Cl^- conductance in the rabbit PCT. PCT were perfused with a high chloride solution, which simulated late proximal tubular fluid from which NaCl is essentially the only solute reabsorbed. Two groups of experiments were performed. The first group of experiments was designed to determine whether active NaCl transport was electroneutral or electrogenic. NaCl transport and PD were examined after elimination of anion gradients and after inhibition of active transport. In the second group of experiments two protocols examined the significance of a basolateral membrane Cl^- conductance. In one

1. *Abbreviations used in this paper:* COMP-ALB, albumin solution that simulated serum; J_v, net volume absorption; PD, transepithelial potential difference; PCT, proximal convoluted tubule.

protocol the effect of depolarizing the basolateral membrane with Ba^{++} on NaCl transport was examined (12–15). Depolarizing the basolateral membrane would decrease the electrical driving force for conductive Cl^- exit and inhibit NaCl transport if Cl^- exit were conductive. In a second protocol, the effect on NaCl transport of adding anthracene-9-CO_2H, a presumptive Cl^- conductance inhibitor (16–18), to the bath solution was measured.

Our results show that active NaCl transport in the PCT is electroneutral, where both Na^+ and Cl^- transport are transcellular. Furthermore, neither depolarizing the basolateral membrane with Ba^{++} nor the addition of anthracene-9-CO_2H had any effect on NaCl transport. These studies provide evidence against a significant basolateral membrane chloride conductance and suggest an electroneutral mechanism for basolateral Cl^- exit in the PCT.

Methods

Isolated segments of rabbit PCT were dissected and perfused as previously described (1, 19, 20). Briefly, kidneys from female New Zealand white rabbits were cut in coronal slices. Individual PCT were dissected in cooled (4°C) ultrafiltrate-like solution containing 104 mM NaCl, 25 mM $NaHCO_3$, 4 mM Na_2HPO_4, 7.5 mM Na acetate, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 5 mM glucose, 5 mM alanine, and 5 mM urea. PCT were identified as juxtamedullary if obtained from immediately above the corticomedullary junction, and superficial if obtained from the remaining cortex.

During the control and recovery periods, tubules were perfused with a high chloride solution simulating late proximal tubular fluid and bathed in an albumin solution that simulated serum (COMP-ALB). The high chloride solution contained 136.5 mM NaCl, 5 mM $NaHCO_3$, 4 mM Na_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, and 5 mM urea. The salt composition of the albumin solution was the same as the ultrafiltrate-like solution except dialyzed albumin (bovine albumin, fraction V, Sigma Chemical Co., St. Louis, MO) was added at 6 g/dl. In two protocols the transepithelial anion gradients were eliminated by bathing the tubules in a solution exactly like the perfusate (Hi Cl^--ALB) except that 6 g/dl of dialyzed albumin and 5 mM alanine and glucose were added. All solutions were adjusted to 290 mosmol/kg H_2O by adding H_2O or NaCl salt. The serumlike bathing solutions were bubbled with 95% O_2 and 5% CO_2 and had a pH of 7.40. High chloride solutions were bubbled with 99% O_2 and 1% CO_2 and had a pH of 7.40. All tubules were perfused at ~10 nl/min at 38°–39°C in a 1.2-ml temperature-controlled bath. The first period began after an equilibration time of 30 min. Subsequent periods were separated by an equilibration time of at least 15 min.

Net volume absorption (J_v, nanoliters/millimeter · minute) was measured as the difference between the perfusion and collection rates (nanoliters/minute) normalized per millimeter of tubular length. Exhaustively dialyzed [*methoxy*-^{3}H] inulin (New England Nuclear, Boston, MA) was added to the perfusate at a concentration of 50 μCi/ml so that the perfusion rate could be calculated. The collection rate was measured with a 50-nl constant-volume pipette. The length, in millimeters, was measured with an eyepiece micrometer.

The transepithelial potential difference (PD, in millivolts) was measured by using the perfusion pipette as the bridge into the tubular lumen. The perfusion and bath solutions were connected to the recording and reference calomel half-cells, respectively, via a bridge containing perfusion or ultrafiltrate-like solution in series with a 3.6 M KCl/0.9 M KNO_3 agrose bridge. This arrangement avoided direct contact of KCl/KNO_3 agrose bridges with the solution that bathed the tubule. In addition, this arrangement eliminates the Donnan potential when the bath contains protein (19). The recording and reference calomel half cells were connected to the high and low impedance side, respectively, of a Vibron Electrometer (model 33B, Richmond, England).

Two groups of experiments were performed. The first group of experiments was designed to determine whether NaCl transport in the PCT was electroneutral or electrogenic. The second group of experiments was performed to determine the significance of a basolateral membrane Cl^- conductance. In all protocols PCT tubules were perfused with a high chloride solution simulating late proximal tubular fluid. During the control and recovery periods, tubules were bathed in a serumlike solution.

Electrical nature of NaCl transport. Three protocols were performed to determine whether NaCl transport was electroneutral or electrogenic. In the first protocol the PD was measured in six juxtamedullary and six superficial PCT in the presence of active transport and when active transport was inhibited with 0.01 mM bath ouabain. In the second protocol the electrical nature of NaCl transport was examined more directly by examining the effect of eliminating anion gradients on J_v and PD. In the experimental period tubules were bathed in a high chloride albumin solution to eliminate anion gradients. After the recovery period 0.01 mM ouabain was added to the serumlike bathing solution to determine the passive component of NaCl transport. In the final protocol examining the electrical nature of NaCl transport tubules were again bathed in a high chloride albumin solution to eliminate anion gradients after the control period. Subsequently, 0.01 mM ouabain was added to the high chloride albumin solution to inhibit active transport.

Significance of a basolateral membrane Cl^- conductance. Two protocols were performed to determine the possible role of basolateral membrane Cl^- conductance in NaCl transport. Tubules were perfused with a high chloride solution that simulated late proximal tubular fluid and were bathed in a serumlike solution during the control and recovery periods. In the first protocol, we examined the effect of depolarizing the basolateral membrane on J_v and PD. During the experimental period, 2 mM $BaCl_2$, a potassium conductance inhibitor (12–14), was added to the bathing media. In the second protocol, we examined the effect of anthracene-9-CO_2H, a presumptive Cl^- conductance inhibitor (16–18), on J_v and PD. During the experimental period, 0.1 mM anthracene-9-CO_2H (obtained from a 0.1 M stock solution dissolved in dimethyl sulfoxide) was added to the albumin bathing solution. During these experiments an equivalent concentration of dimethyl sulfoxide was added to the perfusate and albumin solution in the control and recovery periods.

There were three measurements of each parameter in a given period for each tubule. The mean values for individual periods in a given tubule were used to calculate the mean value for that period. Data are expressed as a mean±SEM. The *t* test for paired or unpaired data was used to determine statistical significance.

Results

Electrical nature of NaCl transport. The effect of inhibition of active transport with 0.01 mM ouabain on transepithelial PD was measured in six superficial and six juxtamedullary PCT to determine if active NaCl transport in these segments was electroneutral or electrogenic. In all tubules the perfusate was a

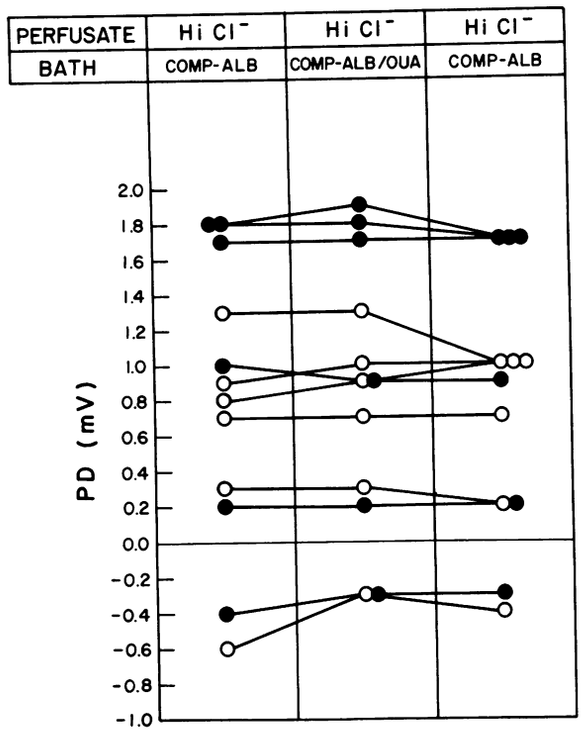

Figure 1. Effect of 0.01 mM ouabain (OUA) on PD. PD for individual superficial (○) and juxtamedullary (●) PCT were plotted during control, experimental, and recovery periods.

high chloride solution that simulated late proximal tubular fluid, and the tubules were bathed in a serumlike solution. The results are shown in Fig. 1. There was no significant difference in transepithelial PD after the addition of 0.01 mM ouabain in either group. The PD in superficial PCT was 0.6±0.3 and 0.6±0.2 mV in the control and recovery periods, respectively, and 0.7±0.2 mV after the addition of ouabain. The PD in juxtamedullary PCT was 1.0±0.4 in the control and recovery periods, and 1.0±0.4 mV after the addition of ouabain. There was also no significant difference between the PD in superficial and juxtamedullary PCT. Failure of the PD to change after inhibition of active transport with ouabain suggests that active NaCl transport is electroneutral and transcellular.

In a second protocol designed to determine if active NaCl transport was electroneutral or electrogenic, we examined the effect of a high chloride bath on J_v (n = 9) and PD (n = 7). All tubules were perfused with a high chloride solution that simulated late proximal tubular fluid and were bathed in a serumlike solution. To eliminate anion gradients responsible for passive transport, the bathing solution was changed to a high chloride albumin solution during the experimental period. In the final period 0.01 mM ouabain was added to the serumlike bathing solution to inhibit active transport. The mean tubular length was 1.3±0.2 mm and the perfusion rate was 12.35±0.19 nl/min. The results of these experiments are shown in Fig. 2. The J_v during the control and recovery period was 0.56±0.07 and 0.51±0.07 nl/mm·min, respectively, and 0.45±0.07 nl/mm·min when the bathing solution was changed to a high chloride albumin solution. The transepithelial PD was 2.2±0.4 and 2.4±0.3 mV during the control and recovery periods, respectively, and 0.0±0.1 mV when anion gradients were eliminated. In the final period, active transport was inhibited in the presence of anion gradients. The remaining transport, that due to passive transport, was 0.08±0.07 nl/mm·min. The PD was 2.5±0.3 mV, a value not significantly different from the control or recovery periods. These data show that active NaCl transport can occur without a transepithelial anion gradient. Furthermore, the active transport PD is zero in the absence of anion gradients,

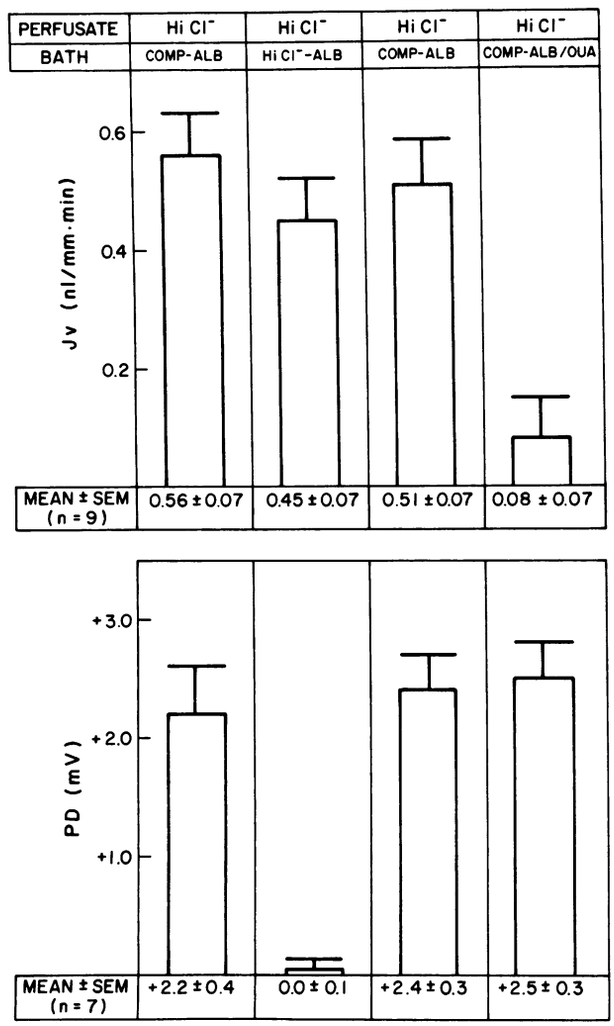

Figure 2. Effect of a high chloride albumin bath (Hi Cl^- ALB) and 0.01 mM ouabain (OUA) on J_v and PD. In the second period, the anion gradients responsible for passive transport were eliminated. In the final period, passive transport was determined by inhibiting active transport in the presence of anion gradients.

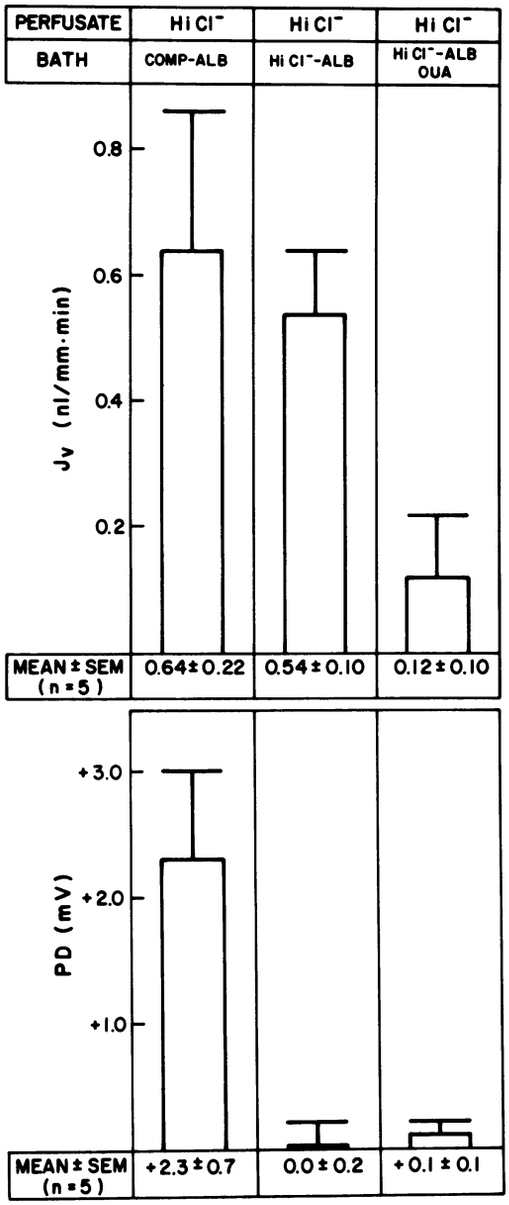

Figure 3. Effect of 0.01 mM ouabain (OUA) on NaCl transport in the absence of anion gradients.

indicating that active NaCl transport is electroneutral and transcellular.

In the final protocol designed to determine the electrical nature of active NaCl transport, we examined the effect of 0.01 mM ouabain on J_v and PD in the absence of anion gradients. Five tubules were perfused with a high chloride solution that simulated late proximal tubular fluid. In the first period, tubules were bathed in a serumlike solution. In the next period the bathing solution was changed to a high chloride solution to eliminate anion gradients. In the final period 0.01 mM ouabain was added to the high chloride bathing solution to inhibit active transport. The mean tubular length was 1.1±0.1 mm and the perfusion rate was 12.48±0.27 nl/min. The results of these experiments are shown in Fig. 3. In the initial period where tubules were bathed in a serumlike solution, the J_v was 0.64±0.22 nl/mm · min and the PD was 2.3±0.7 mV. When anion gradients were eliminated by changing the bathing solution to a high chloride albumin solution, the J_v was 0.54±0.10 nl/mm · min and the PD decreased to 0.0±0.2 mV. During the final period 0.01 mM ouabain was added to inhibit active transport. The J_v was 0.12±0.10 nl/mm · min and the PD was 0.1±0.1 mV; both values were not significantly different from zero. Failure of the PD to change after inhibition of active NaCl transport with ouabain confirms that NaCl transport is electroneutral and transcellular.

Significance of a basolateral membrane Cl^- conductance. Transcellular NaCl transport requires a mechanism for Cl^- exit across the basolateral membrane. The possible contribution of basolateral membrane Cl^- conductance was examined in two protocols. In the first protocol the effect of 2 mM bath Ba^{++}, a compound that has been shown to depolarize the basolateral membrane from ~ −55 mV to −25 mV (12–14), was examined in 10 tubules. All tubules were perfused with a high chloride solution that simulated late proximal tubular fluid and were bathed in a serumlike solution. The mean tubular length was 1.0±0.1 mm and the perfusion rate was 10.70±0.31 nl/min. The transepithelial PD was 1.0±0.2 and 0.9±0.2 mV in the control and recovery periods, respectively, and 1.0±0.2 mV when 2 mM Ba^{++} was added to the bathing solution. These differences were not significant. The effect of 2 mM Ba^{++} on J_v is shown in Fig. 4. The J_v during the control and recovery periods was 0.63±0.08 and 0.72±0.12 nl/mm · min, respectively, and 0.69±0.08 nl/mm · min when 2 mM Ba^{++} was added to the bath. There was no difference between these periods. Thus, decreasing the electrical driving force for Cl^- exit did not decrease NaCl transport.

In the second protocol the effect of 0.1 mM anthracene-9-CO_2H, a presumptive chloride conductance inhibitor (16–18), was studied in six PCT. All tubules were perfused with a high chloride perfusate that simulated late proximal tubular fluid

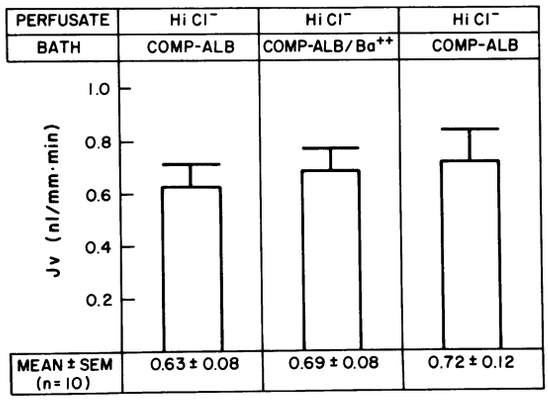

Figure 4. Effect of 2 mM bath Ba^{++} on J_v.

and were bathed in a serumlike solution. The mean tubular length was 1.2±0.2 mm and the perfusion rate was 12.80±0.32 nl/min. The transepithelial PD was 0.9±0.4 and 1.0±0.4 mV during the control and recovery periods, respectively, and 0.9±.3 mV after the addition of 0.1 mM anthracene-9-CO_2H to the bath. These differences were not significant. The effect of 0.01 mM anthracene-9-CO_2H on J_v is shown in Fig. 5. The J_v during the control and recovery periods was 0.9±0.17 and 1.17±0.18 nl/mm · min, respectively, and 1.14±0.16 nl/mm · min after 0.1 mM anthracene-9-CO_2H was added to the bathing solution. The Cl^- conductance inhibitor anthracene-9-CO_2H did not decrease NaCl transport.

Discussion

The present studies were designed to examine transepithelial and basolateral Cl^- transport in rabbit PCT that were perfused with a high chloride solution that simulated late proximal tubular fluid. The electrical nature of active NaCl transport was examined in the absence of anion gradients by bathing PCT with a high chloride albumin solution. Our results show that active NaCl transport exists in the absence of anion gradients and that this transport is electroneutral and transcellular. The presence of a basolateral membrane Cl^- conductance was examined by depolarizing the basolateral membrane with Ba^{++} and by the addition of anthracene-9-CO_2H, a presumptive Cl^- conductance inhibitor. Neither maneuver decreased NaCl transport, which provided evidence against a Cl^- conductance in the basolateral membrane.

Electrical nature of transepithelial NaCl transport. Insight into the electrical nature of the active component of NaCl transport from a high chloride perfusate can be obtained by examining the effect of inhibition of active transport on the transepithelial PD due to active transport. Our reported transepithelial PDs have been corrected for the equilibrium ion diffusion PD (Donnan PD) due to the presence of peritubular protein, and thus reflect only the contribution of active ion transport and non-

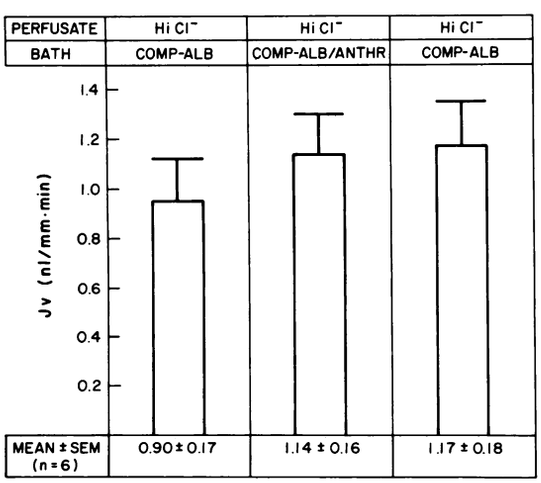

Figure 5. Effect of 0.1 mM anthracene-9-CO_2 H (ANTHR) on J_v.

equilibrium ion concentration gradients (see Methods). Under these experimental conditions, the measured transepithelial PD is the deviation from the Donnan PD and is the sum of the lumen-positive Cl^- diffusion PD and any lumen-negative PD generated by active electrogenic Na^+ transport. Therefore, if active transcellular sodium transport is responsible for passive paracellular Cl^- transport, then the transepithelial PD should become more lumen positive after inhibition of sodium transport with ouabain. We found no significant change in PD in either superficial or juxtamedullary PCT (Fig. 1). If NaCl transport were electrogenic, the expected change in PD after inhibition of active transport would be ~ +2 mV.[2] Failure to find a change in PD strongly indicates that the active component of NaCl transport from a high Cl^- solution is electroneutral and that Na^+ and Cl^- transport are transcellular.

Other studies have examined the electrical nature of transepithelial NaCl transport. Jacobson (21) perfused superficial PCT with subcapsular convolutions and juxtamedullary PCT with a high chloride solution and bathed the tubules in rabbit serum. He found a small but statistically insignificant decrease in J_v and no difference in PD in subcapsular superficial PCT after inhibiting active transport with ouabain. He attributed most of NaCl transport in subcapsular superficial PCT to a passive process. In juxtamedullary PCT all NaCl transport was inhibited with ouabain and the PD, although not statistically significant, depolarized from −1.9 to −0.7 mV. Berry (1) found a depression in J_v with no change in transepithelial PD upon cooling to 20°C in PCT that were perfused with a high chloride solution, which suggested that active NaCl transport is electroneutral and transcellular.

The electrical nature of NaCl transport was examined more directly by eliminating the anion gradients that are responsible for passive transport (Fig. 2). Tubules were perfused with a high chloride solution that simulated late proximal tubular fluid and were bathed in a high chloride albumin solution. Elimination of passive transport caused only a 20% reduction in NaCl transport. The PD in the absence of anion gradients was zero, which indicated that active NaCl transport is electroneutral and transcellular. In the third series which examined the electrical nature of NaCl transport, 0.01 mM ouabain was added to the high chloride bathing solution to inhibit active transport (Fig. 3). Inhibition of active transport caused NaCl transport to decrease to a value not significantly different from zero, but did not significantly affect the PD. This provides further evidence that active NaCl transport is electroneutral and transcellular.

The conclusion that active NaCl transport in the PCT is

2. For a J_v of 0.6 nl/mm · min, approximately two-thirds or 0.4 nl/mm · min will be actively transported. If Cl^- transport were secondary to active Na^+ transport and paracellular, then this paracellular Cl^- flux of 58 pmol/mm · min would generate a current of 1.4×10^{-4} A/cm^2. The resistance of the PCT is 15 Ω cm^2 (1). Thus, from Ohm's law, the predicted change in PD upon inhibition of active transport would be +2.1 mV. This calculation is actually an underestimation of the expected change in the PD, for it assumes that the paracellular sodium permeability is zero.

neutral and transcellular requires a mechanism for apical NaCl entry. Three mechanisms have been proposed (22, 23). Na^+ and Cl^- could enter the cell via parallel conductive pathways that are tightly coupled electrically to maintain equal rates of Na^+ and Cl^- entry. A second proposed mechanism is a neutral NaCl symporter; however, studies using apical brush-border membrane vesicles have not found evidence for a NaCl cotransport system (24, 25). The final proposed mechanism is parallel ion exchangers (4, 24); here the Na^+/H^+ antiporter in combination with a Cl^-/OH^- (or HCO_3^-) exchanger would provide net NaCl entry into the cell. Our data do not address which mechanism is present in the PCT.

Nature of basolateral chloride transport. The conclusion that the active component of NaCl transport is electroneutral and that Na^+ and Cl^- transport are transcellular requires a mechanism for intracellular Cl^- to exit across the basolateral membrane. Several mechanisms including a Cl^- conductance (9), a Cl^-/OH^- (or HCO_3^-) antiporter (9, 26), and a neutral KCl symporter (26–28) in the basolateral membrane have been proposed. In several leaky epithelia (5–9), including the PCT (10, 11), the intracellular Cl^- concentration has been found to be at or above electrochemical equilibrium. An intracellular Cl^- concentration above electrochemical equilibrium in combination with a basolateral membrane Cl^- conductance might easily account for Cl^- transport across the basolateral membrane. We investigated the role of basolateral membrane Cl^- conductance in NaCl transport by depolarizing the basolateral membrane with 2 mM bath Ba^{++}. Ba^{++}, a potassium conductance inhibitor (12–14), depolarizes the basolateral membrane of rabbit proximal tubules from ~ -55 mV to -25 mV, and will decrease the electrochemical gradient for anions which exit the cell through a conductive pathway. Serosal Ba^{++} decreases conductive Cl^- secretion in in vitro frog and piglet gastric mucosa (29) and canine tracheal epithelium (30). In addition, bath Ba^{++} has also been shown to decrease $NaHCO_3$ absorption by $\sim$50% in rabbit PCT perfused in vitro, which suggests that the HCO_3^- exit across the basolateral membrane is via a conductive pathway (15). In the present study, depolarization of the basolateral membrane with 2 mM Ba^{++} did not decrease NaCl transport (Fig. 4). It is possible that Ba^{++} has effects other than to depolarize the basolateral membrane and decrease the driving force for anions that exit conductively. However, our results confirm electrophysiologic studies (12) and provide evidence against a significant Cl^- conductance in the PCT.[3]

The presence of a basolateral membrane Cl^- conductance was also examined by using 0.1 mM anthracene-9-CO_2H. Anthracene-9-CO_2H decreases the Cl^- conductance in skeletal muscle in a dose-dependent fashion (18). At the concentration used in this study, >90% of muscle chloride conductance was inhibited. This concentration has also been shown to decrease the basolateral Cl^- conductance in the rabbit thick ascending limb (16) and amphibian diluting segment (17). However, in the present study, the addition of 0.1 mM anthracene-9-CO_2H to the bathing solution did not inhibit NaCl transport (Fig. 5). These data provide further evidence against a significant Cl^- conductance in the basolateral membrane.

The conclusions that Cl^- absorption from a high chloride solution is transcellular and that Cl^- exit across the basolateral membrane is not conductive suggest that Cl^- exit is electroneutral. Two mechanisms of electroneutral Cl^- exit have been proposed: Cl^-/OH^- (or Cl^-/HCO_3^-) exchange (9, 26) and KCl symporter (26–28). These electroneutral mechanisms, however, can not satisfactorily explain Cl^- exit across the basolateral membrane. The problem with Cl^-/OH^- (or Cl^-/HCO_3^-) antiporter is qualitative. This mechanism would transport Cl^- into, rather than out of, the cell. The driving force for electroneutral ion transporters is the chemical concentration gradient across the membrane. In the proximal tubule, the intracellular Cl^- concentration is $\sim$10 mM (10, 11) and the extracellular Cl^- concentration is $\sim$100 mM. For a Cl^-/OH^- antiporter to transport Cl^- across the basolateral membrane out of the cell, the intracellular pH would have to be <6.4, an unlikely value (31). The problem with the KCl symporter is quantitative. If the Na^+-K^+ ATPase pump system exchanges three Na^+ for two K^+ as has been proposed (32), the KCl symporter can account for only two-thirds of the Cl^- exit across the basolateral membrane. One-third must exit by some other mechanism. Alternatively, if the Na^+-K^+ ATPase pump system could operate in an electroneutral mode, then all Cl^- could exit the cell via the KCl symporter.

In summary, we have shown that active NaCl transport in the PCT from a high chloride perfusate is electroneutral and transcellular. Neither depolarizing the basolateral membrane with 2 mM bath Ba^{++} nor the addition of anthracene-9-CO_2H to the bathing solution inhibited NaCl transport. From these data we conclude that there is not a significant role of basolateral membrane Cl^- conductance in NaCl absorption in the rabbit PCT.

Acknowledgments

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References

1. Berry, C. A. 1983. Lack of effect of peritubular protein on passive NaCl transport in the rabbit proximal convoluted tubule. *J. Clin. Invest.* 71:268–281.

2. Berry, C. A., and F. C. Rector, Jr. 1980. Active and passive sodium transport in the proximal tubule. *Miner. Electrolyte Metab.* 4:149–160.

3. Green, R., J. H. V. Bishop, and G. Giebisch. 1979. Ionic re-

3. Previously, we have shown that Ba^{++} specifically inhibited $NaHCO_3$ absorption from an ultrafiltrate-like perfusate by $\sim$45% (15). Of concern in those studies was whether Ba^{++} might be inhibiting $NaHCO_3$ absorption by a more general mechanism, such as inhibition of Na^+-K^+ ATPase. In the present studies, failure of Ba^{++} to inhibit NaCl transport indicates that Ba^{++} does not inhibit the Na^+-K^+ ATPase pump system.

quirements of proximal tubular sodium transport. III. Selective lumenal anion substitution. *Am. J. Physiol.* 236:F268–F277.

4. Lucci, M. S., and D. G. Warnock. 1979. Effects of anion transport inhibitors on NaCl reabsorption in the rat superficial proximal convoluted tubule. *J. Clin. Invest.* 64:570–579.

5. Duffey, M. E., S. M. Thompson, R. A. Frizzell, and S. G. Schultz. 1979. Intracellular chloride activities and active chloride absorption in the intestinal epithelium of the winter flounder. *J. Membr. Biol.* 50:331–341.

6. Duffey, M. E., K. Turnheim, R. A. Frizzell, and S. G. Schultz. 1980. Intracellular chloride activities in rabbit gallbladder: direct evidence for the role of the sodium gradient in energizing "uphill" chloride transport. *J. Membr. Biol.* 42:229–246.

7. Frizzell, R. A., and M. E. Duffy. 1980. Chloride activities in epithelia. *Fed. Proc.* 39:2860–2864.

8. Spring, K. R., and G. Kimura. 1978. Chloride reabsorption by renal proximal tubules. *J. Membr. Biol.* 38:233–254.

9. Yoshitomi, K., and T. Hoshi. 1983. Intracellular Cl^- activity of the proximal tubule of Triturus kidney: dependence on extracellular ionic composition and transmembrance potential. *Am. J. Physiol.* 245:F359–F366.

10. Cassola, A. C., B. Gebler, and E. Frömter. 1981. Measurements of intracellular Cl^- activity in proximal convolution of rat kidney. *Pflügers Arch.* 391:R17.

11. Sohtell, M. 1978. Electrochemical forces for chloride transport in the proximal tubules of the rat kidney. *Acta Physiol. Scand.* 103:363–369.

12. Bello-Reuss, E. 1982. Electrical properties of the basolateral membrane of the straight portion of the rabbit proximal renal tubule. *J. Physiol. (Lond.).* 326:49–63.

13. Biagi, B., T. Kubota, M. Sohtell, and G. Giebisch. 1981. Intracellular potentials in rabbit proximal tubules perfused in vitro. *Am. J. Physiol.* 240:F200–F210.

14. Nagel, W. 1979. Inhibition of potassium conductance by barium in frog skin epithelium. *Biochim. Biophys. Acta.* 552:346–357.

15. Sasaki, S., and C. A. Berry. 1984. Mechanism of bicarbonate exit across the basolateral membrane of the rabbit proximal convoluted tubule. *Am. J. Physiol.* In press.

16. Greger, R. 1984. The Na^+/K^+2Cl^- system in the diluting segment of rabbit kidney. *Fed. Proc.* In press.

17. Oberleithner, H., M. Ritter, F. Lang, and W. Guggino. 1983. Anthracene-9-carboxylic acid inhibits renal chloride reabsorption. *Pflügers Arch.* 398:172–174.

18. Palade, P. T., and R. L. Barchi. 1977. On the inhibition of muscle membrane chloride conductance by aromatic carboxylic acids. *J. Gen. Physiol.* 69:879–896.

19. Berry, C. A., and M. G. Cogan. 1981. Influence of peritubular protein on solute absorption in the rabbit proximal tubule. *A specific effect on NaCl transport. J. Clin. Invest.* 68:506–516.

20. Burg, M. B., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* 210:1293–1298.

21. Jacobson, H. R. 1979. Characteristics of volume reabsorption in rabbit superficial and juxtamedullary proximal convoluted tubules. *J. Clin. Invest.* 63:410–418.

22. Rector, F. C., Jr. 1983. Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am. J. Physiol.* 244:F461–F471.

23. Warnock, D. G., and J. Eveloff. 1982. NaCl entry mechanisms in the luminal membrane of the renal tubule. *Am. J. Physiol.* 242:F561–F574.

24. Warnock, D. G., and V. J. Yee. 1981. Chloride uptake by brush border membrane vesicles: coupling to proton gradients and K^+ diffusion potentials. *J. Clin. Invest.* 67:103–115.

25. Seifter, J., R. Knickelbein, and P. S. Aronson. 1983. Cl transport in rabbit renal microvillus membrane vesicles: evidence against Cl-OH exchange. *Kidney Int.* 23:266*A*. (Abstr.)

26. Reuss, L., and S. A. Weinman. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membr. Biol.* 49:345–362.

27. Reuss, L., S. A. Weinman, and T. P. Grady. 1980. Intracellular K^+ activity and its relation to basolateral membrane ion transport in Necturus gallbladder epithelium. *J. Gen. Physiol.* 76:33–52.

28. Shindo, T., and K. R. Spring. 1981. Chloride movement across the basolateral membrane of proximal tubule cells. *J. Membr. Biol.* 58:35–42.

29. McLennan, W. L., T. E. Machen, and T. Zeuthen. 1980. Ba^{++} inhibition of electrogenic Cl^- secretion in vitro frog and piglet gastric mucosa. *Am. J. Physiol.* 239:G151–G160.

30. Welsh, M. J. 1983. Barium inhibition of basolateral potassium conductance in tracheal epithelium. *Am. J. Physiol.* 244:F639–F645.

31. Sasaki, S., Y. Iino, T. Shiigai, and J. Takeuchi. 1984. Intracellular pH of isolated perfused rabbit proximal tubule: effects of luminal Na^+ and Cl^-. *Kidney Int.* 25:282*A*. (Abstr.)

32. Goldin, S. M. 1977. Active transport of sodium and potassium ions by the sodium potassium ion-activated adenosine triphosphatase from renal medulla. *J. Biol. Chem.* 252:5630–5642.