Biochimica et Biophysica Acta, 426 (1976) 349—353

BBA Report

BBA 71244

JEJUNAL AND ILEAL D-GLUCOSE TRANSPORT IN ISOLATED BRUSH BORDER MEMBRANES

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(Received December 10th, 1975)

Summary

D-Glucose transport was investigated in isolated brush border membranes from small intestine. The transport properties of membranes from upper jejunum were compared with those from terminal ileum. The jejunal membranes accumulated D-glucose to a greater extent than the ileal membranes when supplied with energy in the form of a NaSCN gradient. This difference in behavior is similar to that of the more intact epithelial preparations and suggests that the isolated membranes actually reflect the state present in intact cells. Ileal membranes transported D-glucose about two to three times slower than the jejunal ones, which can partially explain the lower sugar accumulation.

D-Glucose is absorbed by all regions of the small intestine. Although the transport exhibits Na^+ dependence and inhibition by phlorizin all along the gut, the degree of active sugar absorption varies with the region. The jejunum not only has a higher capacity for sugar absorption than the ileum, but it also can achieve D-glucose concentrations on the serosal side several times that of the luminal fluid. In contrast, it is difficult to demonstrate transport against a concentration gradient in the ileum [1—3]. This difference in absorption cannot be ascribed to a lower amount of surface area per unit weight in the ileum as compared to jejunum [4].

Recently, D-glucose transport has been measured in an in vitro system of isolated brush border membranes [5, 6]. Similarly to intact epithelial preparations, this transport is dependent on the presence of Na^+ and inhibited by low

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concentrations of phlorizin. However, there is very little information on how well the characteristic transport properties of different regions of intestine are preserved during isolation.

In order to evaluate this problem we compared D-glucose transport in isolated brush border membranes from upper jejunum and terminal ileum, and found differences in active D-glucose transport similar to those present in the more intact epithelial preparations.

Membranes were prepared by the methods of Hopfer et al. [5] and Schmitz et al. [7] as modified by Storelli et al.* from jejunum (a 30 cm section proximal to the ileocecal valve) of male Sprague-Dawley rats (200 to 250 g body weight) supplied either by Ivanovas, Kisslegg, Allgäu, Germany, or Hilltop Laboratory Animals, Inc., Scottdale, Pa. The membranes form vesicles after isolation and the Na^+-dependent glucose "carrier" mediates an uphill D-glucose transport into the space within the vesicles, provided osmotic energy is supplied in the form of an electrochemical Na^+ gradient such that the intravesicular Na^+ activity is lower [6]. This property is thought to constitute the biochemical counterpart to active accumulation and active transcellular sugar transport of the intact cell.

The ability of the isolated membranes to accumulate D-glucose was measured using a 0.1 M NaSCN gradient as driving force [6, 8]. For this purpose the membranes were homogenized in 0.1 M D-mannitol, 0.1 mM $MgSO_4$ and 1 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid adjusted with Tris hydroxide to pH 7.5 as described [5]. Subsequently, the membranes were exposed to 0.1 M NaSCN and 1 mM labeled D-glucose and the sugar uptake measured at 25°C as previously reported [6]. The concentrations of D-mannitol, $MgSO_4$, and buffer were kept constant.

Under these conditions D-glucose is initially concentrated within the vesicles several fold above the medium concentration; the accumulated D-glucose is released with continuing incubation until equilibrated between medium and intravesicular space (by 10—12 min of incubation) [6]. In other words, the resulting curve of D-glucose uptake vs. time is characterized by an initial overshoot. The ratio between the maximum of the overshoot and the equilibrium uptake was calculated and taken as a measure of the ability to move D-glucose uphill. Such a normalization circumvents the problem of variation in glucose uptake per mg protein due to possible varying degrees of vesiculation or different protein concentration per surface area. Table I summarizes the results with membranes prepared by the two procedures. The membranes prepared according to Storelli et al. consistently showed higher overshooting sugar uptake than those prepared with the method of Hopfer et al. [5] thus pointing up a definite influence of the isolation procedure. Nevertheless, with both methods of preparation active D-glucose transport was significantly higher in jejunal than in ileal membranes. This difference is similar to that reported for intact jejunum and ileum [1—3] and suggests that the isolated membranes reflect membrane properties originally present in the

*Storelli, C., Kessler, M., Mueller, M., Murer, H., Joss, C. and Semenza, G., unpublished. A brief description of this procedure has been given in ref. 8.

TABLE I

ACCUMULATION OF D-GLUCOSE BY BRUSH BORDER MEMBRANES

Membranes A: prepared according to Hopfer et al. [5]. Membranes B: prepared according to Storelli et al. (see footnote *). *n*, number of experiments;

Intestinal region	Ratio of maximum/equilibrium uptake of D-glucose			
	With membranes A		With membranes B	
	Mean ± S.D.	(*n*)	Mean ± S.D.	(*n*)
Jejunum	1.9 ± 0.2	(6)	6.6 ± 1.1	(5)
Ileum	1.2 ± 0.2	(6)	1.8 ± 0.4	(5)
Significance†	$p < 0.001$		$p < 0.001$	

†Paired *t*-test.

intact cell. The reason for the influence of isolation procedures on the behavior of the membrane is not known.

The time of maximum sugar uptake roughly correlated with the absolute ratio of maximum/equilibrium uptake. Ratios of about 6 were measured at the earliest time point, i.e., after 10 to 12 s of incubation. In those preparations with ratios of 1.2—2.0, the maxima were observed at about 45 s of incubation. Preparations with ratios below 1.3 reached maximum at about 90 s.

Several factors can produce the observed difference between the isolated jejunal and ileal membranes. For example, contamination of ileal membranes with non-brush border plasma membranes would reduce the apparent overshoot of D-glucose uptake because the contaminating membranes would contribute to the equilibrium uptake level of the sugar but would not be capable of active transport. Likewise, greater general "leakiness' of ileal membranes would result in faster dissipation of the imposed NaSCN gradient and thus explain the lower overshoot. However, several findings argue against both these explanations: (1) The uptake of the relatively impermeant solute L-glucose was equally slow in jejunal and ileal membranes (prepared according to Hopfer et al. [5]).with estimated half-equilibration times of 76 and 78 s, respectively (calculated from uptake values after 45 s of incubation and at equilibrium; for method see Table III); (2) Using sucrase as specific marker for intestinal brush border membranes, the ileal membranes were purified at least to the same extent as the jejunal membranes (Table II) at similar yield**. Differences in specific activity of scrapings between the upper and lower region of the small intestine have been noticed previously [9]. Either a higher proportion of cell types without sucrase or a lower specific activity per enterocyte have to be considered as explanations for lowered overall specific sucrase. However, non-enterocyte cell types should not contribute to membranes prepared by the method of Hopfer et al. [5], since an intermediate step in this isolation procedure selects for brush borders present only in enterocytes. The differences in sucrase activity between membranes prepared by the method of Hopfer et al. [5] and Storelli et al. [1] as well as the relatively large standard deviation for the latter membranes in Table II are at least partially caused by the use of different batches of animals differing widely in their specific activity.

**In contrast, the method of Storelli et al. (see footnote*) could not be used for comparison of jejunal and ileal membranes of Sprague-Dawley rats from another supplier (Carworth) since the yield of membranes was four times lower from jejunum than from ileum.

TABLE II

SUCRASE ACTIVITY OF BRUSH BORDER MEMBRANES

Intestinal region	Membranes A[a]			Membranes B[a]		
	Specific activity of sucrase[b]	Enrichment over homogenate		Specific activity of sucrase	Enrichment over homogenate	
	Mean ± S.D. (μmol/min/mg)	Mean ± S.D. (fold)	(n)	Mean ± S.D. (μmol/min/mg)	Mean ± S.D. (fold)	(n)
Jejunum	3.15 ± 0.28	13 ± 0.9	(4)	2.73 ± 2.45	25 ± 12	(3)
Ileum	1.25 ± 0.10	25 ± 1.4	(4)	0.40 ± 0.44	25 ± 6	(3)

[a]See Table I for explanations.
[b]Assayed as described by Hopfer et al. [5].

Alternatively, changes in the D-glucose transport can result from changes of the inherent properties of the membrane, affecting either the D-glucose or the Na^+ permeability. Thus, the kinetics of the glucose-"carrier", the "carrier" concentration in the membrane, or the ability of the membrane vesicles to maintain the driving force, i.e., the imposed Na^+ gradient, will determine the active D-glucose uptake. In order to evaluate the possibility of differences in the kinetics of D-glucose translocation between the two membranes, D-glucose uptake was measured with equal driving force for D-glucose flow (as opposed to equal initial NaSCN gradients as in Table I) in membranes from both intestinal regions. For this purpose the membranes were preincubated with NaSCN until the Na^+ gradient was dissipated (10 min [8]). Subsequently, labeled D-glucose was added to the membranes and the uptake of this sugar measured. Under these conditions the only driving force for net flow is the D-glucose concentration gradient, and hence, the uptake is equilibrating [6, 10] To quantitatively characterize the kinetics the half-time of equilibration ($T_{1/2}$) was estimated from the uptake at the earliest time point (10 to 12 s) and the equilibrium level with the assumption that only one exponential term determines the uptake curve around the early time point. As the apparent $T_{1/2}$ increased with vesicle filling, this method underestimated possible differences. Table III contains the $T_{1/2}$ values for jejunal and ileal membranes prepared by both isolation methods. Interestingly, D-glucose transport was 2 to 3 times slower in ileal than in jejunal membranes, which may explain the lower overshooting sugar uptake in the former ones when a Na^+ gradient is present as the

TABLE III

KINETICS OF D-GLUCOSE TRANSPORT BY BRUSH BORDER MEMBRANES

Intestinal region	$T_{1/2}$ of D-glucose uptake (s)			
	With membranes A[a]		With membranes B[a]	
	Mean[b] ± S.D.	(n)	Mean[c] ± S.D.	(n)
Jejunum	6.6 ± 1.1	(5)	10.4 ± 4.0	(5)
Ileum	15.6 ± 4.0	(5)	29.0 ± 2.6	(5)
Significance[d]	$p < 0.005$		$p < 0.005$	

[a]See Table I for explanations.
[b]Calculated from a plot of log(1 – uptake at 10 s/uptake at equilibrium) versus time.
[c]Calculated from a plot of log(1 – uptake at 12 s/uptake at equilibrium) versus time.

driving force. However, as additional explanation, faster dissipation of the Na^+ gradient in ileal than in jejunal membranes must be considered. It was recently shown that an increase in the Na^+ conductance of brush border membranes, caused by ionophores, results in lowered active D-glucose transport under the same experimental conditions as employed in Table I [10].

In conclusion, these experiments demonstrate with respect to active D-glucose transport close correspondence of the behavior of the isolated brush border membranes and more intact epithelial preparations. This similarity provides good evidence that the isolated membranes reflect the state of the plasma membrane as present in the intact cell.

We thank Drs. N. Robbins and H. Gershman for reading the manuscript. The work was supported by the NIH (Grant No. AM-18265) and Swiss Nationalfonds (Grant No. 3.0090.73).

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