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THE EFFECT OF D-GLUCOSE ON THE ELECTRICAL POTENTIAL PROFILE ACROSS THE PROXIMAL TUBULE OF NEWT KIDNEY

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SUMMARY

1. The effects of intraluminal microinjection of solutions containing actively transported sugar on the electrical potential profile across the proximal tubule of newt kidney were studied.

2. In the absence of sugar in both intraluminal and peritubular fluids, the transtubular potential (E_{tt}) and the transmembrane potential across the basal (peritubular) membrane (E_{bm}) were -4.2 ± 0.1 mV and -73.6 ± 2.4 mV, with respect to the peritubular fluid, respectively.

3. Injection of glucose-containing Ringer's solution caused both a marked depolarization of the basal membrane and an increase in luminal negativity. D-Mannitol at comparable concentrations had no such effects.

4. The size of the glucose-evoked potential changes, ΔE_{bm} and ΔE_{tt}, were dependent on both glucose and Na^+ concentrations in the perfusion fluid. A Michaelis–Menten-type relation was found between the magnitude of the potential changes and the glucose concentration. The maximum values for ΔE_{bm} and ΔE_{tt}, estimated at normal Na^+ concentration, were 12.4 ± 0.5 mV and 1.48 ± 0.13 mV, respectively, ΔE_{bm} being about 8-fold greater than ΔE_{tt}.

5. The results indicate that active transport of sugar markedly alters the potential profile across the proximal tubule, and that the primary event induced by sugar transport may be the depolarization of the luminal membrane. The depolarization of the basal membrane can be interpreted as a secondary phenomenon coupled with the luminal depolarization owing to the existence of an extracellular electrical shunt.

INTRODUCTION

The transmural potential difference of the small intestine is known to increase immediately after the addition of an actively transported sugar into the solution bathing the mucosal surface[1-4]. Although the exact nature of genesis of this potential increment is not fully understood, former studies have revealed that sugar–sodium interaction is well reflected in this potential change, *e.g.* the size of the potential in-

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crement appears to be closely related to sugar influx and possibly to sugar-coupled Na^+ influx across the brush border membrane[2-5]. Na^+ dependence of active sugar transport has also been demonstrated in the kidneys of the frog[6], *Necturus*[7], and rat[8,9]. However, it is not known whether similar sugar-evoked potential changes can be recorded from the renal tubule or not.

The aim of the present study is to try to record the sugar-dependent potential changes from the proximal tubule of the kidney, and should such recording be possible, then to examine their basic properties. For this purpose, various test solutions containing either actively transported or non-transported sugars were injected into single proximal tubules of newt kidney, and changes in electrical potential profile across the tubular wall were investigated with microelectrodes. Special attention was directed to the transmembrane potential across the basal (peritubular) border of the cell, since, in the small intestine, contradictory views have been postulated with regard to the potential change across the serosal (basal) membrane. Gilles-Baillien and Schoffeniels[10], Wright[11], and more recently, Lyon and Sheerin[12] have postulated that this membrane hyperpolarizes in response to the addition of an actively transported sugar or amino acid to the mucosal side, whereas Rose and Schultz[13] and White and Armstrong[14] have presented data showing occurrence of the opposite response (depolarization) at this membrane. As direct access to the basal membrane is much easier in the kidney than in the small intestine, some crucial information for this problem was expected to be obtained from this study.

METHODS

Preparation of animals

Male Japanese newts (*Triturus pyrrhogaster*) were used in all experiments. The animals were kept in a cold room (4–6 °C) and fasted for more than 1 month before use. After destruction of the spinal cord with a thin metal rod, the abdominal wall of the animal was opened wide by the use of an electrosurgical knife. Almost the entire left kidney was exposed by removing the colon and the urinary bladder. The whole body of animal thus prepared was fixed on a cork board attached to the bottom of a small lucite box. The box was then filled with the Ringer's solution until the animal body had been just totally immersed in the solution. The Ringer's solution in the box was continuously bubbled with pure oxygen through a gas-lift system and its temperature was kept constant at 20 ± 1 °C. Experiments were carried out throughout all seasons except summer.

Microinjection of solutions

Early parts of the proximal tubules of the so-called pelvic[15] (or definitive[16]) kidney were selected for puncture sites. Microinjection was carried out in the following sequence. First, mineral oil saturated with Sudan black II was injected through a single-barreled glass pipette into one of the Bowman's capsules under a stereomicroscope (Fig. 1). The outer diameter of the sharpened tip of the pipette was about 20 μm. This injection was made for the purpose of blocking the flow of the glomerular filtrate into the lumen of the tubular segment under investigation. Thereafter, the proximal tubule was punctured by a double- or triple-barreled glass pipette which contained different solutions. The outer diameter of the latter was less than 20 μm.

In most experiments, the basic Ringer's solution, containing D-mannitol at various concentrations, was first infused through one of the barrels, and after a while, the perfusion fluid was suddenly switched to a test solution containing D-glucose at the same concentration as that of D-mannitol in the control solution. The transtubular

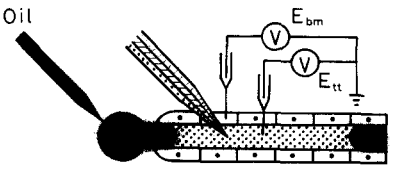


Fig. 1. A schematic presentation of methods for applying various perfusion fluids and recording electrical potential differences. First, colored mineral oil was injected into a Bowman's capsule through a single-barreled micropipette. Then, by using a double-barreled or triple-barreled micropipette, the basic Ringer's solution or one of test solutions was injected with a constant pressure into the oil column to split it. During the perfusion, both the transmembrane potential across the basal membrane (E_{bm}) and the transtubular potential (E_{tt}) were recorded with a microelectrode.

potential (E_{tt}) or the transmembrane potential across the basal membrane (E_{bm}) was recorded continuously during such perfusion with different solutions. The perfusion rate ranged from 10 to 50 nl/min. The basic Ringer's solution used in the present study had the following composition; 105 mM NaCl; 2.65 mM KCl; 1.8 mM $CaCl_2$; 2.25 mM Na_2HPO_4; 0.75 mM NaH_2PO_4; pH 7.2. The osmolality of the solution was 227.5 mosM per kg water. In experiments where the effect of Na^+ concentration was investigated, NaCl in the perfusion fluid was replaced by mannitol. For complete replacement, sodium phosphate was replaced by potassium phosphate.

Electrical potential recording

Ling–Gerard type glass microelectrodes, filled with 3 M KCl *plus* 10 mM potassium citrate by the method of Tasaki *et al.*[17], were used as the exploring electrode. The electrodes having electrical resistance of 12–18 MΩ and tip potential less than 5 mV were selected for use. An Ag–AgCl plate was immersed in the outside solution and used as the reference electrode. The potential difference thus picked up was recorded with a pen-writing recorder (National Model VP-652A) mediated through a FET-differential preamplifier, of which input resistance and gate current were $5 \cdot 10^{10}$ Ω and $7 \cdot 10^{-11}$ A, respectively. In order to obtain an accurate record of E_{tt}, similar procedures to those described by Hoshi and Sakai[18] were employed.

RESULTS

E_{tt} and E_{bm} during perfusion with the basic solution or the solution containing D-mannitol

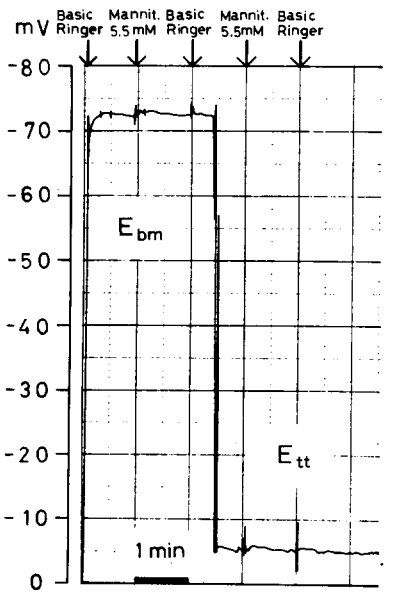
The values for the basal (peritubular) membrane potential (E_{bm}) and the transtubular potential (E_{tt}), estimated during the perfusion with the basic solution, were -73.6 ± 2.4 mV (mean ± S.E., $n = 57$) and -4.2 ± 0.1 mV (mean ± S.E., $n = 23$) with respect to the outside (peritubular) solution, respectively. The value for E_{bm} was slightly greater than that reported by Sakai *et al.*[19] (65 mV) for the proximal tubule of the same animal immersed in the Ringer's solution containing glucose and that reported by Giebisch[20] (65 mV) in the *Necturus* kidney perfused with the Ringer's solution containing glucose.

The effect of D-mannitol was observed by comparing E_{bm} or E_{tt} recorded before and after switching from the basic solution of perfusion fluid to a solution containing D-mannitol at various concentrations, ranging from 0.55 to 11 mM. Fig. 2 shows an example of such experiments where the perfusion fluid was switched to a 5.5 mM mannitol solution. Apparently, no significant change was seen in either E_{bm} or E_{tt} after switching. A very small change in the steady level was sometimes noticed after a small transient irregular change, but fine measurements revealed that the changes in the steady level of E_{bm} were less than 0.5 mV, *i.e.* 0.7 % of the value for E_{bm} above described. The changes in E_{tt} were also very small, being less than 0.2 mV. These small changes were independent of the concentration of D-mannitol. The results of these control experiments indicate that D-mannitol in its concentration range from 0.55 to 11 mM does not cause any specific change in either E_{bm} or E_{tt}.

Effects of perfusion with a solution containing D-*glucose*

In subsequent experiments, the Ringer's solution containing D-mannitol was used as the control solution. D-Mannitol was also added to the outside bathing solution at the same concentration as in the perfusion fluid in order to eliminate any osmotic effect. After measurements of the potentials during perfusion with the control solution, the perfusion fluid was switched to a solution containing D-glucose at the same concentration as that of D-mannitol in the control solution.

Fig. 3 shows an example of the results of such experiments. Immediately after the start of the perfusion with a glucose-containing solution, E_{bm} started to



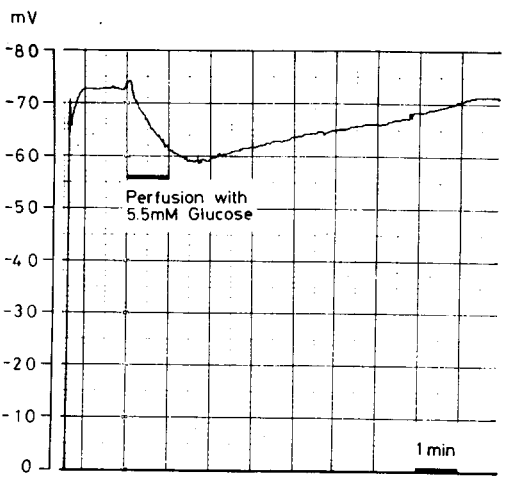


Fig. 2. An example of records of E_{bm} and E_{tt} during successive perfusion with the basic Ringer's solution and the Ringer's solution containing 5.5 mM mannitol. E_{bm} and E_{tt} were recorded successively from the same tubule.

Fig. 3. The change in E_{bm} during the perfusion of glucose-containing Ringer's solution (5.5 mM glucose) and after the cessation of the perfusion. The initial level was obtained by the perfusion of a 5.5 mM mannitol solution. Perfusion with glucose solution lasted for 1 min.

depolarize and it reached a new stable potential level in about 1.5 min. In this particular experiment, the perfusion was continued for 1 min. After stopping the persion, E_{bm} slowly hyperpolarized spontaneously toward the initial level obtained during the perfusion with the control solution.

Fig. 4 shows the changes in both E_{bm} and E_{tt} and also the effect of glucose concentration. At first, 1.375 mM glucose solution was perfused. E_{bm} depolarized immediately by about 7 mV. On the other hand, E_{tt} increased by about 0.2 mV. It should be noticed that the change in E_{tt} was an increase in the luminal negativity. On switching to 5.5 mM glucose solution, a further depolarization occurred in E_{bm} by about 6 mV, and also a further increase in E_{tt} by about 0.5 mV. This clearly shows that the effects of glucose are dependent on its concentration in the perfusion fluid.

Fig. 5 shows the relationship between the magnitude of the depolarization of E_{bm} (ΔE_{bm}) and glucose concentration in the perfusion fluid. Evidently, the response to glucose is saturable in the same fashion as reactions following Michaelis–Menten

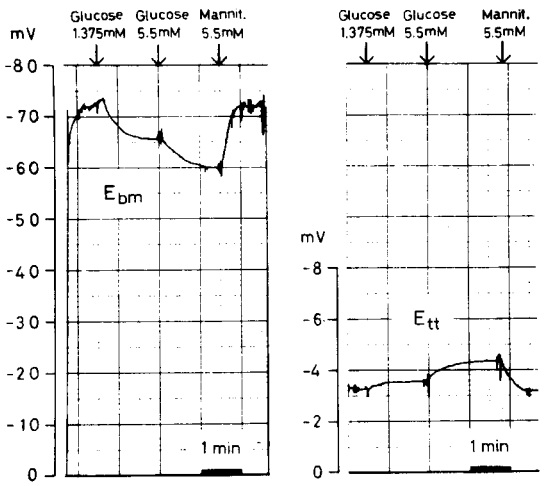


Fig. 4. Changes in E_{bm} and E_{tt} during successive perfusion with 5.5 mM mannitol, 1.375 mM glucose and 5.5 mM glucose solutions. A stepwise depolarization of the basal membrane and a stepwise increase in the luminal negativity were seen when glucose concentration was increased. E_{bm} and E_{tt} were recorded from different tubules.

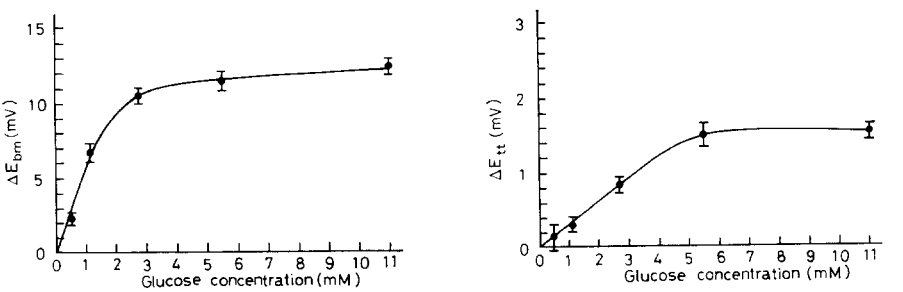


Fig. 5. Relationship between the size of ΔE_{bm} and glucose concentration in the perfusion fluid. Each solid circle with a bar indicates mean $\pm$ S.E. ($n = 7$–19).

Fig. 6. Relationship between the size of ΔE_{tt} and glucose concentration in the perfusion fluid. Each solid circle with a bar indicates mean $\pm$ S.E. ($n = 6$–11).

kinetics. The apparent K_m for glucose calculated from Fig. 5 was 1.15 mM. A similar relationship was also seen in changes in E_{tt} (ΔE_{tt}) (Fig. 6).

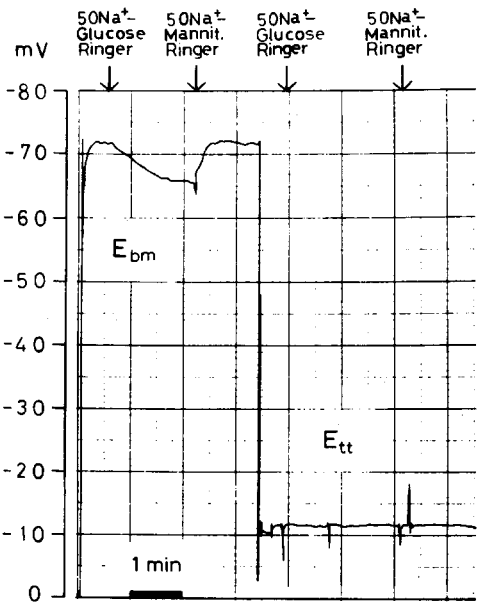
The value for ΔE_{bm}, estimated at 3 mM glucose, where nearly the maximum value was achieved, was 12.4 ± 0.5 mV (mean ± S.E., n = 19), whereas the near maximum value for ΔE_{tt} measured at 5 mM glucose was 1.48 ± 0.13 mV (mean ± S.E., n = 8). Thus, the change in E_{tt} was only about one eighth that of E_{bm}.

Effects of luminal Na^+ concentration

The effects of luminal Na^+ concentration on the glucose effects were observed by reducing the Na^+ concentration by replacing NaCl with mannitol without changing glucose concentration, which was fixed at 5.5 mM. The lower the Na^+ concentration, the smaller the responses to D-glucose which were observed in both ΔE_{bm} and ΔE_{tt}. When Na^+ concentration was lowered to 50 mM, ΔE_{bm} was reduced to about 50% of the control value and ΔE_{tt} was no longer detectable (Figs 7 and 8). The data indicate that both ΔE_{bm} and ΔE_{tt} evoked by D-glucose are also dependent on the luminal Na^+ concentration. In these experiments, no correction was made for conductivity change associated with the reduction in Na^+ concentration.

Effects of phlorizin

The effects of phlorizin, a specific inhibitor of sugar transport, on the responses to glucose were tested in order to know whether glucose-evoked potential changes



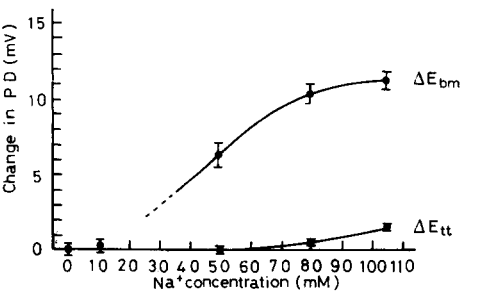


Fig. 7. Changes in E_{bm} and E_{tt} during the perfusion with a low-Na^+ Ringer's solution containing glucose. Na^+ concentration in both the perfusion fluids and outside bathing solution was 50 mM, and glucose concentration in the perfusion fluid was 5.5 mM.

Fig. 8. Effects of Na^+ concentration on the responses of E_{bm} and E_{tt} to injected glucose. D-Glucose concentration in the perfusion fluid was 5.5 mM. Ordinate: Na^+ concentration in the perfusion fluid. Each circle indicates mean ± S.E. (n = 4–19). PD = potential difference.

were directly related to the sugar transport mechanism, *per se*. In most observations, the perfusion was carried out in the following sequence; first, with the control solution (5.5 mM mannitol), second, with the glucose-containing solution (5.5 mM glucose), and finally with the solution containing 5.5 mM glucose *plus* $1.4 \cdot 10^{-4}$ M phlorizin. E_{bm} or E_{tt} was continuously recorded during this successive alteration of perfusion fluid. As shown in Fig. 9, E_{bm}, once having been depolarized by glucose, immediately hyperpolarized after the start of perfusion with the phlorizin-containing solution. Thus, the glucose-evoked change in E_{bm} was largely depressed by phlorizin. After phlorizin, E_{bm} stabilized at a level of -69.7 mV ($n = 3$), which was slightly lower than that attained during perfusion with the control solution. Similarly, glucose-evoked increment of E_{tt} was abolished by phlorizin, in this case the abolition appeared to be complete.

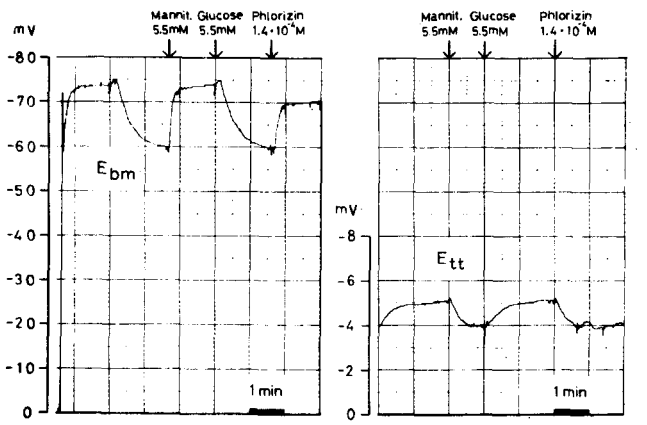


Fig. 9. Effects of phlorizin on glucose-induced changes in E_{bm} and E_{tt}. After the perfusion with 5.5 mM glucose, a solution containing 5.5 mM glucose *plus* $1.4 \cdot 10^{-4}$ M phlorizin was infused. Two records were obtained from different tubules.

DISCUSSION

The results of the present study clearly show that the transtubular potential (the luminal negativity) increases when D-glucose is added to the luminal fluid. This increment can be regarded as a phenomenon closely related to active sugar transport, since D-mannitol, which is not actively transported[21], does not cause a similar potential change. Also, phlorizin, a specific inhibitor of sugar transport, almost completely blocks the generation of the potential change. Moreover, the potential increment was dependent on both sugar and Na^+ concentrations in the luminal fluid, and a Michaelis–Menten-like relation was found between ΔE_{tt} and sugar concentration. These properties observed indicate that the ΔE_{tt} evoked by D-glucose can be regarded as being of the same nature as that of the sugar-evoked potential seen in the small intestines of various animal species (*cf.* refs 1–4). The fact that a similar sugar-evoked potential change can be recorded seems to indicate that the coupling mechanism of sugar with Na^+ in the proximal tubule of the newt kidney is essentially the same as that in the small intestine.

The present study also clearly demonstrated that the basal (peritubular)

membrane depolarized when an actively transported sugar was present in the luminal fluid. Such a depolarization of the basal membrane in response to sugar or amino acid transport was suggested by Rose and Schultz[13] and White and Armstrong[14] from their studies on the changes in the mucosal membrane potential in rabbit and bullfrog small intestines. However, they did not demonstrate this directly, because, in the small intestine, direct exploring of the basal membrane is extremely difficult. In contrast, Gilles-Baillien and Schoffeniels[10], Wright[11] and Lyon and Sheerin[12] concluded that a hyperpolarization of the basal (serosal) membrane might be the origin of the sugar- or amino acid-evoked potential, since they failed to record any potential change across the brush border membrane. Our present results are in accord with the view of the former group of authors and conflict with the interpretation of the latter group.

The depolarization of the basal membrane observed in the present study can not be regarded as the primary event in the genesis of the sugar-evoked potential, since the increment of the luminal negativity cannot be accounted for by this depolarization, unless a some greater depolarization is assumed to occur across the brush border membrane. Therefore, the depolarization of the basal membrane observed in the present study should be interpreted as a secondary phenomenon coupled with a depolarization of the luminal membrane. Such a coupled change may occur if there is an electrical shunt between the luminal and the peritubular fluids. There is good evidence for the existence of vast extracellular shunt pathways for ions within the proximal tubular wall of newt[18] and *Necturus* kidneys[22].

There seems to be accumulating evidence supporting the view that the cotransport of sugar molecules with Na^+ at the luminal membrane is electrogenic. Direct demonstration of the depolarization of this membrane in response to added glucose was made by Rose and Schultz[13] and White and Armstrong[14]. Besides this, Hoshi and Komatsu[23] have demonstrated that phlorizin-induced outflow of sugar from preloaded and metabolically inhibited intestinal cells to the mucosal medium was coupled with Na^+, and this Na^+-coupled sugar outflow was associated with the generation of a distinct potential surge in the direction opposite to that for the sugar entry. Although the electrogenic mechanism at this membrane has not been well understood, it may be probable that the ternary complex (sugar-Na^+–carrier complex) carries a net positive charge or charges and its diffusion within the membrane along its electrochemical potential gradient would produce a charge separation. The fact that the sugar-evoked potential can be recorded even from the intestine incubated in Na^+-free Li^+ media[24] suggests that the electrogenesis is directly coupled with a passive process rather than active ion pump, since Li^+ is not actively transported by the intestinal cells[25,26].

Therefore an equivalent electrical circuit for the tubular wall can be illustrated as shown in Fig. 10. This electrical model is essentially the same as that previously given by Boulpaep[27] and other investigators[14,28] except for an added circuit for the electrogenic cotransport mechanism at the portion corresponding to the luminal membrane. In this circuit, E_1 designates an electromotive force (emf) responsible for the formation of the basic transmembrane potential across the luminal border and R_1 represents the internal resistance of this battery. E_3 and R_3 are the respective parameters for the basal (peritubular) border of tubular cells. R_4 is a transepithelial extracellular shunt resistance. E_2 is the emf generated in association with Na^+-coupled

sugar entry and R_2 is the internal resistance of this battery. In the absence of actively transported sugar in the luminal fluid, E_2 disappears.

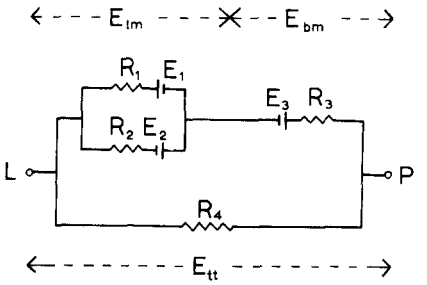


Fig. 10. An equivalent electrical circuit for the proximal tubular wall of newt kidney. L and P represent the luminal and peritubular fluids, respectively. For discussion see text.

The solutions of this circuit for E_{bm}, E_{lm} and E_{tt} are

$$E_{bm} = [E_2R_1R_3 - E_1R_2R_3 - E_3(R_1R_4 + R_2R_4 + R_1R_3)]/R_T(R_1 + R_2) \quad (1)$$

$$E_{lm} = [E_1R_2(R_3 + R_4) - E_2R_1(R_3 + R_4) + E_3R_1R_2]/R_T(R_1 + R_2) \quad (2)$$

$$E_{tt} = [E_1R_2R_4 - E_2R_1R_4 - E_3R_4(R_1 + R_2)]/R_T(R_1 + R_2) \quad (3)$$

where R_T is the total resistance of the closed circuit.

$$R_T = (R_1R_2 + R_1R_3 + R_1R_4 + R_2R_3 + R_2R_4)/(R_1 + R_2) \quad (4$$

The changes in E_{bm}, E_{lm} and E_{tt} due to the generation of E_2 are:

$$\Delta E_{bm} = E_2R_1R_3/R_T(R_1 + R_2) \quad (5)$$

$$\Delta E_{lm} = -E_2R_1(R_3 + R_4)/R_T(R_1 + R_2) \quad (6)$$

$$\Delta E_{tt} = -E_2R_1R_4/R_T(R_1 + R_2) \quad (7)$$

Hence, the relation between ΔE_{bm} and ΔE_{lm} is given by

$$\Delta E_{lm} = -\frac{R_3 + R_4}{R_3} \cdot \Delta E_{bm} \quad (8)$$

This equation means that the generation of E_2 causes concomitant depolarization of both the luminal and basal membranes, and the magnitude of the depolarization of of E_{lm} (ΔE_{lm}) is somewhat greater than ΔE_{bm}, depending on the ratio of $(R_3 + R_4)/R_3$. Similarly, the ratio of $\Delta E_{tt}/\Delta E_{bm}$ is given by

$$\frac{\Delta E_{tt}}{\Delta E_{bm}} = -\frac{R_4}{R_3} \quad (9)$$

The value of $\Delta E_{tt}/\Delta E_{bm}$ found in the present study was about 0.12. This means that the value for R_4 in Fig. 10 is only about one eighth that for R_3. Hoshi and Sakai[18] reported a value of 836 $\Omega \cdot cm^2$ for the specific resistance of the surafce cell membrane of the proximal tubule of the Japanese newt. They also showed that the transtubular

(wall) resistance directly measured was much smaller than that theoretically calculated with this resistance value. Windhager *et al.*[22] also demonstrated that directly measured transepithelial resistance was much smaller than, about one tenth, the value calculated theoretically from data on passive distribution of the electrotonic potential in the wall of the *Necturus* tubules. These studies indicate that the proximal tubule has an extracellular shunt of a considerably low electrical resistance. If we assume that the values of R_1 and R_3 in Fig. 10 are nearly the same and each has a value of 836 $\Omega \cdot cm^2$, R_4 may obtain a value of about 100 $\Omega \cdot cm^2$. Hegel *et al.*[29] also reported a very low value (5–7 $\Omega \cdot cm^2$) for the transepithelial resistance in rat proximal tubule. When we use the value of 100 $\Omega \cdot cm^2$ for the specific resistance of the extracellular electrical shunt, we obtain a value of 13.88 mV for ΔE_{lm}, *i.e.* 12.4·(836 + 100)/836 mV.

On the basis of the present results and above discussion, the changes in potential profile across the renal tubular wall caused by the sugar transport can be illustrated as shown in Fig. 11. The change is essentially the same as that postulated by Rose and Schultz[13] for rabbit small intestine.

Concerning the formation of the transtubular potential, contribution of the electrogenic transport of organic solutes has not been considered so far (see refs 28 and 30), although Boulpaep[31] has suggested a possibility of participation of some active transport mechanism in the formation of the luminal membrane potential. The results of the present study seem to indicate that Na^+-coupled solute transport is a very important factor modifying the potential profile of the proximal tubular cells. Not only sugar transport, but also transport of various amino acids are considered to be significantly contributing to the formation of, not only the transtubular potential, but also the transmembrane potentials across the luminal and basal borders under physiological conditions.

It is generally thought that cell mechanisms of both renal and intestinal sugar transport are essentially the same, as far as the D-glucose–D-galactose system is concerned. In both tissues, uphill movement of the sugars takes place at the brush border membrane[32–34] and the entry mechanisms exhibit similar Na^+ dependence and similar electrogenic properties as discussed above. Na^+ dependence of the binding of

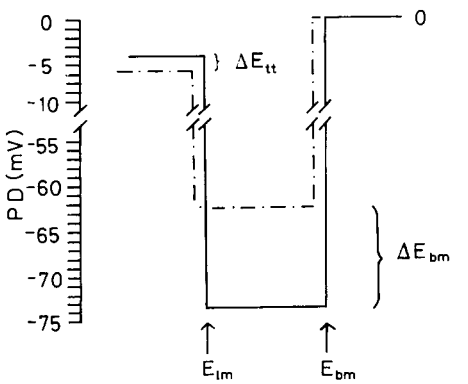


Fig. 11. The electrical potential profile across the proximal tubular wall of newt kidney before (solid line) and after (dashed line) the addition of glucose in the luminal fluid. PD = potential difference.

glucose to the brush border membrane components has also been demonstrated in both rat kidney[35] and hamster small intestine[36]. The similarity of kinetic behavior of the sugar-evoked potential to that of sugar transport has well been recognized in the small intestine[3], and the same seems also to be true in the kidney. Although the exact value for the apparent K_m for glucose transport is not known, to our knowledge, in newt or *Necturus* kidney, the half maximum concentration for glucose in frog kidney has been shown to be about 1.8 mM[37], this value being very similar to the apparent K_m for glucose calculated from the data presented in this paper (1.15 mM).

However, some differences in specificity of sugar transport system have recently been noticed by Kleinzeller[38] between these two tissues. He showed the existence of Na^+-dependent active transport of 2-deoxyhexoses in rat kidney slices, while these sugars are not transported actively in the small intestine[39]. He also showed that fructose was transported through a Na^+-dependent phlorizin-sensitive system in rat renal cortical slices, while this sugar crosses the brush border membrane through a Na^+-independent facilitated diffusion system in rabbit ileum[40]. Further studies seem to be necessary before the nature of these differences becomes more clearly understood.

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