TRANSPORT OF L-PROLINE AND D-GLUCOSE IN LUMINAL (BRUSH BORDER) AND CONTRALUMINAL (BASAL-LATERAL) MEMBRANE VESICLES FROM THE RENAL CORTEX

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SUMMARY: The uptakes of L-proline and D-glucose by brush border and basal-lateral membranes, derived from the rabbit renal cortex tubular cell plasma membrane, represented mostly transport into an intravesicular space rather than membrane binding. A Na^+ electrochemical gradient (medium>vesicle) stimulated the initial rates of uptake of both the imino acid and sugar, effecting their transient movements into the membrane vesicle against concentration gradients. The uptakes of L-proline and D-glucose by basal-lateral membranes were less responsive to Na^+ and the relatively small stimulation could largely be accounted for by contamination of the basal-lateral membrane preparation with brush border membranes. These findings are consistent with the hypothesis that the transcellular "active" transports of L-proline and D-glucose consist of a Na^+ electrochemical gradient-dependent uphill influx at the luminal brush border end and a Na^+-independent downhill efflux at the basal-lateral region of the tubule epithelial cell.

INTRODUCTION: The renal proximal tubule cell, which transports solutes vectorially across the tubular epithelium, is characterized by a plasma membrane that is differentiated into two distinct segments, the luminal brush border and the contraluminal basal-lateral membrane. This asymmetry is demonstrated by the findings that the two membranes differ ultrastructurally, in chemical composition, enzymically, and functionally (1-3). A large electrochemical Na^+ gradient (medium>vesicle) provokes a marked stimulation of D-glucose uptake in the renal brush border membrane, effecting the transient movement of the sugar into the vesicle against its concentration gradient (4-6). In contrast, uptake of D-glucose by basal-lateral membranes is significantly less responsive to Na^+ (5). Moreover, the transport of D-glucose by luminal membranes is highly sensitive to phlorizin (7, 4, 5), but the uptake by contraluminal membranes is only moderately inhibited (5). The uptake of amino acids by

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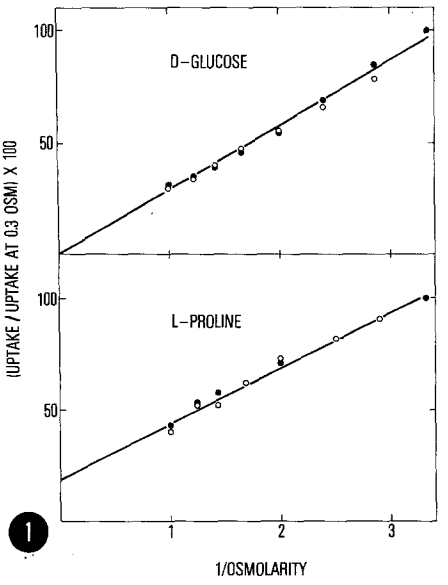
renal brush border membranes has also been investigated. A Na^+-dependent transport system for neutral amino acids has been identified (8, 9). In addition, the luminal membranes possess a Na^+-dependent transport system for imino acids, *i.e.* L-proline, which is distinct from that for neutral amino acids (10, 11). Recently, we described the preparation of the brush border and basal-lateral segments of the plasma membrane from the same kidney cortex (12). In this paper, the uptakes of L-proline and D-glucose by the two membranes are compared.

METHODS: Rabbit renal brush border and basal-lateral membranes were isolated as described previously (12). The specific activities of the marker enzymes trehalase and γ-glutamyl transpeptidase in the brush border membrane preparations were 1.9 and 5.9 μmol/min/mg protein, respectively, approximately 12- to 16-fold those in the cortical homogenate (12). The basal-lateral membrane preparations were enriched in NaK-ATPase and guanylate cyclase, with specific activities of 330 and 0.31 nmol/min/mg protein, respectively, 2.5- and 10-fold greater than those in the luminal membrane (12).

Uptakes of D-glucose (13, 4) and L-proline (10) were measured by the Millipore filtration technique detailed previously. Hitherto reported methods were used for the assays of trehalase (14), γ-glutamyl transpeptidase (15), NaK-ATPase (14), and guanylate cyclase (12). Protein was determined by a standard procedure (16), following 15 hr digestion in 1.0 M NaOH to ensure complete solubilization of the membrane protein; bovine serum albumin was used as the standard. Reagents were of the highest purity available commercially and were obtained from the sources reported previously (4, 10).

RESULTS AND DISCUSSION: Initial (15 sec) rates of uptake of D-glucose or L-proline by renal brush border and basal-lateral membranes were proportional to the concentration of membrane protein, at least within the range of from 10 to 100 μg per incubation mixture. Over 96% of the radioactive sugar or imino acid taken up by the membranes in 2 min, upon extraction, appeared in a single spot on thin layer silica gel plates (7, 9) and cochromatographed with authentic D-glucose or L-proline. These findings indicate that both solutes when taken up by the two membranes were accumulated and not metabolized.

To ascertain that the uptakes of D-glucose and L-proline by both brush border and basal-lateral membranes represented transport into vesicles rather than simply membrane binding, the effect of intravesicular volume on the uptakes was determined (Fig. 1). Since the uptakes of D-glucose and L-proline reached equilibrium after about 1 hr of incubation (see Fig. 2



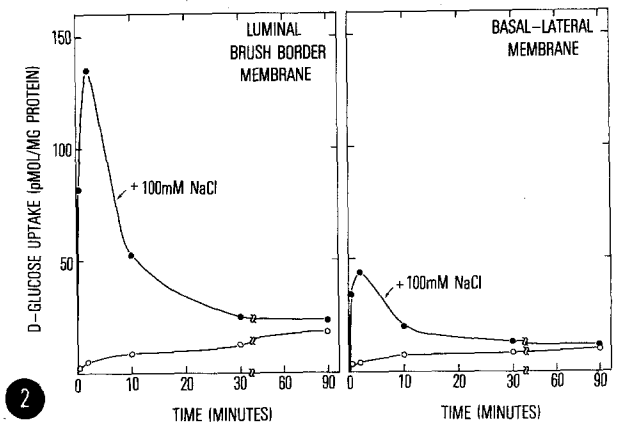


Fig. 1. Effect of the osmolarity of the medium on the uptake of D-glucose (top) or L-proline (bottom) by brush border (•) and basal-lateral (o) membrane vesicles. Uptake was measured after 60 min in a medium containing 50 μM sugar or 25 μM imino acid in 1 mM Tris-Hepes, pH 7.4, and sucrose to give the indicated osmolarity. Each point represents the mean of 3 experiments.

Fig. 2. Comparison of the uptakes of D-glucose by renal brush border and basal-lateral membrane vesicles in 300 mM buffered mannitol (o) or in a medium in which mannitol was replaced isosmotically by 100 mM NaCl (•) at the initiation of incubation. The concentration of D-glucose was 50 μM.

and 3), the amounts taken up at that time should, if they were transported, be dependent on the intravesicular volume. In the experiments shown 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 (17). Uptakes of the sugar and imino acid by both membranes were inversely proportional to medium osmolarity from 0.3 to 1.0 M and, consequently, directly related to intravesicular space. Extrapolation to infinite medium osmolarity (zero intravesicular volume) indicated little, if any, uptake of D-glucose in either brush border or basal-lateral membrane, confirming the earlier conclusion that virtually all D-glucose uptake into the two renal membranes could be accounted for by transport into intravesicular spaces (5, 6). With L-proline,

about 15% of the uptakes by both brush border and basal-lateral membranes at 0.3 M, the final osmolarity in all subsequent uptake measurements, were estimated by extrapolation to infinite medium osmolarity. This relatively small calculated uptake might suggest some binding of L-proline to the membranes. With brush border membranes, previous counter transport experiments also indicated that the bulk of the L-proline taken up was transported into the intravesicular space (10).

The uptake of D-glucose by brush border and basal-lateral membrane vesicles during the time course of incubation, either in a 300 mM buffered mannitol medium or in a medium in which mannitol was replaced isosmotically by 100 mM NaCl at the initiation of incubation, is shown in Fig. 2. With brush border membrane vesicles, the presence of the electrochemical Na^+ gradient induced a marked stimulation of sugar uptake, the initial rate with the gradient being 40- to 50-times that in the absence of Na^+. The final level of uptake in the presence and absence of the Na^+ electrochemical gradient was essentially the same, indicating that equilibrium had been established. At the peak of the "overshoot" (2 min) the uptake of D-glucose was 6- to 10-fold the final equilibrium value, suggesting that the imposition of a large extravesicular to intravesicular Na^+ electrochemical gradient effected the transient movement of the sugar into brush border membrane vesicles against its concentration gradient (uphill transport). Similar observations were reported with renal brush border membranes prepared by a different procedure (4). In contrast to these findings, uptake of D-glucose into basal-lateral membrane vesicles showed less dependence on a Na^+ electrochemical gradient. Although the initial rate of uptake by basal-lateral membrane vesicles was substantially higher in the presence of the Na^+ gradient than in the absence of Na^+, the relative stimulation was only about 30% that found with brush border membranes. With the basal-lateral membrane, as with the brush border membrane, the uptakes of the sugar in the presence and absence of Na^+ were the same at equilibrium. In other experiments, it was found that phlorizin (1 mM) was 3-times more

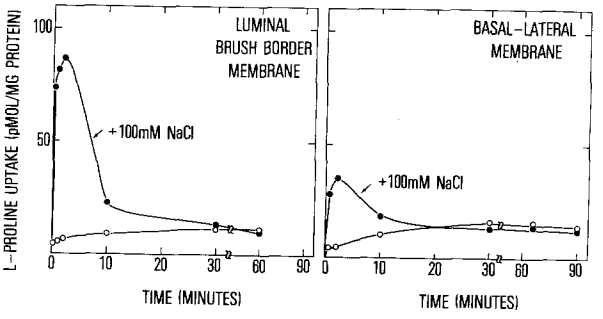


Fig. 3. Comparison of the uptakes of L-proline by renal brush border and basal-lateral membrane vesicles in 300 mM buffered mannitol (o) or in a medium in which mannitol was replaced isosmotically by 100 mM NaCl (•) at the initiation of incubation. The concentration of L-proline was 25 μM.

potent an inhibitor of the Na^+-dependent D-glucose uptake by brush border membranes than by basal-lateral membranes, in agreement with previous findings (5). L-Glucose (1 mM) had no effect on the uptake of 50 μM D-glucose by basal-lateral or brush border membrane vesicles, in the presence or absence of the Na^+ electrochemical gradient, suggesting stereospecific sugar transport in both segments of the renal tubular cell plasma membrane.

Distinction between the transport systems of the brush border and basal-lateral membranes was evident additionally from an examination of the Na^+ electrochemical gradient-dependent uptakes of L-proline (Fig. 3). With brush border membrane vesicles, a 20- to 40-fold stimulation of the initial rate of L-proline uptake was found in the presence of the Na^+ gradient; at the peak of the "overshoot" the uptake was about 8-times the equilibrium value. With basal-lateral membrane vesicles, however, only a 4- to 8-fold increase in initial rate of uptake was found and the maximum transient accumulation was 2-times the equilibrium value. Thus, for the transport of both L-proline and D-glucose the brush border membranes were significantly more responsive to a Na^+ electrochemical gradient than were the basal-lateral membranes. Indeed, in view of the known contamination of the basal-lateral membrane preparation with brush border membranes (12), the question arose as to whether the small Na^+ dependence seen with the basal-lateral membrane preparation was, in fact, due to

TABLE I

COMPARISON OF THE Na^+-DEPENDENT AND -INDEPENDENT RATES OF D-GLUCOSE AND L-PROLINE UPTAKE IN BRUSH BORDER AND BASAL-LATERAL MEMBRANE VESICLES

MEMBRANE	D-GLUCOSE ($+Na^+/-Na^+$)	L-PROLINE ($+Na^+/-Na^+$)	TRANSPEPTIDASE (μmol/min/mg protein)
BRUSH BORDER	40.3 ± 7.6	23.0 ± 1.5	5.28 ± 0.31
BASAL-LATERAL	12.7 ± 2.3	5.52 ± 0.78	1.63 ± 0.26
BRUSH BORDER/ BASAL-LATERAL	3.17 ± 0.39	4.16 ± 0.69	3.23 ± 0.43

Membranes, suspended in 300 mM mannitol, 2 mM tris-hepes, pH 7.2, were incubated in 300 mM mannitol, 2 mM tris-hepes, pH 7.2, 1 mM EDTA, 1 mM $MgCl_2$ and 50 μM D-glucose or 25 μM L-proline. When present, 100 mM NaCl replaced mannitol isosmotically. Incubation time was 15 s.

contamination. Therefore, the ratios of the Na^+ gradient-dependent:-independent initial rates of L-proline and D-glucose uptake by the two membranes were calculated and compared to the specific activities in the two membrane preparations of γ-glutamyl transpeptidase, a brush border membrane enzyme marker (Table I). Such ratios for L-proline and D-glucose were about 4 and 3, respectively. If the basal-lateral membrane preparation was contaminated with brush border membranes to the extent of 25% the relatively small Na^+ gradient-dependent stimulations observed with the basal-lateral membranes would be accounted for. The specific activities of γ-glutamyl transpeptidase in the two preparations suggest this level of contamination. Thus, the apparent enhancements of L-proline and D-glucose uptake by Na^+ with basal-lateral membrane preparations may not represent an intrinsic property of the basal-lateral membrane transport systems. The present findings, therefore, are consistent with the hypothesis that the transcellular "active" transports of

L-proline as well as D-glucose consist of a Na^+ electrochemical gradient-dependent uphill influx at the luminal brush border end and a Na^+-independent downhill efflux at the basal-lateral region of the renal tubule epithelial cell.

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