

Proximal Tubule Potential Difference

DEPENDENCE ON GLUCOSE, HCO₃, AND AMINO ACIDS

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ABSTRACT The effect of various intraluminal substrates on the magnitude of the transepithelial potential difference (PD) across the proximal convoluted tubule (PCT) of the mammalian kidney was investigated in two ways. First, the transepithelial PD was measured before and after the removal of glucose, bicarbonate, and alanine from the lumen. Second, the effects of specific transport inhibitors—ouabain, phloridzin, and acetazolamide—was ascertained when placed either on luminal or blood side.

Isolated segments of rabbit PCT were perfused in vitro. Tubules perfused with isosmolar ultrafiltrate (UF) at rates > 10 nl/min had a mean PD of -5.8 ± 0.2 mV (lumen negative). Normal UF was simulated by an artificial perfusion solution. Using the latter, observed PD was -5.4 ± 0.2 mV. A significant reversible decrease in PD was noted when the following constituents were removed singly: glucose (from -5.7 ± 0.4 to -3.5 ± 0.4 mV); alanine (from -5.8 ± 0.4 to -4.7 ± 0.3 mV); and bicarbonate (from -5.3 ± 0.3 to -3.3 ± 0.5 mV). The combined removal of alanine and glucose (replaced with mannitol) reduced the transepithelial PD to -0.5 ± 0.1 mV with removal of glucose and alanine (replaced with mannitol) and decrease of NaHCO₃ to 5.6 meq/liter (replaced with NaCl), as normally occurs in early part of in vivo PCT, resulted in reversible change of PD from -5.1 ± 0.2 to $+3.2 \pm 0.2$ mV. Ouabain (10^{-6} M) reversibly decreased the negative control PD from blood side, but had no effect from luminal side. Phloridzin (10^{-5} M) reversibly decreased PD from -6.4 ± 0.3 to -3.7 ± 0.4 mV when placed on luminal side but had minimal effect from blood side, -6.3 ± 0.4 to -5.8 ± 0.4 mV. Acetazolamide (Diamox) was without effect from either side. Reversal of bulk flow of water by addition of 31 mosmol/liter raffinose to perfusion ultrafiltrate did not significantly decrease the PD.

It is concluded that specific pumps for transport of glucose, amino acids, and bicarbonate exist on the lu-

minal surface. All three constituents are necessary for expression of maximum PD. Removal of these substrates by transport changes PD from -5.1 mV to $+3.2$ mV (lumen positive). This 3.2 mV positive PD is secondary to a chloride diffusion potential and is not effected by ouabain from the blood side.

INTRODUCTION

We recently reported that isolated segments of rabbit proximal convoluted tubules had a mean transmembrane potential difference (PD)¹ of -5.8 ± 0.3 mV (lumen negative) when perfused in vitro with ultrafiltrate of rabbit serum at flow rates greater than 10 nl/min (1). However, when the perfusion rate was decreased below 2 nl/min there was a marked reduction in PD towards zero. Several possibilities for this phenomena were proposed. One such possibility was that the PD was dependent on the presence of certain essential transportable constituents in the luminal fluid (e.g., glucose, bicarbonate, and amino acids), and at low flow rates depletion of these substances by reabsorption resulted in a marked fall in PD.

The present investigations were explicitly designed to investigate the effects of the transport of these specific constituents on the generation of the transmembrane PD by the proximal convoluted tubule. In these investigations it was first necessary to develop an artificial perfusion solution which gave approximately the same PD as that obtained under similar conditions by isosmolar ultrafiltrate of rabbit serum. Once this was achieved, then a single constituent could be removed selectively in order to determine its specific effect on the observed transmembrane PD. The removal of glucose, amino acids, and bicarbonate were all associated with a significant decrease in the transmembrane PD. When the tubule was perfused with a solution free of all three of these con-

¹ Abbreviations used in this paper: PCT, proximal convoluted tubule; PD, potential difference.

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stituents, then the orientation of the PD was reversed with the lumen averaging $+3.2 \pm 0.2$ mV. The effect of reducing the transport rate of these constituents was also examined by studying the effects of appropriate inhibitors (phloridzin, ouabain, and acetazolamide) when added separately to the luminal fluid or to the bathing media. On the basis of the results obtained, a schematic model of those factors modulating transmembrane PD across the proximal convoluted tubule (PCT) is proposed.

METHODS

Isolated segments of PCT obtained from female New Zealand rabbits were perfused by the exact same techniques previously described (1). The only modification used in these studies was the development of a technique by which the intraluminal perfusion fluid could be changed during the experiment. This involved sealing a standard plastic intravenous (i.v.) three-way stop cock to the lucite piece holding the perfusion pipet. The stop cock could then be turned in such a way as to allow the passage of PE 10 tubing down to the tip of the pipet filled with the perfusion fluid. The pipet is then emptied by suction, flushed, and refilled through the same PE tubing with the desired new perfusion fluid. The entire process by which perfusion fluids are exchanged generally takes less than 2 min. The perfusion rate was controlled by varying the height of the perfusion chamber connected to the other outlet of the stop cock via polyethylene tubing. In all instances the tubules were initially perfused with control isosmolar ultrafiltrate of same rabbit serum as used in the bath. After the PD had stabilized, then the desired exchanges were performed. In all cases the bath was regular commercially available rabbit serum and kept at 37°C and at pH of 7.4 by continuous bubbling with 95% O_2 and 5% CO_2 .

In all of these studies the same electrical circuit was used. Equivalent bridges of 300 mosmol/liter Ringer's in 4% agar (PE tubing size 240) were connected to the end of the perfusion pipet 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 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 the experiments (2-6 h). This circuit is completely symmetrical when perfusion fluid has the same electrolyte concentration as the bath. However, when the NaHCO_3 in the perfusion fluid was replaced by NaCl , then a liquid junction potential must be calculated and added in the appropriate polarity to the observed PD. The circuit diagram can be represented by the scheme below.

The measured PD is equal to sum of all the separate PD's, however, $E_1 = -E_6$ and $E_2 = -E_5$, thus $E_{\text{measured}} =$

$E_3 + E_T + E_4$. Since rabbit serum and 300 mosmol/liter Ringers have nearly same electrolyte concentrations, E_4 is essentially zero, as is E_3 when ultrafiltrate is used as the perfusion fluid, then the observed PD is equal to the PD generated by the tubule. In those experiments when tubules were perfused with low bicarbonate (5.6 meq/liter), high chloride (143.6 meq/liter), see Table I, the liquid junction potential, E_j , was calculated by a general junction potential equation based on Nernst-Planck equation as derived by Barry and Diamond (2), and is equal to:

$$E_j = -\frac{RT}{F} \ln \frac{\mu_1(a_1'' - a_1') + \mu_2(a_2'' - a_2') - \mu_3(a_3'' - a_3')}{\mu_1(a_1'' - a_1') + \mu_2(a_2'' - a_2') + \mu_3(a_3'' - a_3')} \times \ln \frac{\mu_1 a_1'' + \mu_2 a_2'' + \mu_3 a_3''}{\mu_1 a_1' + \mu_2 a_2' + \mu_3 a_3'}$$

where subscripts 1, 2, and 3 refer, respectively to Cl, HCO_3 , and Na; a is the activity of each constituent, and μ refers to mobility of each respective ion and equals 76, 44, and 50 (3) while '' and ' refer respectively to luminal and bath fluids. Substituting these values, the calculated liquid junction potential equals:

$$E = -\frac{RT}{F} \ln \frac{(76 \cdot 143.6) + (44 \cdot 5.6) + (50 \cdot 149.6)}{(76 \cdot 110.4) + (44 \cdot 24.2) + (50 \cdot 147.9)} = -2.7 \text{ mV.}$$

It is to be noted that the method by which the magnitude of the liquid junction potential correction was calculated is only a first order approximation and should not be considered exact. There are a number of sources of error, however, these are considered minimal. First, the estimation of exact liquid junction PD correction requires that the two dissimilar solutions form a static junction in which the two solutions form sharp boundaries. The only way that this can be accomplished is to allow the two solutions flow next to each other to form free-flowing junctions. This, however, is technically impossible with the methods by which single nephrons are perfused in vitro. Therefore, a 4% agar salt bridge was used. Use of agar can be criticized since it is not free of charges and the mobilities of each ion can be influenced by the agar. In a separate series of studies² equivalent bridges of 300 mosmol/liter Ringers in Agarose were used. Agarose has the advantage that it is essentially charge free. The observed PD using solution B as perfusate was not statistically different ($n=6$) from those in which agar was used for the bridge. From these

² Imai, M., and J. P. Kokko. Unpublished observation.

HgCl ₂ -Hg	: Sat	: 4% Agar	: Perfusion	: Tubule	: Rabbit	: 4% Agar	: Sat	: HgCl ₂ -Hg
	KCl	300 mosmol/liter	Fluid		Serum	300 mosmol/liter	KCl	
		Ringers				Ringers		
E_1	E_2		E_3	E_T	E_4		E_5	E_6

TABLE I
Composition of Artificial Perfusion Solutions Used*

	A	B	C	D	E	F
	<i>mmol/liter</i>					
NaCl	105	135	105	105	105	105
KCl	5	5	5	5	5	5
NaHCO ₃	25	5.6	25	25	25	5.4
NaCH ₃ SO ₄	—	—	—	—	—	20
CaCl ₂	1.8	1.8	1.8	1.8	1.8	1.8
MgSO ₄	1	1	1	1	1	1
Na ₂ HPO ₄	4	2	4	4	4	4
Na acetate	10	5	10	10	10	10
Glucose	8.3	—	—	8.3	—	—
Alanine	5	—	5	—	—	—
Mannitol	—	12	8	5	12	12
Osmolality	298	296	297	297	299	303

* Gravimetrically measured, $\pm 1\%$. Osmolality measured by standard freezing point depression techniques.

experiments it is concluded that use of agar vs. agarose does not introduce significant sources of error in estimation of the magnitude of liquid junction potential. To be thermodynamically correct, activities should be used in calculation of E_j and not concentrations as in this text. However, the activity coefficients for HCO₃⁻ are not available, and for this reason liquid junction potential was calculated using concentrations. This potential source of error also can be considered minimal since all the measurements were done with dilute solutions, approximately 300 mosmol/liter, and in addition, activity coefficients of all univalent ions are approximately the same (3). A third source of minimal inaccuracy in calculation of the liquid junction PD arises from use of equivalent conductances at 25°C instead of free mobilities at 37°C. This approximation is necessitated by the fact that free mobility of HCO₃⁻ at 37°C is not known. Thus, though our liquid junction potential correc-

tions are not exact (it is estimated that true E_j may be as much as $\pm 20\%$ of the E_j calculated in this manuscript), they are thought to represent adequate and necessary corrections which were applied in the appropriate circumstances as will be discussed in the result section.

Table I summarizes the concentrations of constituents in the various perfusion fluids used. The bicarbonate-free ultrafiltrate and its control were made up by titrating regular serum to pH of 6.1 with HCl and bubbling 95% O₂/5% room air through this solution for 48 h. The control perfusate was made up by addition of appropriate amounts of 300 mosmol NaHCO₃ solution to give a final NaHCO₃ concentration of 25.6 meq/liter. The bicarbonate-free perfusate was made up identically except 300 mosmol/liter NaCl solution was added in equal amounts to the bicarbonate-free ultrafiltrate in place of the NaHCO₃ solution. These solutions thus were identical except for the concentrations of HCO₃ and Cl.

Ouabain (10⁻⁵ M), phloridzin (10⁻⁵ M), or acetazolamide (10⁻⁴ M) were added either to the perfusion fluid or to the bath in those experiments in which the effect of various inhibitors of transmembrane PD were studied.

RESULTS

At perfusion rates greater than 10 nl/min the control transmembrane PD across the PCT in this study was -5.8 ± 0.3 ($n = 18$) (lumen negative) when isosmolal ultrafiltrate was used as the perfusion solution. The -5.8 ± 0.3 mV is in good agreement with our previously published result of -5.8 ± 0.2 mV (1). In experiments in which the tubules were perfused in random order with the control artificial solution and isosmolal ultrafiltrate, the mean observed PD was -5.4 ± 0.2 mV with the former as compared to -6.1 ± 0.3 mV obtained using isosmolal ultrafiltrate as perfusion solution, Table II.

TABLE II
Transmembrane Potential Differences Using Various Perfusion Solutions

Control perfusion solution	Experimental perfusion solution	Control PD	Experimental PD (n)
Ultrafiltrate	HCO ₃ free ultrafiltrate	-5.3 ± 0.3	-3.3 ± 0.5 (6)
Ultrafiltrate	Ultrafiltrate 31 mosmol L ⁻¹ raffinose	-5.7 ± 0.3	-5.5 ± 0.3 (6)
Ultrafiltrate	Control artificial solution (A)	-6.1 ± 0.2	-5.4 ± 0.2 (12)
Artificial solution (A)	Artificial solution less glucose (C)	-5.7 ± 0.2	-3.5 ± 0.4 (6)
Artificial solution (A)	Artificial solution less alanine (D)	-5.8 ± 0.2	-4.7 ± 0.4 (6)
Artificial solution (A)	Artificial solution less alanine and glucose, HCO ₃ = 5.6 meq/liter (B)	-5.1 ± 0.3	$+3.2 \pm 0.2$ (6)
Artificial solution (A)	Artificial solution less alanine and glucose, HCO ₃ = 26 meq/liter (E)	-4.9 ± 0.3	-0.5 ± 0.1 (6)
Artificial solution (A)	Artificial solution less alanine and glucose; HCO ₃ = 5.4 meq/liter; CH ₃ SO ₄ = 20 meq/liter (F)	-4.9 ± 0.3	-1.1 ± 0.2 (6)*

* The transtubular PD of -1.1 ± 0.2 mV is the observed PD without liquid junction correction. The purpose of these experiments was to see if there was an effect of HCO₃ per se on the PD when the Cl concentrations on the two sides of the membrane were kept unchanged. Therefore, these experiments were conducted after perfusing with solution (E) with an observed PD of -0.5 ± 0.1 mV. It is impossible to make an accurate calculation of the difference between the true PD between these two perfusates since the relative mobility of CH₃SO₄⁻ to HCO₃⁻ is not known. It is assumed in the text that the mobilities of these two ions do not differ greatly, and therefore, the liquid junction correction between these two solutions would be small.

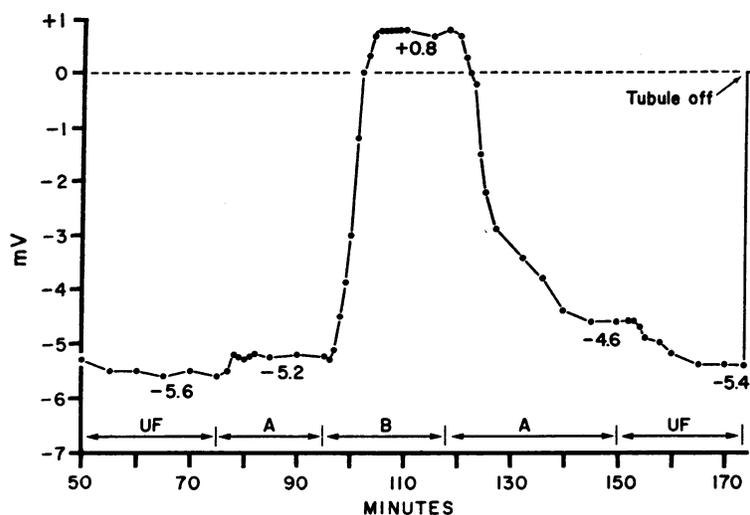


FIGURE 1 Transmembrane potential difference in an isolated proximal convoluted tubule perfused with various solutions: UF, ultrafiltrate; A, control artificial solution; B, artificial solution less glucose, alanine with $\text{HCO}_3^- = 5.6$ meq/liter, see Table I.

This represents a 13% decrease in the transmembrane PD and is statistically significant. The reason for this difference is not apparent, but probably represents the lack of some necessary constituent in our artificial solution. If glucose is taken out of this control solution, then there is a reversible and a significant ($P < 0.001$) drop in the potential from -5.7 ± 0.2 mV to -3.5 ± 0.4 . A selective removal of the alanine decreases this control PD of -5.8 ± 0.2 mV to -4.8 ± 0.4 mV which also is reversible and statistically significant ($P < 0.001$, by paired t). In those experiments in which the HCO_3^- was removed from the ultrafiltrate and replaced with chloride, there was a reversible decrease of PD from -5.3 ± 0.3 to -3.3 ± 0.5 (including the imposed liquid junction PD of 1.2 mV). If both glucose and alanine are removed from the perfusate and replaced by isosmolar quantities of mannitol there is a decrease in PD from -4.9 ± 0.3 to -0.5 ± 0.1 . If glucose and alanine are removed from the artificial solution and replaced by isoosmotic amounts of mannitol and bicarbonate reduced to 5 meq/liter by chloride substitution, then the PD decreases reversibly and changes polarity from control of -5.1 ± 0.3 mV to $+3.2 \pm 0.2$ mV (Fig. 1); the $+3.2$ mV is the observed PD of $+0.5 \pm 0.2$ mV corrected for the -2.7 mV liquid junction PD. On the other hand, when bicarbonate of solution E (Tables I and II) was replaced by methyl sulfate, keeping chloride concentration the same on both sides of the membrane (solution F), the PD changed minimally from -0.5 ± 0.1 mV to -1.1 ± 0.2 mV and did not become positive (Table II). The addition of 31 mosmol/liter raffinose to ultrafiltrate had a minimal effect on PD decreasing it by 0.2 mV when this solution was used as the perfusion solution (Table II).

When 10^{-5} ouabain was added to the bath there was a reversible decrease of PD from -5.5 ± 0.3 to -1.0 ± 0.1 mV. Similar concentrations of ouabain added to the luminal side had no effect on PD. When tubules were perfused with solution B, the $+3.2$ mV PD was not influenced by the addition of ouabain to the bath. If 10^{-5} M phloridzin is added to the bath, there was only a small decrease in PD of 0.5 ± 0.1 mV, from 6.3 ± 0.4 to 5.8 ± 0.4 , however, when same concentration of phloridzin was added to the perfusion fluid, there was a decrease of PD from -6.4 ± 0.3 to -3.7 ± 0.4 mV. This change was immediately reversible when phloridzin was removed from the perfusion fluid. 10^{-4} M acetazolamide had no effect when added either to the luminal or to the blood side.

DISCUSSION

Considerable difference of opinion exists concerning the magnitude and polarity of the transmembrane PD across the mammalian kidney. Initially, many investigators found the transmembrane PD to be about -20 mV (lumen negative). In 1966 Fromter and Hegel (4) reported that they were unable to find any measurable PD across the rat PCT, and attributed the previous values to various technical artifacts. More recently, Boulpaep and Sealy (5) found transmembrane PD of -2.0 mV across the PCT of the autoperfused dog kidney.

In studies in which isolated segments of rabbit PCT were perfused in vitro with isosmolar ultrafiltrate of same rabbit serum as used in the bath, Burg and Orloff (6) found the PD of PCT to be -3.8 mV (lumen negative), while Kokko and Rector (1) reported that mean PD of -5.8 ± 0.3 mV existed across the PCT. In the latter

studies (1) it was further noted that the PD was dependent on the rate of tubular perfusion. These studies suggested that depletion of some intraluminal constituents as a result of transport might influence the magnitude of the observed PD. The present studies were designed to examine this possibility.

These studies show that the transmembrane PD across the PCT is generated by ouabain-sensitive transport processes, and clearly indicate that the magnitude of this PD is dependent on intraluminal glucose, amino acids, and bicarbonate. When any one of these three substrates is removed singly from the perfusate there is a reduction in the transepithelial PD of 1 to 3 mV. Inhibition of glucose transport with phloridzin reversibly reduced the PD to a comparable level as glucose removal. Removal of glucose and amino acids together reduced the PD virtually to zero (-0.5 to ± 0.1 mV) while the additional removal of bicarbonate with chloride substitution converts the PD to $+3.2$ mV. When the bicarbonate was removed by methyl sulfate substitution in order to avoid transtubular chloride concentration gradient the PD, in the absence of glucose and amino acids, did not become positive. These results indicate that only glucose and alanine participate in the generation of negative PD and that there is no specific effect of bicarbonate. The fact that positive PD is generated when bicarbonate is substituted by chloride, and not by substitution with methyl sulfate, would suggest that the positive PD is a chloride diffusion potential. In support of this latter view is the fact that ouabain had no effect on the positive PD.

If these *in vitro* results are representative of *in vivo* conditions, then they would suggest that a potential gradient profile exists down the length of the proximal tubule as shown in Fig. 2. Near the glomerulus, where bicarbonate, glucose, and amino acids are in same con-

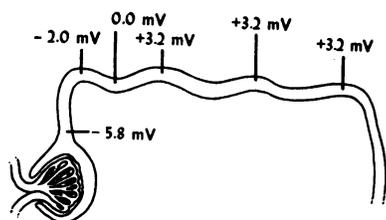


FIGURE 2 The proposed potential gradient existing in the renal proximal tubule. It is important to note that the depicted PD gradients are the consequence of varying intraluminal constituent concentrations as the fluid courses from the glomerulus to more distal segments of the proximal tubule. The magnitude of the positive PD is principally a function of diffusion potential secondary to the generated high intraluminal chloride concentrations, see text. There are no direct *in vivo* measurements of rabbit proximal tubule chloride concentrations; however, in the rat the measured intraluminal proximal tubule chloride concentrations have been just over 140 meq/liter (15, 16) which are similar to the concentrations used in the current studies utilizing solution B as the perfusate.

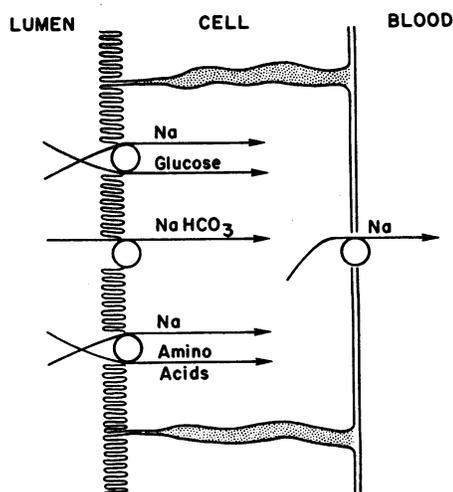


FIGURE 3 Schematic of constituents modulating transmembrane potential difference across PCT.

centrations as in circulating plasma water, the transtubular PD would be maximally negative (lumen negative relative to blood). As the fluid courses down the tubule all of these intraluminal constituents are decreased in concentration by transport processes, and accordingly, the transmembrane PD would decrease proportionately in magnitude. Since most of the amino acid, glucose, and bicarbonate reabsorption takes place in the early part of the proximal tubule (7-11), associated with a rise in intraluminal chloride concentration, it is suggested that the transmembrane PD down the remainder of the tubule is oriented in such a fashion that the lumen is positive to the blood side. Indeed, Fromter (personal communication) has recently found that the lumen of free flow proximal tubule of rat is 1.5 to 2.0 mV positive with respect to the blood side. This positive PD, which appears to be a chloride diffusion potential, would facilitate cation reabsorption.

There are two basic mechanisms by which organic solute transport can be coupled to transmembrane PD (12). In the first, the osmotic gradient theory, active transport of impermeant solute generates water flow and sieving of NaCl. As a consequence local concentration gradients of NaCl are developed with the concentration higher at the luminal surface. In order for these local concentration gradients to generate a negative PD would require that the diffusion permeability for Na^+ be greater than that for Cl^- . In fact, we have previously shown that the permeability coefficient of Na^{23} is greater than Cl^{35} in the isolated tubule (13). To test the osmotic gradient model we reversed the direction of net fluid movement by addition of 31 mosmol/liter raffinose to the perfusate. By this maneuver, local NaCl concentrations would be higher on the periluminal surface and it might be expected that the transepithelial PD would reverse in po-

larity if the osmotic gradient theory is correct. However, no significant change in PD was noted when raffinose was added to the perfusate. It should be pointed out that this constitutes evidence against the osmotic gradient theory only if raffinose pulls water through the same channels as would be associated with net efflux of fluid secondary to active transport of glucose, alanine, and bicarbonate. That these results do not exclude the osmotic gradient model with certainty is based on the possibility that different channels of water flow are utilized when an osmotic gradient is imposed on the tubule as compared to the normal conditions when the tubule transport fluid secondary to generated local osmotic gradients (14).

A second mechanism by which transport of organic solute can influence PD is the coupled transport of sodium and organic solute (Fig. 3). In this model glucose, amino acids, and bicarbonate are in some way coupled to sodium movement across the luminal membrane of the cell. According to this model the presence of glucose, amino acids, and bicarbonate in the luminal fluid would facilitate the entrance of Na into the cell where it can have access to a "potential generating pump" in the peritubular membrane. A decrease in transport of any of these, whether by selective removal of these from the lumen or by addition of specific metabolic inhibitors, would decrease net transport of the respective intraluminal constituent, and accordingly, would decrease the net entry of Na⁺ into the cell.

The observation that removal of glucose or alanine from the perfusate decreased the transepithelial PD would support the model in which Na gains access to the electrogenic pump as a consequence of organic solute transport. Against this model, however, are the findings that the transmembrane PD is close to zero when bicarbonate transport continues in absence of alanine and glucose, Table II. However, this latter finding would not negate the coupled Na/organic solute transport model if it is theorized that the transported sodium gains access to a different intracellular pool when it is associated with bicarbonate transport (via H⁺ secretion) as contrasted to transport of Na associated with glucose and amino acid transport. Currently our data do not permit us to answer with certainty as to which of the two proposed models are correct.

In the model depicted in Fig. 3 the active glucose transport step is placed on the luminal surface since it was shown that phloridzin rapidly and reversibly decreases the PD when applied on the luminal side, but had minimal effects when added to the bathing media. These results are consistent with observations of Tune and Burg (8) demonstrating that glucose concentration of proximal tubule cells was higher than the ambient surroundings when the tubules were perfused; however, glucose concentration gradients were not demonstrated

in nonperfused tubules. The sodium-coupled amino acid transport is also placed on the luminal surface since it was observed that selective removal of alanine from the perfusion fluid reversibly decreased the observed transmembrane PD.

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