Energetics of the Na\(^+\)-dependent Transport of \(\text{D-Glucose}\) in Renal Brush Border Membrane Vesicles

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The energetics of the Na\(^+\)-dependent transport of D-glucose into osmotically active membrane vesicles, derived from the brush borders of the rabbit renal proximal tubule, was studied by determining how alterations in the electrochemical potential of the membrane induced by anions, ionophores, and a proton conductor affect the uptake of the sugar.

The imposition of a large NaCl gradient (medium > vesicle) resulted in the transient uptake of D-glucose into brush border membranes against its concentration gradient. In the presence of Na\(^+\) salts of isethionate or sulfate, both relatively impermeable anions, there was no accumulation of D-glucose above the equilibrium value. With Na\(^+\) salts of two highly permeable lipophilic anions, NO\(^–\) and SCN\(^–\), the transient overshoot was enhanced relative to that with Cl\(^–\). With Na\(^+\) salts whose mode of membrane translocation is electroneutral, i.e. acetate, bicarbonate, and phosphate, no overshoot was found. These findings suggest that only anions which penetrate the brush border membrane and generate an electrochemical potential, negative on the inside, permit the uphill Na\(^+\)-dependent transport of D-glucose.

Valinomycin, which mediates electrogenic K\(^+\) movements, enhanced the Na\(^+\)-dependent accumulation of D-glucose, provided a K\(^+\) gradient (vesicle > medium) was present. In contrast, nigericin, which mediates an electroneutral exchange of Na\(^+\) for K\(^+\) did not. Na\(^+\)-dependent D-glucose uptake was diminished by ionophores that allow Na\(^+\) to pass through the membrane via another channel, either electrogenically, e.g. gramicidin, or electroneutrally, e.g. nigericin. The electrogenic proton conductor, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, enhanced D-glucose uptake in the presence of a proton gradient (vesicle > medium). These findings demonstrate that changes in the electrochemical potential across the vesicular membrane, i.e. making the interior negative, stimulates the Na\(^+\)-dependent transport of D-glucose.

These results indicate that the Na\(^+\)-dependent transport of D-glucose into renal brush border membrane vesicles is an electrogenic process. It is suggested that in the intact kidney the asymmetric distribution of Na\(^+\) across the proximal tubular cell and the electrochemical potential across the luminal membrane provide the energy required to transport D-glucose against its concentration gradient.

The stimulation by Na\(^+\) of the transport of D-glucose across the renal proximal tubule luminal (brush border) membrane has been shown in intact animals (1, 2), renal cortical slices (3), and isolated brush border membrane vesicles (4, 5). The precise mechanism by which Na\(^+\) enhances D-glucose transport in the kidney is not known. A Na\(^+\) gradient hypothesis, formulated for intestinal sugar transport, first by Crane (6) and later elaborated upon by Schultz and Curran (7), proposes that the Na\(^+\) electrochemical gradient across the cell membrane drives the uptake of D-glucose, the translocation of the sugar being coupled in some manner to the flux of Na\(^+\). The coupling of the fluxes of Na\(^+\) and D-glucose in the Na\(^+\) gradient hypothesis bears on the mechanics of the transport process, but not on the energetics. An important aspect of the driving force is the question of whether Na\(^+\)-dependent D-glucose transport is electroneutral, i.e. the positive charge associated with Na\(^+\) flux is compensated by the co-transport of an anion or the counter movement of a cation via the same carrier, or electrogenic, i.e. charge compensation is not made via the glucose carrier but at a different site in the membrane. In the latter case, Na\(^+\)-dependent D-glucose transport should be influenced by an electrochemical potential across the membrane. This question, as it relates to the transport of sugar in the renal tubule, is examined in this paper.

Previous studies with isolated renal brush border membranes have demonstrated that the imposition of a large extravesicular to intravesicular NaCl gradient effects the transient uphill movement of D-glucose into the membrane vesicles, attaining at the peak of the overshoot levels of D-glucose several fold that of the final equilibrium value (5). In the present study, the uptake of D-glucose is determined when the membrane potential across the brush border membrane is...
regulated experimentally by anions of varying modes of permeability, specific ionophores, and proton conductors. The findings have impact on the energetic relationship between the electrochemical potential of the renal luminal membrane and the Na\textsuperscript{+}-dependent transport of sugar in the kidney.

**Experimental Procedures**

*Preparation of Membrane Vesicles—* Rabbit renal brush border membranes were isolated as described (8) with modification (5). After isolation, the membranes were washed three times in a medium containing 300 mM mannitol, buffered with 1 mM Tris-Hepes\textsuperscript{1} (1 mM Hepes adjusted with Tris hydroxide) to pH 7.5, by centrifuging at 27,000 \( \times g \) for 5 min. Following the last centrifugation the membranes were suspended in the buffered mannitol medium and the protein concentration was adjusted to 10 to 15 mg/ml.

*Transport Measurements—* Uptake of D-glucose was measured by the Millipore filtration technique detailed previously (9) and modified (5). Unless otherwise specified, the brush border membranes were incubated at 20\textdegree C in 50 \( \mu l \) of a mixture containing 100 mM mannitol, 100 mM Na\textsuperscript{+} salt, 50 \( \mu l \) D-[\textsuperscript{14}C]glucose (approximately 0.1 \( \mu Ci \), and 1 mM Tris-Hepes, pH 7.5. Concentration of the membranes varied between 2 and 3 mg of protein/ml. Within this range, D-glucose uptake was linearly related to protein concentration. All incubations were carried out in triplicate with freshly prepared brush border membranes. Each experiment was repeated at least three times with different membrane preparations. The results are expressed as the mean \( \pm \) the S.E.M.

*Potassium-loaded Vesicles—* After isolation, the brush border membranes were washed three times in 50 mM KCl and 200 mM mannitol buffered with 1 mM Tris-Hepes, pH 7.5, as described above. In some experiments, loading was also accomplished by preincubating 10 \( \mu l \) of a suspension of membranes (100 to 150 \( \mu g \) of protein), that had been washed in the standard manner, with 1 \( \mu l \) of 550 mM KCl buffered with 1 mM Tris-Hepes, pH 7.5, for 20 min at 20\textdegree C. After preincubation, the solution of D-[\textsuperscript{14}C]glucose was added and uptake measured as described. Similar results, as reflected by Na\textsuperscript{+}-dependent D-glucose uptake, were obtained with both loading procedures.

*Proton-loaded Vesicles—* After isolation, the brush border membranes were washed three times in 225 mM mannitol buffered with 50 mM Tris-Mes (50 mM Mes adjusted with Tris hydroxide) to pH 5.5. The final osmolarity of the suspension was 300 mosM. These membranes were assayed using a radioactive glucose solution buffered with 50 mM Tris-Hepes, pH 7.5. Control membranes, i.e. those with no gradient, were washed three times in 200 mM mannitol buffered with 50 mM Tris-Hepes, pH 7.5 (final osmolarity = 300 mosM). Preliminary experiments showed that the uptake of D-glucose by membranes incubated in mannitol containing either Tris-Hepes or Tris-Mes buffer was the same.

*Protein Assay—* Protein concentrations were determined by the method of Lowry et al. (10) using bovine serum albumin as the standard.

*Chemicals—* D-[\textsuperscript{14}C]Glucose (240 Ci/mol) was obtained from Schwarz BioResearch. Valinomycin and gramicidin D were purchased from Sigma Chemical Co. and FCCP from Pierce Chemical Co.; nigericin was donated by Eli Lilly Laboratories. The ionophores and FCCP were added in 95% ethanol; control suspensions received ethanol alone.

**Results**

*Transport of D-Glucose into Osmotically Active Renal Brush Border Membrane Vesicles—* Before studying how effectors of the membrane electrochemical potential may influence the transport of D-glucose in renal brush border vesicles it was important to establish that the sugar is, indeed, transported across the membrane into an intravesicular space. Previous findings (4), that preloading the renal membrane with D-glucose accelerated exchange diffusion of the sugar, and that phlorizin was a potent inhibitor of efflux as well as influx, suggested that the uptake of D-glucose by the brush border membranes represents transport into vesicles rather than merely membrane binding. This suggestion was tested further by examining the effect of intravesicular volume on the uptake of D-glucose. Since the uptake of the sugar reaches equilibrium after 60 min (5), the amount of D-glucose taken up at this time should be dependent on the intravesicular volume. In the experiments illustrated in Fig. 1, intravesicular space was decreased by increasing the medium osmolarity with sucrose, a relatively impermeable solute which is not hydrolyzed in the kidney (11). Uptake of D-glucose was found to be inversely proportional to medium osmolarity from 0.3 to 1.0 mM sucrose and, thus, directly related to intravesicular space. Less than 5% of the uptake at 0.3 mM sucrose, the final osmolarity in all subsequent uptake measurements, was estimated by extrapolation to infinite medium osmolarity. This relatively small calculated uptake may suggest a slight binding of D-glucose to the membrane (12), or, perhaps more likely, the slow leak of sucrose into the vesicle, thus negating zero intravesicular volume. Other experiments, not shown, with cellobiose, another relatively impermeable disaccharide, when tested at concentrations from 100 to 350 mM, yielded the same results as did sucrose. Moreover, uptakes of D-[\textsuperscript{14}C]glucose in 300 mM mannitol, sucrose, or cellobiose were the same. This indicates the absence of significant D-glucose contaminating the sucrose solution, as used in the experiments described in Fig. 1, and the lack of an effect of sucrose on D-glucose uptake. Thus, virtually all D-glucose uptake into the isolated renal brush border membranes can be accounted for by transport into an intravesicular space, thereby confirming the earlier suggestion (4).

Effect of Anions on Na\textsuperscript{+}-dependent D-Glucose Uptake—*Fig. 2 describes paired experiments in which different anions were tested relative to Cl\textsuperscript{−} for their effectiveness in supporting the Na\textsuperscript{+} gradient-dependent uphill transport of 50 \( \mu M \) D-glucose. In agreement with previous findings (5), a 100 mM Na\textsuperscript{+} gradient (extravesicular > intravesicular) with Cl\textsuperscript{−} as the accompany-

![Fig. 1. Effect of medium osmolarity on glucose uptake](http://www.jbc.org/Downloaded_from)
In the proximal tubule of rabbit kidney, the permeability coefficients for Na+ and Cl- are $0.23 \times 10^{-4}$ and $0.73 \times 10^{-4}$ cm s$^{-1}$, respectively (15). Thus, Cl$^-$ is three times more permeable than Na$^+$, in the present experiments with membranes from the rabbit kidney proximal tubule it is reasonable to assume that Cl$^-$ enters the intravesicular space more rapidly than Na$^+$ and permits the development of an electrochemical potential (interior negative). Moreover, SCN$^-$ and NO$_3^-$, which are probably more permeable than Cl$^-$, will facilitate the more rapid development of this electrochemical potential. Thus, with the isolated membrane preparations, transport of these anions into the vesicles would tend to make the membrane more negative on the inside and, if the Na$^+$-stimulated uptake of d-glucose were electrogenic, enhance the transport of the sugar. On the other hand, both SO$_4^{2-}$ and the isethionate anion are relatively impermeable to the renal proximal tubule. The permeability of SO$_4^{2-}$ is approximately 25% that of Cl$^-$ (16), whereas the permeability coefficient for the isethionate ion is only 3% that of Cl$^-$.

With either of these anions, one would predict little development of an electrochemical potential across the membrane to drive an electrogenic Na$^+$-stimulated uphill uptake of the sugar.

Acetate crosses mitochondrial membranes as the undissoociated free acid (17); presumably the same mode of translocation is operant in the renal brush border membranes. If this be the case, the transport of acetate across the brush border membrane will not develop an electrochemical potential.

As shown in Fig. 3A, when a salt gradient with 100 mM sodium acetate was established, there was no accumulation of D-glucose above equilibrium. Instead, the uptake of the sugar reached its equilibrium value in about 2 min. Bicarbonate passes the proximal tubule luminal membrane predominately as dissolved CO$_2$ (18), generating little electrochemical potential. When D-glucose uptake was assayed with 100 mM NaHCO$_3$ (Fig. 3B), there was only a small, if any, uptake above the equilibrium value, clearly different from the results with NaCl.

Phosphate is present at physiological pH mostly as HPO$_4^{2-}$ and HPO$_4^{2-}^-$. However, H$_2$P$_O_4^-$ is postulated to be more permeable through the luminal membrane of the proximal tubule (19). If H$_2$PO$_4^-$ is translocated across this membrane for OH$^-$, as is the case with mitochondrial membranes (17), then there would be little development of an electrochemical potential. As illustrated in Fig. 3C, supplying a salt gradient with 100 mM sodium phosphate at pH 7.5 and assaying the uptake of D-glucose by brush border membrane vesicles resulted in no accumulation of sugar above equilibrium, although as in the experiments with acetate and bicarbonate there was initially (30 s) significant Na$^+$ stimulation (3- to 4-fold) above the 30-s mannitol control. Thus, the results presented in Fig. 3 lead to the conclusion that Na$^+$ salts of permeant anions, whose mode of membrane translocation is presumed to be electrochemically neutral, do not develop a membrane electrochemical potential (inside negative) and, therefore, do not support the uphill transport of d-glucose.

The possibility that H$_2$PO$_4^-$/OH$^-$ exchange does not occur in the renal brush border membrane, but that a significant membrane potential is not developed with sodium phosphate because of the low permeability of H$_2$PO$_4^-$ relative to Na$^+$, has not been ruled out.

**Effect of Ionophores on Na$^+$-dependent d-Glucose Uptake**—The role of the electrochemical membrane potential was examined additionally by determining d-glucose uptake by membrane vesicles under conditions in which membrane

\[ T. E. Andreoli, \text{personal communication.} \]
Membrane potentials were induced experimentally and predictably by ionophores. Gramicidin increases the cation permeability of biological and artificial membranes (20–22). The ionophore was used in the present experiments to enhance membrane conductance for Na⁺ electrogenically. As shown in Fig. 4 when gramicidin was added to an incubation containing a NaCl gradient (extravesicular > intravesicular), the ionophore decreased the D-glucose uptake overshoot by 60 to 65%, presumably by dissipating the electrochemical membrane potential by accelerating the electric entrance into the vesicle of the positively charged Na⁺. A relatively small transient accumulation of sugar twice the equilibrium value was still seen, however, compared to an overshoot of four to five times in the absence of the ionophore. Increasing the concentration of gramicidin from 8 to 20 µg/mg of membrane protein did not increase the inhibition nor abolish completely the overshoot. The data in Fig. 4 also show that gramicidin had no effect on the equilibrium value; the uptakes for D-glucose at 60 min were identical for NaCl ± gramicidin and the mannitol control.

Nigericin binds K⁺ (or Na⁺) and permits cations to exchange freely across biological membranes in an electroneutral fashion (23–26). Therefore, the movement of ions, induced by nigericin, will not generate an electrochemical membrane potential. The effect of this ionophore on the Na⁺-dependent D-glucose uptake by brush border membrane vesicles was tested by preloading the vesicles with protons followed by incubating the membranes in a medium establishing a large gradient of NaCl. As illustrated in Fig. 5, nigericin inhibited the overshoot uptake of D-glucose by 40 to 50%, a result consistent with the action of the ionophore in facilitating an electroneutral Na⁺/H⁺ exchange. Accumulation of sugar above equilibrium was not completely abolished, however. Nigericin did not affect the final equilibrium value.

Valinomycin binds K⁺ and mediates the electrogenic movement of K⁺ down its concentration gradient, thus setting up an electrochemical potential across the membrane (22, 24, 27). Experiments in which brush border membranes were preloaded with K⁺ (vesicle > medium) and then assayed for Na⁺-dependent glucose transport in the presence of a Na⁺ gradient (medium > vesicle), with and without valinomycin, are described in Fig. 6. In the absence of valinomycin, an accumulation of D-glucose above equilibrium (about 2- to 3-fold) was evident. Thus, when KCl rather than mannitol was initially on the inside of the vesicle the magnitude of the overshoot generated by extravesicular NaCl was reduced (compare with Figs. 2 to 5). Presumably, intravesicular K⁺ partially minimized the development of an electrochemical membrane potential (negative inside) induced by the Na⁺ gradient (medium > vesicle). Preliminary findings measuring Na⁺ influx into brush border membrane vesicles indicate that preloading with K⁺ did, indeed, reduce Na⁺ uptake into the vesicles. In the presence of valinomycin, however, a greatly enhanced overshoot above equilibrium (about 7- to 8-fold) was seen. Valinomycin added to K⁺-loaded brush border membrane vesicles induced the influx of K⁺ down its concentration gradient with concomitant generation of an electrochemical potential, interior negative. The development of this membrane potential accelerated the influx of Na⁺ and D-glucose into the membrane vesicle resulting in the marked transient accumulation of the sugar. In contrast, when nigericin was added to K⁺-loaded brush border membranes, there was no uptake of D-glucose above the equilibrium value (Fig. 6). In this case, nigericin mediated the electroneutral exchange of intravesicular K⁺ for extravesicular Na⁺. Thus, an electrochemical membrane potential was not generated by the efflux of K⁺. Therefore, sugar uptake was not stimulated. In fact, the rate of

\[ A. \text{ Chernoff and B. Sacktor, unpublished observations.} \]
sugar uptake in the presence of nigericin was less than that found for K⁺-loaded vesicles in the absence of ionophores.

Effect of Proton Conductors on Na⁺-dependent D-Glucose Uptake—The proton conductor, FCCP, increases the permeability of membranes to protons (22, 28). In experiments analogous to those with valinomycin, FCCP was used to generate an electrochemical membrane potential, interior negative, with brush border membranes that had been preloaded with protons. The results are described in Fig. 7. As shown, in the absence of FCCP, there was only a 2-fold accumulation of d-glucose above the equilibrium value when there was both a H⁺ gradient (vesicle > medium) and a Na⁺ gradient (medium > vesicle). When the same experiment was carried out in the presence of FCCP, a rapid overshoot of d-glucose uptake, six times the equilibrium value, was reached in 30 s. Thus, FCCP by inducing a rapid electrogenic efflux of protons generated a membrane potential, negative on the inside. This resulted in the enhanced influx of Na⁺, coupled to the influx of d-glucose. Other experiments, not shown, revealed that the concentration of FCCP used in the experiments illustrated in Fig. 7, i.e. 10 μM, produced the maximum overshoot without inhibiting the equilibrium value.

**DISCUSSION**

From the experimental findings presented in this study, it is proposed that the Na⁺-dependent transport of D-glucose against its concentration gradient into renal proximal tubule luminal membrane vesicles is an electrogenic process and that the driving force is provided by the electrochemical potential.
across the brush border membrane. This interpretation is supported by several lines of evidence.

Anions which penetrate the brush border membrane and generate an electrochemical potential, negative on the inside, permit the uphill Na⁺-dependent transport of D-glucose. D-Glucose accumulation above equilibrium is not supported by impermeant anions and anions whose mode of translocation is electroneutral. Experiments with intestinal microvillus membrane vesicles also demonstrated that a concentration gradient of NaSCN enhances Na⁺-coupled D-glucose uptake (29).

The use in the present studies of ionophores to induce electrical potential differences across membranes is derived from the findings of Cockrell et al. (25) with other membrane systems. With our preparations of renal brush border membrane vesicles, Na⁺-dependent D-glucose accumulation is supported by an ionophore (valinomycin), which catalyzes electrogenic K⁺ movements, but not by one (nigericin), which mediates an electroneutral exchange. Na⁺-dependent D-glucose uptake is diminished, but not abolished, by ionophores which allow Na⁺ to pass through the membrane via another channel, be it an electrogenic one, i.e. induced by gramicidin, or an electroneutral one, i.e. induced by nigericin. It has been reported that gramicidin inhibits the Na⁺ gradient-stimulated transport of 2-aminoisobutyric acid into membrane vesicles derived from Ehrlich ascites cells (30). With the ascites cell preparation gramicidin does not completely abolish accumulation of the amino acid against its concentration gradient, as is true for the transport of D-glucose with our renal membrane vesicles.

Lastly, Na⁺-dependent D-glucose uphill transport into renal brush border membrane vesicles is enhanced by the proton conductor, FCCP, in the presence of a pH gradient (vesicle > medium). Murer and Hopfer (29) have also shown with intestinal microvillus membranes that the efflux of protons induced by proton conductors will contribute to the driving force for Na⁺-dependent D-glucose uptake.

The accumulation of substrates, linked to a membrane potential, has been indicated recently in other systems. Some examples include the uptake of β-galactosides by Streptococcus lactis (31) and Escherichia coli (32), the uptake of amino acids by Streptococcus faecalis (33) and E. coli membrane vesicles (34), the Na⁺-dependent transport of D-glucose by rat intestinal microvillus membranes (29), and the Na⁺-dependent uptake of amino acids by Ehrlich ascites cell membrane vesicles (30). In the present study, the crucial role of the membrane potential for the uphill Na⁺-dependent transport of D-glucose across the luminal membrane of the renal proximal tubule is demonstrated. The charge associated with the Na⁺-coupled D-glucose translocation is not accompanied by the co-movement of an anion or counter movement of a cation via the sugar carrier. Moreover, considerable evidence has been presented suggesting the importance of the anion in the development of the electrochemical potential.

The response of the Na⁺-dependent D-glucose transport system to a membrane potential in isolated renal membrane vesicles is consistent with what is known about the physiology of the transport system. Rose and Schultz (35, 36) have shown that in the rabbit ileum, the interior of the cell is electrically negative with respect to the mucosal solution. Addition of D-glucose or alanine to the mucosal solution decreases the transmembrane electrical potential. Similar findings have been reported for bull frog small intestine (37). Microperfusion studies with the proximal tubule of the newt kidney have also shown that the active transports of D-glucose and Na⁺ depolarize the luminal membrane (38).

Fig. 8 illustrates diagrammatically a tentative model for the Na⁺-coupled D-glucose uphill transport in the renal proximal tubule. Na⁺ and d-glucose are translocated from the glomerular filtrate across the luminal membrane into the cells by an electrogenic process, with the transmembrane electrochemical potential (interior cell negative) providing the driving force. This electrochemical membrane potential may be maintained in part by an active Cl⁻ pump transporting the anion across the cell (39), the extrusion of H⁺ from the cell across the luminal membrane by a HCO₃⁻-stimulated ATPase, and by the extrusion of Na⁺ from the cell across the anti-luminal basal-lateral membrane by a ouabain-sensitive (Na⁺-K⁺)-ATPase localized in this membrane (40). D-Glucose exits from the cell via the basal-lateral membrane, presumably by a Na⁺-independent process (41). Thus, in the intact kidney the asymmetric distribution of Na⁺ across the proximal tubular cell and the electrochemical potential across the luminal membrane provide the energy needed to transport D-glucose against its concentration gradient.

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J C Beck and B Sacktor


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