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Proximo-distal gradient of Na^+-dependent D-glucose transport activity in the brush border membrane vesicles from the human fetal small intestine

Christiane Malo and Alfred Berteloot

Membrane Transport Research Group, Department of Physiology, Faculty of Medicine, University of Montreal, CP 6208, succursale 'A', Montreal, Quebec H3C 3T8, Canada

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Brush-border membrane vesicles were isolated from the jejunum and ileum of 17–20-week-old normal human fetuses and found to be highly enriched in sucrase activity with less than 5% contamination by basolateral membranes. Time course studies of D-glucose uptake clearly showed a transient, phlorizin-sensitive, and Na^+-dependent accumulation of sugar into these vesicles. Higher maximum overshoot values and initial rates of D-glucose uptake were recorded in jejunal as compared to ileal vesicles while low substrate binding to the membranes, identical intravesicular volumes and equivalent dissipation of the Na^+-gradient were found in the two preparations. It was concluded that a fully functional Na^+-D-glucose cotransport system is present with a proximo-distal gradient of activity during the early gestation period.

Ontogeny; Sugar transport; (Small intestine; Human)

1. INTRODUCTION

The functional differentiation of the human fetal small intestine occurs very early during the gestation period as evidenced by the appearance of brush-border membrane (BBM) enzymes like disaccharidases and alkaline phosphatase by 8–10 weeks of intrauterine life [1,2]. Active D-glucose transport has also been reported to be present in both jejunum and ileum of 11- to 19-week-old fetuses [3] and it would appear that the jejunum of the human fetus has developed an electrogenic hexose and amino acid transfer mechanism by the 12th week of gestation [4]. Since these early studies have analysed intracellular accumulation of glucose [3] or variation in electrical potential differences across the intestinal wall [4] follwing the mucosal addition of sugars to everted sacs of fetal small intestine, difficulties in the interpretation of the results were reported [4] and no hypothesis was made as to the nature of the transport system involved. However, inhibition of the changes in transmural potential differences by phlorizin, anoxic conditions and low Na^+ concentrations [4] are all compatible with the presence of an Na^+-D-glucose cotransport system in the early fetal small intestine. Such a hypothesis would agree with the recent demonstration of secondary active transport of sugars in the developing jejunum [5] and colon [6] of the rat but was never tested in the human fetus, probably due to the difficulty in obtaining sufficient amounts of tissue for such studies.

In the present paper, we have tested this hypothesis directly using BBM vesicles isolated

Correspondence address: C. Malo, Membrane Transport Research Group, Department of Physiology, Faculty of Medicine, University of Montreal, CP 6208, succursale 'A', Montreal, Quebec H3C 3T8, Canada

Abbreviations: BBMV, brush border membrane vesicle; BLM, basolateral membrane

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from 17- to 20-week-old human fetal small intestine as this technique has by now been widely used to study the characteristics of sugar cotransport systems in animals [7] and successfully applied to the adult human small intestine [8,9]. Our studies first justify this approach by showing that BBM vesicles can be easily purified from both the proximal and distal parts of the human fetal small intestine and then demonstrate aboral changes in Na^+-dependent cotransport of D-glucose using these vesicles.

2. MATERIALS AND METHODS

2.1. *Preparation of BBMVs*

Fresh, 17- to 20-week-old normal human fetal small intestines were kindly provided by Dr Michel Potier from Ste-Justine Hospital where social abortions have been performed. The jejunum and ileum were separated [4] and the mucosa of each segment was scraped, weighed and frozen in liquid nitrogen. An average of 6 tissues were pooled for each transport experiment. BBMs were purified by $CaCl_2$ precipitation [10] and vesicles prepared according to Hopfer et al. [11] as modified by Berteloot [12]. The purity of these preparations was routinely determined by assaying enzyme marker activities for the apical (sucrase) and basolateral (Na^+,K^+-ATPase) membranes in both the starting homogenates and the vesicle fractions.

2.2. *Transport studies*

Time course studies of D-[^{14}C]glucose or ^{22}Na uptake were performed using the rapid filtration technique of Hopfer et al. [11] as modified by Berteloot [12]. Freshly purified BBMVs were resuspended to a final protein concentration of 6–8 mg/ml in the final resuspension buffer. Aliquots were added to the incubation medium kept at room temperature to start the transport experiments. The exact compositions of resuspension buffers and incubation media are given in the legends to the figures. Uptake was terminated by the addition of quenched ice-cold stop-solution as described [12] except for the addition of 200 μM phlorizin. Results are expressed as nmol solute uptake/mg protein. Linear regression analysis was performed using an Apple IIe desk computer and a curve fitter program (P.K. Warme Copyright (C) 1980, Interactive Microware Inc.).

2.3. *Assays*

Sucrase activity was assayed by the method of Dahlqvist [13] as modified by Lloyd and Whelan [14] and Na^+,K^+-ATPase activity was determined according to Schwartz et al. [15]. Results are expressed as μmol of substrate hydrolysed $\cdot h^{-1} \cdot$ mg protein^{-1}. Protein was assayed according to Lowry et al. [16] using bovine serum albumin as standard.

2.4. *Chemicals*

Labeled compounds, namely D-[U-^{14}C]glucose (spec. act. 315 mCi/mmol) and D-[1(n)-^{3}H]mannitol (spec. act. 19.1 mCi/mmol) were purchased from New England Nuclear Corporation. ^{22}Na, carrier free (spec. act. 100–1000 mCi/mg), was purchased from Amersham. All salts and chemicals for buffer preparation were of the highest purity available.

3. RESULTS AND DISCUSSION

3.1. *Purity of BBMV from the human fetal small intestine*

The specific activity of the BBM marker enzyme, sucrase, showed 19.0- and 11.9-fold enrichment over the starting homogenates in BBMV fractions isolated from the jejunum and ileum, respectively (table 1). At the same time, the specific activity of a BLM marker enzyme Na^+,K^+-ATPase was decreased to 0.88 and 0.57, respectively, thus suggesting little contamination by BLMs. In fact, it can be calculated that this contamination represents less than 5% in both proximal and distal vesicle fractions. These results can be advantageously compared to those obtained in the adult small intestine [8] where enrichments of 11- and 1.6-fold have been reported for alkaline phosphatase and K^+-stimulated *p*-nitrophenyl phosphatase, respectively. These results clearly justify the suitability of our vesicle preparations for transport studies.

3.2. *Transport studies*

Time course studies of D-glucose uptake have been determined in 3 preparations of both jejunal and ileal BBMVs and the results of one typical experiment are shown in fig.1. In the presence of an Na^+ gradient (200 mM out), there was a transient overshoot of D-glucose uptake that peaked at 3 min in both proximal and distal small intestines.

Table 1

Enrichment of marker enzymes in BBMVs from human fetal jejunum and ileum

	Jejunum		Ileum	
	Mucosa	BBMV	Mucosa	BBMV
Proteins (mg)	454 ± 18[a]	3.3 ± 0.2	603 ± 31	6.1 ± 0.9
Sucrase (μmol sucrose hydrolyzed·h^{-1}·mg protein^{-1})	4.8 ± 0.6	91.2 ± 8.4 (19.0 ± 2.9)[b]	4.7 ± 0.3	54.6 ± 6.6 (11.9 ± 1.8)
Na^+,K^+-ATPase (μmol ATP hydrolyzed·h^{-1}·mg protein^{-1})	1.76 ± 0.72	1.47 ± 0.62 (0.88 ± 0.36)	0.80 ± 0.19	0.31 ± 0.16 (0.57 ± 0.43)

[a] Means ± SE
[b] Enrichment factor ± SE

However, the maximum intravesicular accumulation of sugar was significantly greater in the jejunum when compared to ileum with calculated ratios over equilibrium values of 9.4 and 4.7, respectively. When 200 μM phlorizin was present in the incubation medium or when outside NaCl was replaced by an equivalent concentration of KCl, the glucose overshoot completely disappeared and glucose uptake was identical in both proximal and distal segments of the small intestine. Similar results were obtained in vesicles loaded with mannitol instead of KCl (not shown). All together, these data indicate that the Na^+ (electro)chemical gradient provided the driving force for active D-glucose transport and thus demonstrate the presence of an Na^+-D-glucose cotransport system in the human fetal small intestine.

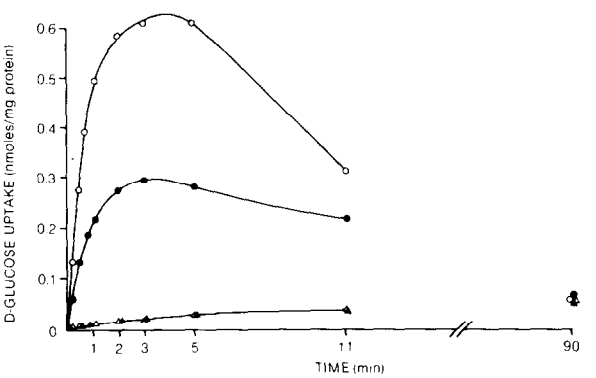

Fig.1. Time course of D-glucose uptake into BBMVs isolated from human fetal jejunum (○, △) and ileum (●, ▲). Vesicles were resuspended in 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, and 250 mM KCl. Incubation media contained 50 μM D-[^{14}C]glucose, 50 mM Tris-Hepes buffer, 0.1 mM $MgSO_4$, and either 250 mM KCl (△, ▲), or 200 mM NaCl and 50 mM KCl without (○,●) or with 200 μM phlorizin (△, ▲). Points shown are individual data points from the same preparation of vesicles and are representative of the three experiments performed under identical conditions.

The difference in the magnitude of the overshoot between jejunal and ileal BBMVs could be explained by different properties between the 2 preparations of vesicles like (i) substrate binding, (ii) Na^+ permeabilities, (iii) intravesicular volumes, (iv) kinetic characteristics, or (v) any combination of these.

To evaluate glucose binding contribution to total uptake, we have analysed the effect of decreasing the intravesicular space by increasing the medium osmolarity with mannitol on the equilibrium uptake of D-glucose. The results of this study are reported in fig.2 and clearly it appears that the amount of glucose taken up by the vesicles was inversely related to the medium osmolarity for both jejunal and ileal BBMVs. Also, extrapolation of the straight lines to infinite osmolarity (zero intravesicular space) only showed minimum binding of D-glucose in both preparations. These findings indicate that transport of D-glucose into an osmotically reactive intravesicular space occurred, and rule out substrate binding as an explanation for the differences in overshoot values observed in fig.1.

The Na^+ permeability of jejunal and ileal

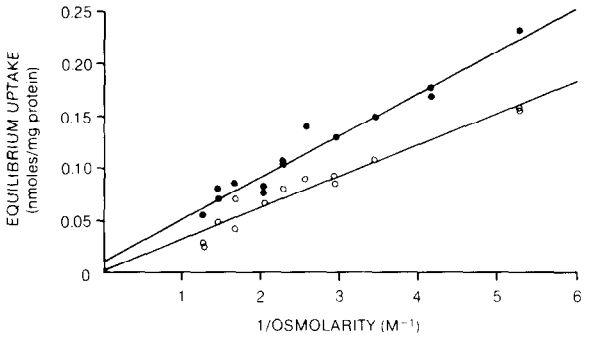

Fig.2. Effect of medium osmolarity on D-glucose uptake. Vesicles were resuspended in 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 50 mM NaCl, and 200 mM mannitol. Uptake studies were performed at 90 min of incubation in media containing 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 50 mM NaCl, 50 μM D-[^{14}C]glucose, and various concentrations of mannitol as to give the desired medium osmolarity. Linear regression analysis gave y intercepts, slopes and coefficients of correlation of 0.0020, 0.0302, 0.972 for jejunum (○) and 0.0124, 0.0406, 0.976 for ileum (●), respectively.

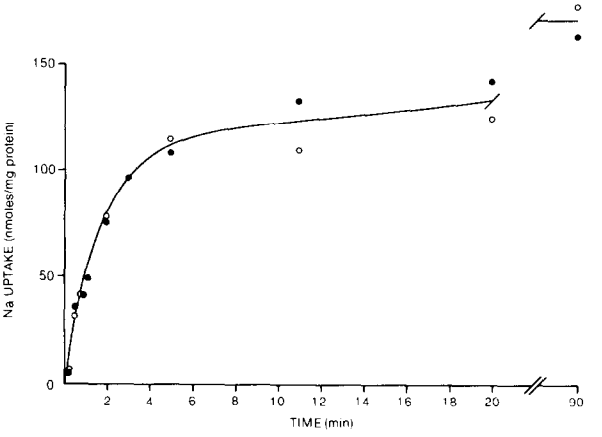

Fig.3. Time course of ^{22}Na uptake into jejunal (○) and ileal (●) BBMVs. Incubation media contained 50 μM D-glucose, 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, and 200 mM NaCl (0.3 μCi $^{22}Na^+$ per assay). The resuspension medium was as described in fig.1.

BBMVs was directly evaluated by studying the time course of ^{22}Na uptake under conditions similar to those of fig.1 and the results of this study are shown in fig.3. No significant differences between the 2 preparations could be observed over the whole time course, thus demonstrating equivalent dissipation of the Na^+-gradient across jejunal and ileal BBMVs and ruling out a faster collapse of the Na^+-gradient in ileal vesicles as an explanation for the differences in overshoot values observed in fig.1.

Intravesicular volumes of BBMVs can be estimated from the equilibrium uptake values of D-glucose (fig.1) or ^{22}Na (fig.3) and clearly it appears that identical values were observed for both proximal and distal vesicles in these 2 experiments. The comparison between the intravesicular volumes calculated from fig.1 (1.2–1.4 μl/mg protein) and fig.3 (0.82–0.89 μl/mg protein) also showed the absence of Na^+ binding to the vesicles as the small difference observed between these 2 values may just reflect the variations between different preparations of vesicles. Intravesicular volumes of BBMVs can also be estimated from the slopes of the straight lines obtained in fig.2. Assuming that BBMVs behaved as perfect osmometers, intravesicular volumes of 0.76 and 1.02 μl/mg protein can be calculated for proximal and distal BBMVs, respectively. Such values agree with those determined from equilibrium uptake values. All together, these results show clearly that differences in intravesicular volumes between jejunal and ileal vesicles cannot account for more than 8–25% variations in transport studies. For the same reasons, and for the identical Na^+ uptake (fig.3), one can exclude that the differences in Na^+-dependent glucose uptake between BBMVs from jejunum and ileum (fig.1) are due to different degrees of purity of the BBMVs from the two segments of intestine.

It thus appears that the differences observed for glucose uptake between jejunal and ileal BBMVs (fig.1) are best explained by different kinetic properties of the Na^+-dependent transport system(s) present in the proximal and distal parts of the human fetal small intestine. A final proof for this conclusion is shown in fig.4 where initial rates of Na^+-dependent D-glucose uptake (corrected for the phlorizin-insensitive component of uptake) have been determined in both preparations of vesicles. In both cases, linearity of uptake was observed for up to 30 s incubation with the substrate. However, the initial rate of D-glucose uptake in the ileum was 56% lower than that determined in the jejunum, a value that agrees closely

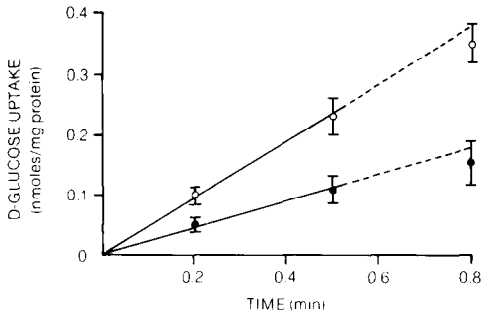

Fig.4. Initial rates of D-glucose uptake into proximal (○) and distal (●) BBMV. Experimental conditions were as described in fig.1. Results have been corrected for phlorizin-insensitive uptake and represent the means ± SD of three different experiments. Linear regression analysis gave y intercepts, slopes and coefficients of correlation of 0.011, 0.428, 0.986 for jejunum (○) and 0.007, 0.189, 0.948 for ileum (●), respectively.

with the 50% decrease in maximum overshoot values found in fig.1.

In summary, we have demonstrated for the first time that the 17–20-week-old human fetal small intestine does possess a functional Na^+-dependent transport system and that regional differences in the activity of this transport system are already established at this early period of gestation. This conclusion agrees with the observations reported by Koldovsky and co-workers [3,4] as to the presence of active sugar transport in the fetal small intestine with lower activity in the ileum when compared to the jejunum [3]. Our results have also established that the aboral differences observed in the human fetal small intestine are best explained by different kinetic properties of the transport system in proximal and distal segments. Assuming that only one carrier is present in the fetal small intestine [17], then differences in the maximum transport capacity of this system and/or differences in its density in the BBM would best explain our results. Kinetic studies are now in progress in our laboratory to elucidate this point.

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