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Na^+-dependent D-mannose transport at the apical membrane of rat small intestine and kidney cortex

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Abstract

The presence of a Na^+/D-mannose cotransport activity in brush-border membrane vesicles (BBMV), isolated from either rat small intestine or rat kidney cortex, is examined. In the presence of an electrochemical Na^+ gradient, but not in its absence, D-mannose was transiently accumulated by the BBMV. D-Mannose uptake into the BBMV was energized by both the electrical membrane potential and the Na^+ chemical gradient. D-Mannose transport vs. external D-mannose concentration can be described by an equation that represents a superposition of a saturable component and another component that cannot be saturated up to 50 μM D-mannose. D-Mannose uptake was inhibited by D-mannose ≫ D-glucose > phlorizin, whereas for α-methyl glucopyranoside the order was D-glucose = phlorizin ≫ D-mannose. The initial rate of D-mannose uptake increased as the extravesicular Na^+ concentration increased, with a Hill coefficient of 1, suggesting that the Na^+:D-mannose cotransport stoichiometry is 1:1. It is concluded that both rat intestinal and renal apical membrane have a concentrative, saturable, electrogenic and Na^+-dependent D-mannose transport mechanism, which is different from SGLT1.

Keywords: Intestine; Kidney; Brush-border membrane vesicle; Sodium/D-mannose

1. Introduction

Mannose is a C2 epimer of D-glucose and its transport across epithelial cells was thought to take place via the glucose transporters. Recently, Halaihel et al. [1] reported that D-mannose is transported across pig intestinal brush-border membrane by a Na^+-independent, diffusive transport system. However, a Na^+/D-mannose cotransporter was reported to be present at the apical membrane of dog kidney [2,3], Caco-2 cells [4] and, while we were preparing the current manuscript, in the apical membrane of rat kidney [5]. In both rat and dog kidney, the Na^+/D-mannose cotransport system is saturable and electrogenic and has a substrate specificity and kinetic properties different from the SGLT. The Na^+-dependent D-mannose transport system of Caco-2 cells was not characterized. We have recently shown that the apical membrane of chicken enterocytes has an active and electrogenic Na^+/D-mannose transport system [6].

In the current work, we demonstrate that the apical membrane of rat kidney proximal tubule and rat small intestine present a Na^+/D-mannose cotransport

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system, with properties similar to those described for the Na^+/D-mannose cotransporter of chicken enterocytes.

A preliminary account of the data described here was given at the 3th 'Congreso de la Fesbe' (Spain, 2000).

2. Material and methods

2.1. Brush-border membrane vesicle (BBMV) preparation

BBMV were isolated from the kidney cortex of male Wistar rats following the method of Biber et al. [7]. Briefly, kidney cortex slices from one rat were homogenized in 15 ml isolation buffer (in mM, 300 mannitol, 5 EGTA, 12 Tris-HCl, pH 7.4) with the Ystral Polytron on setting 5 for 2 min. 21 ml of cold bi-distilled water and $MgCl_2$, up to a final concentration of 10 mM, were added to the homogenate. The suspension was gently stirred for 20 min and then centrifuged at $1900 \times g$ for 15 min. The resultant supernatant was centrifuged at $30\,000 \times g$ for 30 min and the resultant pellet was resuspended in 30 ml of 150 mM mannitol, 6 mM Tris-HCl (pH 7.4), 2.5 mM EGTA and homogenized with a glass-Teflon potter. $MgCl_2$, at a final concentration of 10 mM, was added to the homogenate. The suspension was gently stirred for 20 min and then centrifuged at $1900 \times g$ for 10 min. The resultant supernatant was centrifuged at $30\,000 \times g$ for 30 min and the resultant pellet was resuspended in 0.5 ml of the appropriated loading buffer. The suspension was made homogeneous by passing it through a 20-gauge needle several times and diluted up to 30 ml of the loading buffer. The suspension was centrifuged at $30\,000 \times g$ for 30 min. The isolated apical membranes were made homogeneous by passing them through a 25- and a 28-gauge needle several times, and stored in liquid nitrogen until use. All the steps were carried out at 4°C.

Small intestinal BBMV were obtained following the method described by Stieger and Mürer [8]. The jejunum of two rats were rinsed with ice-cold 0.9% NaCl solution and everted. The mucosa was scraped off and put in 60 ml of 300 mM mannitol, 5 mM EGTA, 12 mM Tris-HCl pH 7.1 and the mixture diluted six times with ice-cold water and then homogenized in a Ystral Polytron on setting 4 for 2 min. $MgCl_2$ was added to a final concentration of 10 mM and after 20 min the homogenate was centrifuged for 15 min at $2300 \times g$. The supernatant was centrifuged for 30 min at $21\,000 \times g$. The resulting pellet was resuspended in 35 ml of 60 mM mannitol, 5 mM EGTA-Tris pH 7.1 and homogenized with a glass-Teflon potter. After the addition of $MgCl_2$ to a final concentration of 10 mM, the solution was allowed to stand for 20 min and then the two centrifugations above were repeated. The vesicles were resuspended in 35 ml of the buffer needed for the experiment, homogenized with a glass-Teflon potter and centrifuged for 40 min at $21\,000 \times g$. Finally, the vesicles were resuspended in a small volume of the loading buffer and made homogeneous by passing them through a 25- and a 28-gauge needle several times, and stored in liquid nitrogen until use. All the steps were carried out at 4°C.

Unless otherwise stated the BBMV were loaded with a pH 7.5 buffer consisting in 140 mM mannitol, 50 mM K-gluconate and 50 mM HEPES-Tris.

Protein was measured by the method of Bradford [9], using γ-globulin as the standard.

2.2. Sugar uptake studies

Sugar uptake was measured at 25°C by a rapid filtration technique as described [10]. Except where indicated otherwise, the uptake buffer consisted of 140 mM mannitol, 50 mM Na-gluconate, 50 mM HEPES-Tris (pH 7.5) and either 0.1 μM D-[^{3}H]mannose or the desired α-[^{14}C]methyl glucopyranoside (α-MG) concentration. The amount of protein in the assay tube ranged from 100 to 150 μg/100 μl of uptake buffer.

2.3. Materials

D-[^{3}H]Mannose and α-[^{14}C]methyl glucopyranoside (carrier free) were purchased from Amersham. The other compounds and salts used were obtained from Sigma (Madrid, Spain).

2.4. Statistical analysis

Individual experiments were carried out in triplicate. Data are presented as mean ± S.E.M. In the

figures vertical bars, that represent the S.E.M., are absent when they are less than symbol height. Comparison between different experimental groups was evaluated by two-tailed Student's *t*-test.

3. Results

3.1. Effect of osmolarity on D-mannose uptake

The binding of D-mannose to the membranes was calculated as the uptake of D-mannose by the BBMV at infinite osmolarity, that is, when there is no intravesicular space. Uptake of D-mannose in both intestinal and renal BBMV was a linear function of the inverse of the extravesicular osmolarity (Fig. 1), indicating that D-mannose is transported into an osmotically active space. The binding represents 32% and 13% of the D-mannose uptake measured in the standard conditions in intestinal and renal BBMV, respectively.

3.2. Mannose uptake vs. time

The time course of D-mannose uptake into either renal or intestinal BBMV (Fig. 2) revealed that D-mannose transiently accumulates in the vesicular space. Mannose uptake overshot the final steady-state equilibrium value by a factor of approx. 2 in both jejunum and kidney cortex. No overshoot was observed when uptake was measured in nominally Na^+ free conditions (Na^+ was replaced by K^+).

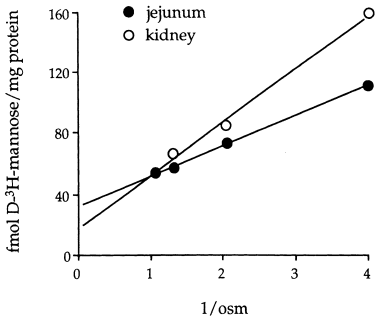

Fig. 1. External osmolarity and D-[^{3}H]mannose uptake into BBMV. Medium osmolarity was increased by the addition of mannitol. Uptake of 0.1 μM D-[^{3}H]mannose was measured during 30 min in the presence of extravesicular Na^+. The composition of the buffers is given in Section 2. Mean values ± S.E.M., $n = 3$.

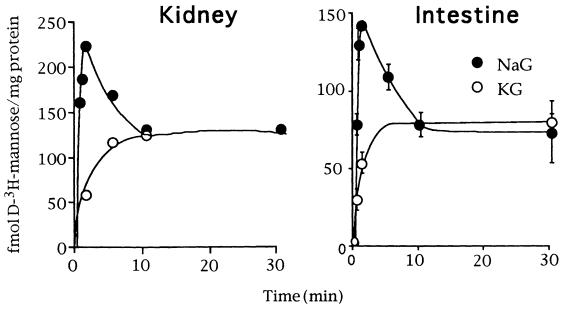

Fig. 2. Time course of D-[^{3}H]mannose uptake into BBMV. 0.1 μM D-[^{3}H]mannose uptake was measured in either the absence (Na^+ isosmotically replaced by K^+) or the presence of extravesicular Na^+ as a function of time. Mean values ± S.E.M., $n = 3$.

3.3. Effect of sugars and sugar transport inhibitors on either D-mannose or α-methyl glucopyranoside uptake into BBMV

D-[^{3}H]Mannose and α-[^{14}C]MG uptake into intestinal and renal BBMV was measured in the absence and presence of the compounds listed in Table 1. The results show that D-[^{3}H]mannose uptake into renal and intestinal BBMV was inhibited by D-mannose > D-glucose > phlorizin, whereas α-MG uptake decreased as follows: phlorizin = D-glucose > D-mannose.

Table 1
Effects of several compounds on either D-mannose or α-MG uptake

Modifiers	Relative uptake (%)	
	D-[^{3}H]mannose	D-α-[^{14}C]MG
Kidney		
None	100	100
Mannose (5 mM)	20 ± 2.0*	87 ± 2$^{\#}$
Glucose (5 mM)	39 ± 3.0*	13 ± 0.1*
Phlorizin (0.1 mM)	71 ± 6.0$^{\#}$	7 ± 0.05*
Intestine		
None	100	100
Mannose (5 mM)	46 ± 6.0*	96 ± 2
Glucose (5 mM)	52 ± 6.0*	7 ± 0.01*
Phlorizin (0.1 mM)	87 ± 5.0	4 ± 0.01*

Uptake of either 0.1 μM D-[^{3}H]mannose or 0.1 μM D-α-[^{14}C]MG was measured during 30 s in the absence and presence of the indicated unlabeled modifiers. Labeled sugar uptake obtained in the absence of modifiers was set at 100%. Values are means ± S.E.M. of four separate membrane vesicle preparations. *$P < 0.001$, $^{\#}P < 0.05$ as compared with control (100% uptake).

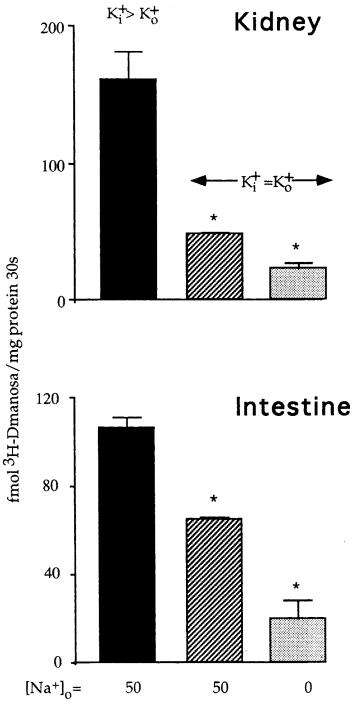

Fig. 3. 30 s sugar uptake into BBMV in the presence and absence of electrical membrane potential and/or Na^+ chemical gradient. The BBMV were loaded with a buffer consisting of (in mM): 140 mannitol, 50 KG and 50 HEPES-Tris (pH 7.5). The uptake buffer contained (in mM): 50 HEPES-Tris (pH 7.5), 0.045 valinomycin, 10^{-4} D-[^{3}H]mannose, and either 140 mannitol, 50 NaG (electrochemical Na^+ gradient, black bar); 40 mannitol, 50 NaG, 50 KG (chemical Na^+ gradient, hatched bar) or 140 mannitol, 50 KG (absence of electrochemical Na^+ gradient, stippled bar). Mean values ± S.E.M., $n=4$. *$P<0.001$, as compared with electrochemical Na^+ gradient conditions. NaG, Na-gluconate; KG, K-gluconate. K_i and K_o above the bars indicate the relative [K^+] (i.e. membrane potential).

3.4. Effect of Na^+ chemical gradient and electrical membrane potential on sugar uptake into BBMV

D-[^{3}H]Mannose uptake into BBMV was measured in the presence and absence of an inwardly directed electrochemical Na^+ gradient. Electrical membrane potential was created by an outwardly directed K^+ gradient in the presence of valinomycin. When required, membrane voltage across the membranes was brought to zero by equal internal and external K^+ concentrations in the presence of valinomycin. An inside directed Na^+ gradient was created by adding 50 mM Na^+ to the extravesicular buffer, being the intravesicular buffer nominally Na^+ free. Na^+ chemical gradient was abolished by Na^+ removal from the extravesicular solution.

Fig. 3 shows that in both intestinal and renal BBMV membrane voltage clamped conditions inhibited D-mannose uptake and this inhibition was further increased by Na^+ free conditions.

3.5. Kinetic study of the D-mannose uptake

D-Mannose uptake into BBMV was measured in the presence of increasing concentrations of unlabeled D-mannose, varying from 0.1 to 80 μM. The shape of the curve (Fig. 4) suggests the existence of a saturable process and the values fit best ($r=0.997$) a transport model describing a single saturable transport system plus a nonsaturable diffusion component:

$$v = (V_{max}S/K_m + S) + K_dS$$

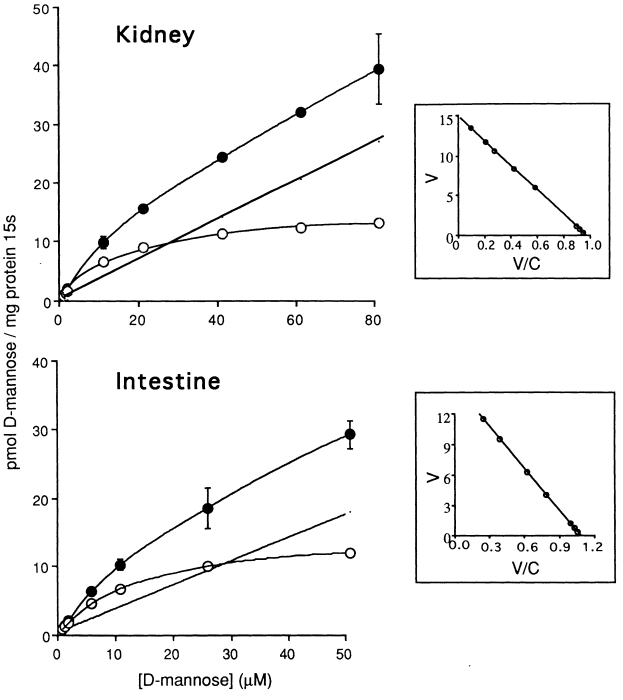

Fig. 4. Initial rate (15 s) of D-mannose uptake vs. increasing concentrations of external D-mannose. The composition of the intra- and extravesicular buffers was as described in Section 2. The values for the nonsaturable component (———) were determined by fitting the total transport data (●) to a transport model describing a single saturable system plus a nonsaturable component. The theoretically derived nonsaturable component was subtracted from total transport to calculate the saturable component (○). (Inserts) Eadie-Hofstee plots of the difference data. Mean values ± S.E.M., $n=4$.

where v is initial rate of uptake, S is the external D-mannose concentration, V_{max} is the maximal initial uptake rate, K_m is the Michaelis-Menten constant, and K_d is the apparent diffusion constant. The calculated apparent K_m and V_{max} values for D-mannose are given in Table 2.

The difference between total D-mannose uptake and the nonsaturable uptake follows first-order kinetics. Analysis with an Eadie-Hofstee plot (Fig. 4) yielded a linear relationship, consistent with the existence of a single saturable D-mannose transporter in both renal and intestinal BBMV.

3.6. Na^+:D-mannose stoichiometry

Na^+:D-mannose stoichiometry has been evaluated by measuring D-[^{3}H]mannose uptake into intestinal and renal BBMV as a function of extravesicular Na^+ concentration, with the membrane voltage clamped to zero (equal internal and external [K^+] in the presence of valinomycin). The Na^+ concentration ranged from 0 to 100 mM, with mannitol replacing Na-gluconate to maintain the extravesicular osmolality constant. The results (see Fig. 5 and Table 2) revealed that the Hill coefficient values were below 1, indicating 1:1 Na^+:D-mannose stoichiometry in both intestinal and renal BBMV.

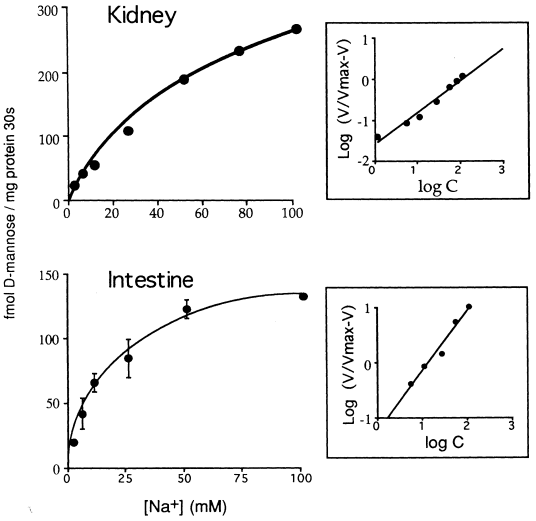

Fig. 5. Stoichiometry of Na^+/D-mannose cotransport. 0.1 μM D-[^{3}H]mannose uptake was measured for 30 s as a function of external Na^+ concentration. The BBMV were loaded with a buffer containing, in mM: 200 mannitol, 50 KG and 50 HEPES-Tris (pH 7.5). The uptake buffer contained (in mM): 50 KG, 0.045 valinomycin, 50 HEPES-Tris (pH 7.5), 10^{-4} D-[^{3}H]mannose, and 0–100 NaG with mannitol replacing NaG isosmotically. The Na^+-dependent D-mannose uptake vs. extravesicular Na^+ concentration is shown. Mean values ± S.E.M., $n=4$. (Inserts) Hill (log-log) plots of the data.

4. Discussion

The epithelial transport of D-mannose has not received much attention and in the small intestine D-mannose has been considered to be transported by the D-glucose transporters, but at a low rate [11]. Recent reports, however, have demonstrated the presence of a Na/D-mannose cotransport system in the apical membrane of Caco-2 cells [4], dog [2,3], rat kidney [5] and chicken small intestine [6].

The following observations agree with those reported for dog kidney [2,3] and are consistent with the presence in the brush-border membrane of rat small intestine and kidney cortex of a Na^+/D-mannose cotransporter, which is active, saturable and electrogenic: (i) D-mannose was taken by an osmotically active space; (ii) D-mannose was transiently accumulated by the BBMV only in the presence of Na^+; (iii) D-mannose uptake was decreased when either Na^+ was removed (substituted by mannitol) from the bathing solutions or when the electrical membrane potential was clamped to zero, and (iv) the carrier-mediated D-mannose uptake vs. D-mannose concentration in the buffer solution follows first order kinetics. The K_m values are similar to those reported for D-mannose transport in dog kidney [2,3] and chicken small intestine [6], but an order of magnitude lower than that reported for rat kidney [5]. The Eadie-Hofstee plots of the carrier-mediated D-mannose uptake (Fig. 4) yielded a linear relationship, consistent with the existence of a single D-man-

Table 2
Kinetic parameters of the D-mannose uptake into BBMV isolated from either rat small intestine or rat kidney cortex

	V_{max}	K_m	Hill coef.
Intestine	14.3 ± 0.06	14 ± 0.05	1.09
Kidney	14.4 ± 0.01	15 ± 0.02	0.77

The apparent K_m (in μM), V_{max} (nmol/mg/15 s) and the Hill coefficient (coef.) values were calculated as indicated in Figs. 4 and 5. Mean values ± S.E.M., $n=4$.

nose transporter in the two BBMV preparations. As reported for dog kidney [2], the stoichiometry of the intestinal and renal Na^+/D-mannose cotransport, described in the current work, is 1:1. Blasco et al. [5] found a 2:1 stoichiometry for Na^+-D-mannose interaction in the BBMV isolated from rat kidney.

The characteristics above described for both intestinal and renal D-mannose transport could indicate that D-mannose is transported by the SGLT transporters. However, the following observations clearly differentiate the Na/D-mannose transporter from the SGLTs. Thus, the inhibition of D-[^{3}H]mannose uptake induced by either cold D-mannose, D-glucose or phlorizin differs from that induced by the same compounds on α-MG uptake (see Table 1). Also, the evaluated V_{max} for D-mannose (see Table 2) is about 1000 times lower than the V_{max} of D-glucose by SGLT1 (nanomolar range). Another fundamental difference is that SGLT1 has a 2:1 Na^+:D-glucose stoichiometry [12,13], whereas we found a 1:1 Na^+:D-mannose stoichiometry. The SGLT2 and SAAT1 have a 1:1 Na^+:D-glucose stoichiometry [14–17], but they are low affinity glucose transporters (K_m for α-MG is around 2 mM). It is unlikely, however, that D-mannose is transported by the low affinity glucose transporters, because phlorizin inhibits the SGLT1, SGLT2 and SAAT1 with equal potency (K_i close to 18 μM) [14–17] and the Na^+/D-mannose transporter described here has low affinity for phlorizin (see Table 1).

In conclusion, the current work shows that the apical membrane of rat small intestine and kidney cortex presents a transporter that catalyzes the Na^+/D-mannose transport, which is different from the SGLT1. This transporter has substrate specificity and kinetic properties similar to those described for the Na^+/D-mannose transporter present at the apical membrane of chicken enterocytes and dog kidney cortex.

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References

[1] N. Halaihel, D. Gerbaud, M. Vasseur, F. Alvarado, Am. J. Physiol. 277 (1999) C1130–C1141.
[2] D.C. Mendelssohn, M. Silverman, Am. J. Physiol. 257 (1989) F1100–F1107.
[3] M. Silverman, L. Ho, Biochim. Biophys. Acta 1153 (1993) 34–42.
[4] E. Ogier-Denis, A. Blais, J.J. Houri, T. Voisin, G. Trugnan, P. Codogno, J. Biol. Chem. 269 (1994) 4285–4290.
[5] T. Blasco, J.J. Aramayona, A.I. Alcalde, N. Halaihel, M. Sarasa, V. Sorribas, J. Membr. Biol. 178 (2000) 127–135.
[6] M. Cano, M.L. Calonge, M.J. Peral, A.A. Ilundáin, Pflug. Arch. 441 (2001) 686–691.
[7] J. Biber, B. Stieger, W. Haase, H. Mürer, Biochim. Biophys. Acta 647 (1981) 169–176.
[8] B. Stieger, H. Mürer, Eur. J. Biochem. 135 (1983) 95–101.
[9] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[10] M.J. Peral, M. Cano, A.A. Ilundáin, Eur. J. Biochem. 231 (1995) 682–686.
[11] U. Hopfer, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, 2nd edn., Raven Press, New York, 1987, pp. 1499–1526.
[12] G.A. Kimmich, J. Randles, Am. J. Physiol. 247 (1984) C74–C82.
[13] W.-S. Lee, Y. Kanai, R.G. Wells, M.A. Hediger, J. Biol. Chem. 269 (1994) 12032–12039.
[14] M.A. Hediger, Y. Kanai, G. You, S. Nussberger, J. Physiol. 482 (1995) 7S–17S.
[15] B. Mackenzi, M. Panayotova-Heiermann, D.D. Loo, J.E. Lever, E.M. Wright, J. Biol. Chem. 269 (1994) 22488–22491.
[16] N. Oulianova, A. Berteloot, J. Membr. Biol. 153 (1996) 181–194.
[17] G. You, W.S. Lee, E.J. Barros, Y. Kanai, T.L. Huo, S. Khawaja, R.G. Wells, S.K. Nigam, M.A. Hediger, J. Biol. Chem. 270 (1995) 29365–29371.