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Effect of hyperglycemia on D-glucose transport across the brush-border and basolateral membrane of rat small intestine

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Experimental hyperglycemia leads to an increase in the capacity of the rat small intestine to absorb glucose. This effect occurs within hours from the onset of hyperglycemia and is thought to involve an induction of glucose transport in the brush-border and/or basolateral membrane of the intestinal epithelium. We devised a protocol for the simultaneous preparation of brush-border vesicles and basolateral vesicles from rat small intestine to determine the locus for the induction of glucose transporter in hyperglycemic rats. A 6 h period of intravenous infusion with a 30% glucose solution had no effect on the initial rate of glucose uptake across jejunal or ileal brush-border vesicles when measured in the absence of a Na^+ gradient, suggesting that enhanced glucose uptake is not dependent on an increase in the number of Na^+-dependent secondary active glucose transporters in the brush-border. Hyperglycemia did not effect the rate of glucose uptake across ileal basolateral vesicles but did cause a 78% increase in the initial rate of carrier-mediated D-glucose uptake across jejunal basolateral vesicles. The induction of glucose transport in the jejunal basolateral membrane was characterized by a rapid rate of glucose equilibration across the vesicles ($t_{1/2} = 46$ s sorbitol infused controls, 18 s hyperglycemia) and a 75% increase in the V_{max} for carrier-mediated glucose uptake with no significant change in K_t. When the rats were pretreated with cycloheximide prior to intravenous infusion, the initial rate of D-glucose uptake dropped to 13% of that seen in jejunal basolateral vesicles prepared from untreated rats. These results suggest a rapid turnover rate for the Na^+-independent glucose transporter in the basolateral membrane of the enterocyte. An increase in the number of functioning glucose transporters in the basolateral membrane may play an important role in the short-term induction of glucose absorption by the jejunum of the hyperglycemic animal.

Introduction

Diabetic animals, those fed a high carbohydrate diet or animals made hyperglycemic by an intravenous infusion of glucose show an increase in glucose absorption by the intestine [1–9]. The membrane locus and mechanism of this induction of glucose transport in the enterocyte is uncertain. Thomson [10] reported a 260% elevation in the V_{max} for active glucose uptake by the intestine of the diabetic rat. This induction may result from an increase in the number of Na^+-dependent glucose transporters in the brush-border or an increase in the down-hill electrochemical gradient for Na^+ across the apical membrane. Hopfer [12] suggested that the increased glucose accumulation observed in brush-border vesicles prepared from alloxan-treated rats was due to a decrease in the glucose-independent Na^+ leakage across the brush-border, leading to a greater driving force for the existing hexose carriers. Recently, short-term hyper-

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glycemia has been shown to increase the transcellular flux of glucose across the guinea-pig intestinal mucosa without affecting tissue levels of glucose, indicating that the increased transport which occurs in the brush-border is matched by a similar enhancement of transport across the basolateral membrane [7,11].

We have devised a procedure for the simultaneous preparation of brush-border and basolateral membrane vesicles from rat small intestinal mucosa. These vesicles were used to provide more direct information on the membrane locus of the induction of glucose transport following short-term hyperglycemia in the rat so that the role of the basolateral membrane in the regulation of transport across the small intestine could be properly assessed.

Materials and Methods

Preparation of membrane vesicles. Brush border vesicles and basolateral vesicles were prepared from rat intestinal mucosal scrapings using a modification of the technique of Scalera et al. [3]. Jejunal or ileal segments of the small intestine were removed from two to four male Sprague-Dawley rats (300–350 g) and the lumen was rinsed with 10 ml ice-cold phosphate-buffered saline (pH = 7.4). Mucosal scrapings were taken with a glass slide and placed in 60 ml ice-cold sucrose buffer (250 mM sucrose/2 mM Tris-HCl (pH 7.4)/0.1 mM phenylmethylsulfonyl fluoride). The suspended scrapings were homogenized using a polytron homogenized (4×30 s pulses) and centrifuged at $2500 \times g$ for 15 min. The resulting supernatant was removed and centrifuged at $20\,500 \times g$ for 20 min. This centrifugation produced a double pellet consisting of a hard brown lower pellet and a fluffy white upper pellet. The fluffy upper pellet was isolated by pouring off the supernatant, adding 10 ml of sucrose buffer and gently swirling the centrifuge tube such that only the fluffy pellet was dislodged. The resuspended fluffy pellet was poured off and used to purify basolateral vesicles while the remaining hard brown pellet was resuspended in 10 ml sucrose buffer and used to purify brush-border vesicles.

To purify basolateral vesicles, the resuspended fluffy pellet was subjected to glass-Teflon homogenization (10 strokes; 1500 rpm) mixed with percoll and sucrose buffer (final volume 35 ml; 12% v/v percoll) and centrifuged at $48\,000 \times g$ for 1 h to obtain a density gradient. The gradient was separated from top to bottom into 19 fractions and fractions 5, 6 and 7 were pooled as basolateral vesicles. The vesicle suspension was diluted with sucrose buffer to a volume of 35 ml and centrifuged at $48\,000 \times g$ for 30 min to obtain the basolateral vesicle pellet.

The resuspended lower brown pellet was subjected to glass-Teflon homogenization and mixed with $CaCl_2$ and sucrose buffer (final volume 35 ml; 10 mM $CaCl_2$). This mixture was incubated for 20 min on ice to allow preferential precipitation of non-brush-border components. The precipitated material was then pelleted by centrifugation at $3000 \times g$ for 10 min. The supernatant was centrifuged at $48\,000 \times g$ for 30 min to obtain the final brush-border vesicle pellet.

Enzyme assays. The purity and recovery of the brush-border and basolateral membranes were determined by measuring marker enzyme activities. Alkaline phosphatase and ouabain-sensitive ($Na^+ + K^+$)-ATPase were assayed according to Parkinson et al. [15]. Cytochrome *c* oxidase was assayed following the procedure of Cooperstein and Lazarow [16]. NADPH-cytochrome *c* reductase was assayed by the method of Sottocasa et al. [17].

Plasma glucose was determined using an automated glucose analyzer (Beckman instruments).

Transport assays. The uptake of the glucose into the vesicles was initiated by mixing a volume of vesicles with an equal volume of uptake media containing D-glucose + D-[^{3}H]glucose (final concentration 1.41 mg vesicle protein/ml; D-glucose + 100 μCi D-[^{3}H]glucose/μmol D-glucose). 40-μl samples were extracted from the mixture at the desired times, diluted with 1.125 ml of ice-cold stop solution (125 mM NaCl/2 mM Tris-HCl (pH 7.4)/0.1 mM $HgCl_2$) and 1.0 ml of the diluted mixture was transferred to a cellulose acetate filter (0.45 μm pore size). The filter was washed with 5 ml ice-cold stop solution then placed in scintillation vials for liquid scintillation counting. Glucose actually held by the vesicles was calculated by correcting for D-[^{3}H]glucose retained on the filter in the absence of vesicles.

For determining glucose uptakes using short

incubation periods (i.e., 10 s of less) a 20 μl drop of vesicles and a 20 μl drop of uptake media were placed in close proximity on the bottom of a polycarbonate tube. Uptake was initiated by vortexing the tube and, at the conclusion of the uptake period, 1.125 ml of ice-cold stop solution was rapidly injected into the mixture. Glucose held by the vesicles was determined as previously described.

Intravenous infusions. Venous infusion experiments were carried out as described by Czáky and Fisher [7]. Male Sprague-Dawley rats (300–350 g) were anesthetized with pentobarbital (50 mg/kg body weight) and a segment of silastic tubing was inserted into the jugular vein and pushed into the heart. The tubing was brought under the skin and exposed through an incision on the back of the animal. On the day of use, the animal was placed in a small cage and connected to an infusion tube. A 2 ml bolus of a 60% glucose or 60% sorbitol solution was initially infused followed by a constant infusion of a 30% glucose or 30% sorbitol solution at a rate of 2.0 ml/h. At the conclusion of the infusion period, the animal was killed and brush-border and basolateral vesicles were prepared from either the jejunum or ileum.

Materials. D-[^{3}H]Glucose and L-[^{14}C]glucose were obtained from New England Nuclear. Cycloheximide, D-glucose and L-glucose were purchased from Sigma Chemical Co.

Results

Purification and glucose transport characteristics of the brush-border and basolateral membrane preparations

The purity of the brush-border and basolateral membrane preparations was determined by marker enzyme assays (Table I). Using alkaline phosphatase as a marker for the brush-border membrane, we found a 17-fold increase in specific activity in the final brush-border pellet relative to the initial homogenate. There was little contamination of the brush-border preparation by basolateral membrane, mitochondria or microsomes. The basolateral membrane pellet was purified 18-fold as judged by the increase in the specific activity of oubain-sensitive ($Na^+ + K^+$)-ATPase and was essentially free of contamination by other membranes.

TABLE I

MARKER ENZYME ANALYSIS OF MUCOSAL HOMOGENATE (MH), BRUSH-BORDER MEMBRANE (BBM) AND BASOLATERAL MEMBRANE (BLM) PREPARATION

Alkaline phosphatase: spec. act., μmol *p*-nitrophenol produced/h per mg protein; ouabain-sensitive ($Na^+ + K^+$)-ATPase: spec. act., μmol PO_4 produced/h per mg protein; cytochrome *c* oxidase: spec. act., μmol cytochrome *c* oxidized/h per mg protein; NADPH-cytochrome *c* reductase: spec. act., μmol cytochrome *c* reduced/h per mg protein.

	MH	BBM	BLM
Alkaline phosphatase			
specific activity	11.3	192.1	0.9
enrichment factor		17.0	0.1
Ouabain-sensitive ($Na^+ + K^+$)-ATPase			
specific activity	10.8	3.2	194.8
enrichment factor		0.3	18.0
Cytochrome *c* oxidase			
specific activity	85.0	15.3	2.1
enrichment factor		0.2	0.00
NADPH-cytochrome *c* reductase			
specific activity	3.47	0.91	0.03
enrichment factor		0.26	0.01

The protocol for the simultaneous purification of brush-border and basolateral vesicles was used to determine the characteristics of glucose uptake across vesicles prepared from the jejunum and ileum. It is generally accepted that glucose uptaken into jejunal brush-border vesicles occurs by accumulation within an intravesicular aqueous compartment and does not include any binding of glucose to the vesicle membrane. However, there is little information on glucose binding to ileal brush-border vesicles, or basolateral vesicles prepared from the ileum or jejunum. Glucose binding can be determined by allowing glucose to equilibrate across the vesicles then adding increasing amounts of a poorly transported solute such that the osmolarity of the media is increased. This procedure will shrink the vesicles and displace some of the glucose held within the intravesicular aqueous compartment. By extrapolating to infinite osmolarity (i.e., zero intravesicular space) it is possible to estimate the binding component of glucose uptake by brush-border or basolateral vesicles. Fig. 1 shows the effects of increasing media osmolarity on D-glucose content of jejunal

and ileal brush-border and basolateral vesicles. In all cases the y-intercept of the regression line is not significantly different from 0 ($P > 0.05$), indicating that the glucose associated with brush-border and basolateral vesicles represents accumulation within an intravesicular aqueous space and does not include any binding component.

The time-dependent profile for D-glucose uptake across jejunal and ileal brush-border and basolateral vesicles in the presence of an initial NaSCN gradient (100 mM outside; 0 inside) are shown in Fig. 2A. In agreement with Wright et al. [18], we found that pretreatment of the vesicles with $HgCl_2$ poisoned the carrier-mediated glucose transport and resulted in a D-glucose uptake profile that resembled the uptake of L-glucose. Further, the passive permeability of the basolateral

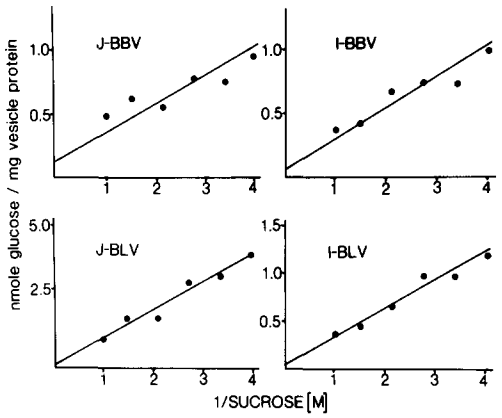
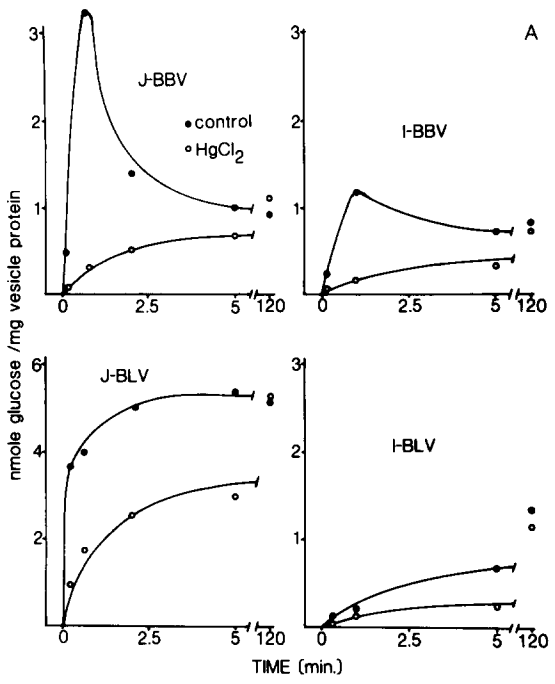


Fig. 1. Effect of media osmolarity on the equilibrated glucose content of brush-border vesicles (BBV) and basolateral vesicles (BLV) prepared from rat jejunum (J) or ileum (I). Vesicles were prepared as described in Materials and Methods and the final vesicle pellets were resuspended in 250 mM sucrose/2 mM Tris-HCl (pH 7.4) to give a concentration of 2.82 mg vesicle protein/ml. The vesicle resuspensions were mixed with an equal volume of uptake media containing 2 mM D-glucose + D-[^{3}H]glucose/250 mM sucrose/2 mM Tris-HCl (pH 7.4). Glucose was allowed to equilibrated across the vesicles during a 10 min incubation at room temperature. The equilibrated mixtures were then divided into six fractions and sucrose was added to each fraction to give the indicated final concentration. After a 10 min re-equilibration period, samples were taken to assay vesicle glucose content using the microporous filtration technique. Each point represents the mean glucose content obtained from triplicate assays of the fraction. In all cases the y-intercept of the regression line is not significantly different from 0 nmol glucose/mg vesicle protein ($P > 0.05$).

vesicles as assessed by the uptake of L-glucose was unaffected by pretreatment with $HgCl_2$ (Fig 2B). As such, we employed $HgCl_2$ treatment as a more convenient and economical method of estimating the carrier-independent component of D-glucose uptake. Samples were taken at time intervals up to 5 min from initiation to determine the profile of D-glucose uptake and at 120 min to determine the final equilibrated glucose content of the vesicles. The Na^+ gradient drove an overshoot of D-glucose into basolateral vesicles that was higher in the jejunum (3.7-fold over equilibrated levels) compared to the ileum (1.4-fold over equilibrated levels). In contrast, the Na^+ gradient did not cause any overshoot in D-glucose accumulation across basolateral vesicles. The equilibrated glucose levels of basolateral vesicles were generally between 3 and 4-fold greater in the jejunum compared to the ileum. In addition, the rate of Hg-sensitive D-glucose uptake was rapid across jejunal basolateral vesicles (complete equilibration at 2.5 min from initiation of uptake) while there was very little carrier-mediated glucose uptake across ileal basolateral vesicles as indicated by comparing the uptake profiles in untreated versus $HgCl_2$-treated vesicles.

In order to detect changes in D-glucose transport across intestinal vesicles it is necessary to accurately measure the initial rate of carrier-mediated D-glucose translocation. In these and all subsequent experiments, the final brush-border and basolated vesicle pellets were resuspended in a solution containing 125 mM NaSCN and allowed to equilibrate prior to initiation of glucose uptaken. As such, NaSCN is present on both sides of the vesicle membrane and changes in vesicle leakiness to Na^+, such as reported by Hopfer [12], will not effect glucose uptake rates. This procedure was adopted so that conclusions could be drawn as to whether there was an intrinsic change in the behaviour of the glucose carriers in the apical membrane rather than a less specific alteration in sodium conductivity.

Fig. 3 shows the results obtained in measuring the rapid time course for D-glucose uptake across brush-border and basolateral vesicles. By sampling the uptake mixture at 10 s or less with brush-border vesicles and 5 s or less with basolateral vesicles it is possible to determine the initial rate of glucose



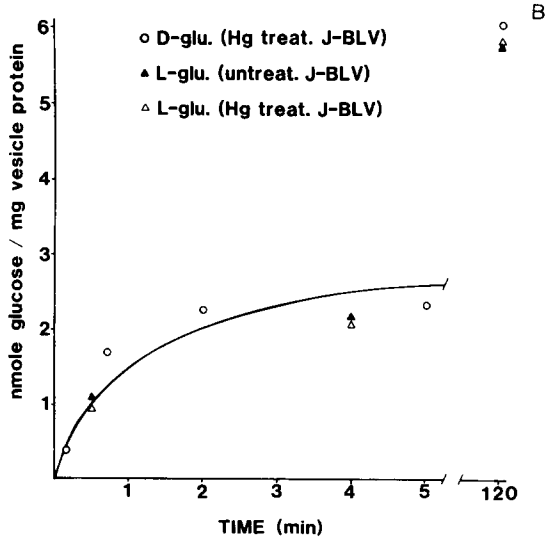


Fig. 2A. Time course for D-glucose uptake across the jejunal and ileal brush-border vesicles (BBV) and basolateral vesicles (BLV) in the presence of a NaSCN gradient. Vesicles were prepared and resuspended as described in Fig. 1. The vesicle resuspensions were divided into control and Hg-treated fractions (final concentration 1.0 mM $HgCl_2$) and incubated for 10 min at room temperature. Glucose uptake was initiated (time = 0) by mixing the vesicle suspension with an equal concentration of uptake media (2 mM D-glucose + D-[^{3}H]glucose/200 mM NaSCN/250 mM sucrose/2 mM Tris-HCl (pH 7.4). Uptakes were performed in triplicate and the points represent the mean glucose content of the vesicles at the indicated time from initiation of uptake. B. Effect of Hg-treatment on the passive permeability of jejunal basolateral vesicles. Time course for D-[^{3}H]glucose and L-[^{14}C]glucose uptake and $HgCl_2$ pretreatment of basolateral vesicles was performed as described in A.

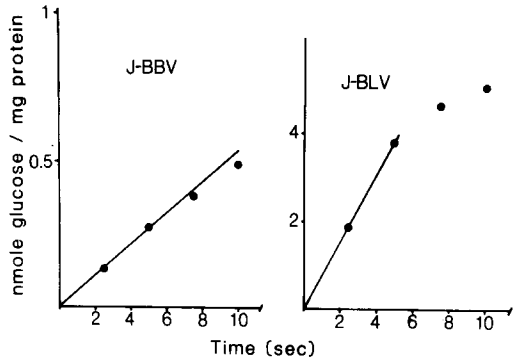


Fig. 3. Initial rate of D-glucose equilibration across jejunal brush-border vesicles (BBV) and basolateral vesicles (BLV). The vesicle pellets were resuspended in 125 mM NaSCN/2 mM Tris-HCl to give a concentration of 2.82 mg vesicle protein/ml and allowed to equilibrate overnight at 4°C. Glucose uptakes were initiated by mixing a 20 μl drop of vesicle suspension with a 20 μl drop of uptake media (2 mM D-glucose + D-[^{3}H]glucose/125 mM NaSCN/2 mM Tris-HCl (pH 7.4). The drops were placed in close proximity on the bottom of a polycarbonate tube and uptake was performed as described in Materials and methods. Each point represents the mean value determined from three assays of vesicle glucose content at the indicated time from initiation of uptake.

translocation across the vesicles. In order to be certain that we consistently measured initial rates, the vesicle mixture was sampled at 2 s after initiation of uptake for all subsequent experiments.

Effect of short-term hyperglycemia and cycloheximide pretreatment on D-glucose uptake across jejunal and ileal brush-border and basolateral vesicles

In this series of experiments, a sorbitol or glucose solution was infused into the jugular vein of rats for a 6 h period. Glucose infusion resulted in a significant hyperglycemia compared to sorbitol-infused controls rats (plasma glucose = 371 ± 19 mg/dl glucose infused; 185 ± 35 mg/dl sorbitol infused). After the infusion, brush-border and basolateral vesicles were prepared from the

jejunum or ileum and the vesicles were pre-equilibrated with NaSCN prior to transport assays.

Under the conditions of this experiment, glucose uptake is composed of carrier-mediated translocation plus diffusion. It is possible to estimate diffusion by measuring glucose uptake across Hg-poisoned vesicles. The initial rate of carrier-mediated D-glucose uptake can then be estimated by subtracting the Hg-insensitive component from the total uptake rate.

Pretreatment of rats with cycloheximide and/or hyperglycemia did not effect the rate of glucose uptake across Hg-treated basolateral or brush-border vesicles compared to glucose uptake across Hg-treated vesicles prepared from sorbitol-infused control rats (average initial rates = 23.5 and 18.8 mol/mg protein per s across basolateral and brush-border vesicles, respectively). These data indicate that cycloheximide and/or hyperglycemia does not effect the rate of glucose diffusion across the vesicles.

Hyperglycemia had no effect on the Hg-sensitive component of glucose uptake across jejunal brush-border vesicles. However, we did find a 78% increase in the initial rate of transport-mediated glucose uptake across jejunal basolateral vesicles prepared from hyperglycemic rats when compared to uptake rates across vesicles prepared from sorbitol-infused controls (Table II).

Treatment of rats with cycloheximide is thought to inhibit protein synthesis and has been reported to block the rapid induction of glucose uptake across rat jejunum following hyperglycemia [7] or perfusion of the ileum with a glucose solution [11]. We pretreated rats with cycloheximide and repeated our intravenous infusion experiments. Cycloheximide pretreatment apparently had no effect on the rate of Hg-sensitive uptake across brush-border vesicles prepared from sorbitol-infused or hyperglycemic rats (Table II). However, cycloheximide had substantial effects on glucose uptake rates across basolateral vesicles. In sorbitol-infused rats, the rate of Hg-sensitive uptake across basolateral vesicles was 13% of that seen without cycloheximide treatment and in hyperglycemic rats uptake rates across basolateral vesicles dropped to 19% of the rate determined in the absence of cycloheximide treatment. In addition, there was no statistically significant differences in glucose uptake rates across basolateral vesicles prepared from sorbitol-infused versus hyperglycemic rats which had been pretreated with cycloheximide. However, the mean uptake across the basolateral vesicles prepared from cycloheximide-treated hyperglycemic animals is at least double that for untreated hyperglycemic ones, suggesting that there may well be induction of transport in the absence of protein synthesis, al-

TABLE II

GLUCOSE UPTAKE ACROSS VESICLES PREPARED FROM SORBITOL- AND GLUCOSE-INFUSED RATS

Six rats were infused with sorbitol or glucose as described in Materials and Methods and brush-border vesicles (BBM) and basolateral vesicles (BLV) were prepared from the jejunum or ileum. Vesicles were pre-equilibrated with NaSCN as described in Fig. 3 and the initial rates of. 1.0 mM D-glucose uptake were measured in the presence and absence of 1.0 mm $HgCl_2$. Each infusion was repeated three times and the values represent the mean ± S.E. of the Hg-sensitive component of D-glucose. Common letter indicates significant difference between means ($P = 0.05$). Cycloheximide-treated rats were those animals pretreated with 3 mg/kg cycloheximide (intravenous injection) prior to infusion.

	Initial rates (pmol/mg protein per s)			
	sorbitol-infused		glucose-infused	
	untreated	cycloheximide-treated	untreated	cycloheximide-treated
Jejunal				
BLV	123.0 ± 14.3 [ab]	15.8 ± 9.1 [a]	219.5 ± 23.1 [ab]	42.4 ± 8.9 [c]
BBV	48.1 ± 5.1	32.7 ± 11.2	37.4 ± 9.2	62.9 ± 11.7
Ileal				
BLV	19.5 ± 1.5	–	16.0 ± 1.5	–
BBV	30.8 ± 4.9	–	16.5 ± 2.7	–

though the high standard errors for these two values make it difficult to make firm conclusions on this point.

Table II also shows the effect of short-term hyperglycemia on the initial rate of glucose uptake across ileal brush-border and basolateral vesicles. The comparatively low rates of glucose uptake across ileal vesicles were not altered by short-term hyperglycemia.

Effect of hyperglycemia on the time course of glucose equilibration across jejunal basolateral vesicles

An accelerated initial rate of uptake should result in a more rapid equilibration of glucose across a membrane vesicle. We compared the time course of glucose equilibration across jejunal basolateral vesicles prepared from hyperglycemic and sorbitol-infused rats. Hyperglycemia induced a rapid equilibration of glucose that was complete by 2 min from initiation of uptake with an estimated time to half equilibration ($t_{1/2}$) of 18 s. In comparison, the rate of glucose equilibration across basolateral vesicles prepared from sorbitol-infused rats was markedly slower with an estimated $t_{1/2}$ of 46 s (Fig. 4).

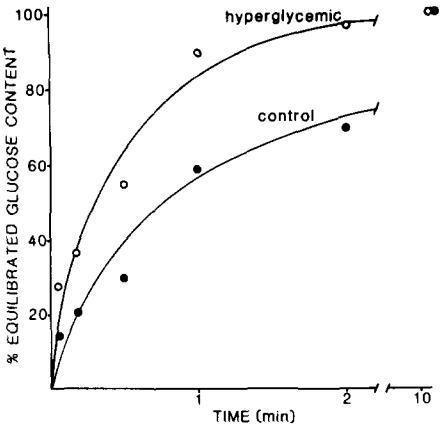


Fig. 4. Time course of D-glucose equilibration across jejunal basolateral vesicles prepared from hyperglycemic and sorbitol-infused rats. Vesicles were pre-equilibrated with 125 mM NaSCN as described in Fig. 3. and the time course for equilibration of 1.0 mM D-glucose was determined by sampling the uptake mixture at the indicated times. Triplicate uptakes were performed on each of three preparations of vesicles obtained from sorbitol- or glucose-infused rats. The points represent the mean basolateral vesicle glucose content expressed as a percentage of the final (10 min) equilibrated glucose level.

Kinetic parameters of glucose uptake across jejunal basolateral vesicles prepared from hyperglycemic and control rats

A change in the rate of glucose equilibration could be due to an effect on V_{max} and/or K_t. We measured initial rates using varying concentrations of glucose to determine the effects of hyperglycemia on the kinetic parameters of glucose transport across jejunal basolateral vesicles.

The initial rates of glucose uptake across basolateral vesicles prepared from hyperglycemic rats were greater than controls at all concentrations of glucose tested (Fig. 5). At concentrations over 50 mM there is a linear relationship between glucose concentration and initial rate of uptake. At these high concentrations, transport-mediated processes are saturated and the slope of the line can be used as an estimate of the diffusion component of glucose uptake. In agreement with the data obtained using Hg-treated vesicles, we found that hyperglycemia did not affect the rate of glucose diffusion across the vesicles (16.6 mol glucose/mg protein per sec per mM glucose, control basolateral vesicles; 20.5 mol glucose/mg protein per s per mM glucose, test basolateral vesicles).

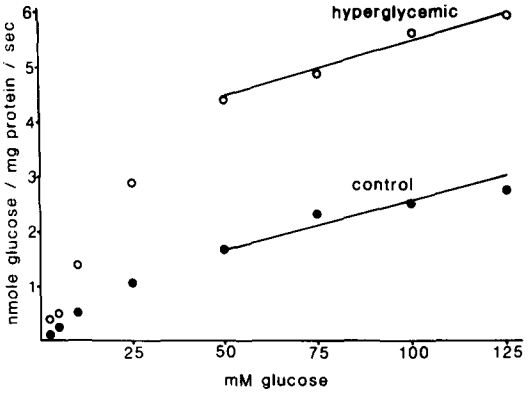


Fig. 5. Initial rates of D-glucose uptake across jejunal basolateral vesicles prepared from hyperglycemic and sorbitol infused rats. Basolateral vesicles were pre-equilibrated with 125 mM NaSCN and the initial rates of glucose uptake were determined as described in Fig. 3. The uptake mixture contained 125 mM NaSCN + D-[^{3}H]glucose + varying concentrations of D-glucose and mannitol. In all cases the final concentration of D-glucose plus mannitol equaled 125 mM. Points represent the mean values of initial rates as determined in triplicate on each of three preparations of vesicles obtained from glucose- or sorbitol-infused rats.

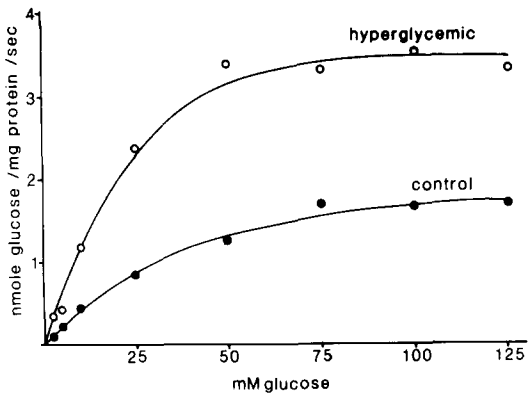


Fig. 6. Initial rates of carrier-mediated D-glucose uptake across jejunal basolateral vesicles prepared from hyperglycemic and sorbitol-infused rats. The linear portion of the curves in Fig. 5 were used to estimate the rate of diffusion of D-glucose across the vesicles as a function of glucose concentration. The data were then corrected for diffusion to determine the initial rates of carrier-mediated D-glucose uptake.

Fig. 6 shows the initial rate of transport-mediated glucose uptake after correcting for diffusion. The data are indicative of a Michaelis-Menten relationship between initial rate of uptake and glucose concentration. We calculated the V_{max} and K_t of transport-mediated uptake using an Eadie-Hofstee transformation. Short-term hyperglycemia induced a 75% increase in the V_{max} of glucose transport across jejunal basolateral vesicles when compared to corresponding control vesicles (Table III). There was no statistically significant differences in the K_t values for glucose transport across vesicles prepared from hyperglycemic versus sorbitol-infused rats.

TABLE III

KINETIC PARAMETERS OF CARRIER-MEDIATED D-GLUCOSE TRANSPORT ACROSS JEJUNAL BASOLATERAL VESICLES PREPARED FROM SORBITOL- AND GLUCOSE-INFUSED RATS

Eadie-Hofstee transformations were performed on the data presented in Fig. 6 to determine the V_{max} and K_t of D-glucose transport across basolateral vesicles. Values represent mean ± S.E. and * indicates a significant difference using basolateral vesicles prepared from hyperglycemic rats when compared to the corresponding value obtained with vesicles prepared from sorbitol-infused controls.

	Sorbitol-infused	Glucose-infused
V_{max} (nmol/mg protein per s)	2.57 ± 0.26	4.51 ± 0.50 *
K_t (mM)	53.4 ± 7.6	28.3 ± 7.6

Discussion

The overall process of glucose absorption by the intestine involves a Na^+-dependent secondary active transport across the brush-border followed by a Na^+-independent facilitated exit across the basolateral membrane.

The jejunal and ileal brush-border vesicles showed the characteristic Na^+-driven overshoot of glucose uptake. The magnitude of the overshoot was considerably larger in jejunal brush-border vesicles and, in the absence of a Na^+-gradient, the initial rate of glucose uptake across jejunal brush-border vesicles was twice that obtained with those prepared from the ileum. These results are similar to those obtained by other workers [19,20] and provide additional supportive evidence that there is less Na^+-dependent D-glucose transport activity per mg brush-border protein in the ileum than in the jejunum.

There is less information available on the glucose transport characteristics of purified basolateral vesicles. Earlier preparations of basolateral vesicles suffered from contamination by the brush-border which made interpretation of uptake data difficult [21]. With the use of density gradient purification it is now possible to prepare basolateral vesicles that are essentially free of brush-border contamination [13,18,22]. Our results are comparable with those obtained by other laboratories [18,22] in that the carrier-mediated D-glucose uptake across purified jejunal basolateral vesicles was rapid with no Na^+-driven glucose overshoot. In contrast to the rapid glucose translocation across jejunal basolateral vesicles, there is little evidence of carrier-mediated D-glucose transport across ileal basolateral vesicles. We found only marginal Hg-sensitive D-glucose uptake and Wright et al. [18] found no difference between the uptake profiles for D and L-glucose across ileal basolateral vesicles.

There is considerable evidence that signal(s) associated with experimental diabetes, hyperglycemia and elevated lumenal carbohydrate lead

to enhanced active glucose uptake across the apical pole of the intestinal mucosa. Fischer and Lauterbach [8] found an increased flux of glucose across both the apical and serosal pole of the isolated guinea-pig mucosa taken from animals made hyperglycemic for 12 h. Alloxan treatment causes a dramatic increase in the V_{max} for active glucose transport [10] and the enhanced glucose uptake by the rat jejunum following glucose perfusion through the lumen of the ileum is sensitive to inhibition by phlorhizin [11]. Brush-border vesicles prepared from alloxan-treated rats show an increase in the Na^+-driven glucose overshoot but no change in the estimated half-time for glucose equilibration in the absence of a Na^+ gradient [12]. We were able to measure actual initial rates for glucose uptake across brush-border vesicles in the absence of a sodium gradient and our data indicate that, at least for hyperglycemic animals, there is no change in the capacity for glucose transport across the brush-border.

In this study, hyperglycemia induced an increase in the V_{max} of carrier-mediated glucose uptake across jejunal basolateral vesicles, suggesting an increase in the number of functioning glucose transporters in the basolateral membrane. Hyperglycemia in guinea-pigs is known to increase glucose flux across the serosal pole of isolated jejunal mucosa [8]. Czáky and Fisher [7] found that the acute increase in glucose uptake across the jejunum of hyperglycemic rats was more sensitive to inhibition by phloretin than by phlorizin, suggesting to these authors that induction of transport occurs in the basolateral membrane. Cycloheximide completely blocked this enhanced glucose uptake by the jejunum. In our study, pretreating the rats with cycloheximide prior to the 6 h infusion period caused a substantial drop in the initial rate of carrier-mediated glucose uptake across jejunal basolateral vesicles prepared from sorbitol- or glucose-infused animals, indicating a marked reduction in the number of functioning transporters per mg basolateral membrane protein. This suggests a rapid rate of turnover for the Na^+-independent glucose transporter in the basolateral membrane of the enterocyte. Induction of glucose transporter in the basolateral membrane could work in conjunction with an improved efficiency of active transport across the brush-border to maximize net glucose absorption by the jejunum of the hyperglycemic animal.

The regulation of ion transport across epithelial has been shown to involve both the apical and basal membranes to minimize intracellular concentration changes of the transported solutes [20,21]. Similarly, for non-ionic substrates such as hexoses, the lack of increased intracellular accumulation following induction of transport and the now clearly demonstrated increased flux across the basolateral membrane indicate that this regulation also involves both poles of the enterocyte.

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