Biochimica et Biophysica Acta, 1107 (1992) 86–92

BBAMEM 75642

Streptozotocin diabetes and sugar transport by rat ileal enterocytes: evidence for adaptation caused by an increased luminal nutrient load

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(Received 8 November 1991)

Key words: Diabetes mellitus; Intestinal sugar transport; Intestinal mucosa

Preparations of villus enterocytes and brush border membrane vesicles have been used to study the effects of streptozotocin-induced diabetes mellitus in rats on sugar transport across the brush border and basolateral membranes of ileal epithelial cells. In isolated cells, diabetes increased Na^+-dependent galactose transport across the brush border of mid-villus but not upper villus cells. Galactose transport across the basolateral membrane was, however, enhanced by diabetes in both cell populations. Kinetic analysis of vesicle data suggested the presence of two transporters for Na^+-dependent glucose transport. Diabetes induced a 5-fold increase in both K_T and V_{max} of the high-affinity/low-capacity system together with a 2-fold increase in the V_{max} of the low-affinity/high-capacity transporter. Glucose was almost undetectable in the lumen of the upper and lower ileum in control animals but was present at high levels (26.1 ± 4.3 mM and 6.5 ± 1.3 mM) in diabetic rats. The possible significance of these changes in luminal sugar concentration in relation to the adaptation of transport across ileal enterocytes is discussed.

Introduction

Diabetes mellitus in rats has a stimulatory effect on Na^+-dependent sugar transport across the jejunal epithelium [1]. Recent studies in our laboratory using streptozotocin-diabetic rats have shown this response to be a combination of enterocyte hyperplasia [2], subtle alterations in the distribution of Na^+-dependent transport along the villus axis [2,3], and an enhanced electrochemical gradient for Na^+-dependent transport at the brush border membrane [4,5].

The signals which initiate these adaptive changes are unclear. An altered endocrine status is undoubtedly important and there is much evidence suggesting that hyperglycaemia per se directly influences sugar movement from lumen to blood [6,7]. An additional important factor may be the level of the transported sugar in the intestinal lumen [8–11] and the simplest explanation is that glucose within the lumen acts directly on enterocytes to induce the synthesis of its own transporter.

Since diabetic rats are hyperphagic, the resulting increased nutrient load entering the small intestine may affect luminal glucose concentration particularly in more distal regions. In non-diabetic animals, the efficiency of absorption in the jejunum ensures only very low levels of the sugar in the ileal lumen [12,13]. Using isolated enterocytes and purified brush border membrane vesicles, the aim of this present study was to investigate the effects of experimental diabetes mellitus in rats on sugar transport across the brush border and basolateral membranes of enterocytes in the lower small intestine and to discuss the changes observed in relation to alterations in the level of luminal glucose.

Methods

Animals

Experiments utilised male Sprague-Dawley rats (initial weight 220–250 g). Diabetes was induced by a single tail vein injection of streptozotocin (65 mg/kg, dissolved in pH 4.5 citrate buffer) with the animal under light ether anaesthesia. Prior to their use 20–25 d later, they were allowed free access to a standard maintenance diet. Blood samples for the measurement of plasma glucose concentration were obtained by cardiac puncture carried out immediately before removal of intestinal tissue whilst the animal was anaesthetised with pentobarbitone sodium (90 mg/kg i.p). A glucose oxidase method (Sigma, kit No. 510) was utilised for assay of the sugar.

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Intraluminal glucose concentration

Segments of intestine 5 cm in length at two positions (10 cm and 30 cm from ileo-caecal junction) were tied off in vivo. The contents of each section were collected in a cooled tube and centrifuged at $600 \times g$ for 20 s. The glucose concentration of the supernatant was measured using a glucose oxidase method.

Intestinal histology

Ileal sections approximately 1 cm long taken from a position 20 cm from the ileo-caecal junction were placed in 10% formol saline. The tissue was later blocked in paraffin wax, sectioned longitudinally (5 μm) and stained with Haematoxylin and Eosin. Measurements of villus height and enterocyte column size (the number of cells along one side of the villus) were determined under light microscopy.

Isolated enterocytes

The method of Weiser [14] with minor modifications [2] was used to isolate upper villus and mid-villus enterocytes. In brief, a 25 cm length of ileum ending 10 cm from the ileal-caecal junction was removed and washed through with ice-cold NaCl (154 mM) containing dithiothreitol (DTT, 0.5 mM). One end was ligated and the segment filled with an oxygenated buffer (pH 7.3) containing (in mM): NaCl, 96; KCl, 1.5; KH_2PO_4, 8; Na_2HPO_4, 5.6; sodium citrate, 27; β-hydroxybutyric acid, 0.5; and bovine serum albumin (BSA), 1 mg/ml. The other end was ligated and the sac was incubated for 15 min at 37°C. The luminal solution was discarded and the segment filled with a second oxygenated buffer containing (in mM): NaCl, 137; KH_2PO_4, 11.5; Na_2HPO_4, 8; KCl, 2.2; β-hydroxybutyrate, 0.5; EDTA, 1.5; DTT, 0.5; and BSA (1 mg/ml). The segment was incubated at 37°C for 8 min (control) or 12 min (diabetic) and upper villus enterocytes were collected by manually dislodging the cells and washing through with ice-cold bicarbonate saline containing BSA (1 mg/ml). The sac was refilled with the EDTA buffer and incubated at 37°C for a further 6 (control) or 8 min (diabetic) in order to harvest mid-villus cells. Cell fractions were washed twice in bicarbonate-BSA buffer and finally resuspended in this buffer to a protein concentration of 12–18 mg/ml. Cells were kept in plastic beakers on ice until their use 10–60 min later. We have previously shown that enterocytes prepared using these techniques effectively exclude Trypan blue dye and are able to accumulate D-galactose against a concentration gradient [2].

For the measurement of sugar uptake, 1 ml of cell suspension was added to 2 ml of gassed (95% O_2: 5% CO_2) bicarbonate-BSA buffer and the cells pre-incubated for 10 min at 37°C. Phlorizin (1 mM) was present in some experiments. The uptake process was initiated by adding 1 ml pre-warmed bicarbonate-BSA buffer containing D-galactose and D-[^{3}H]galactose (final concentration, 10 mM, 0.28 Ci/mol) together with [^{14}C]inulin (0.23 μCi/ml) as an extracellular space marker. The mixture was shaken (70 oscillations/min) during the incubation to ensure adequate mixing of the cell suspension. The uptake process was terminated by adding 250-μl aliquots of the suspension to 500 μl ice-cold saline containing phlorizin (1 mM) and the cells separated by centrifugation ($12000 \times g$, 3 min) through a 250 μl layer of oil (dl-*n*-butyl phthalate/dinonyl phthalate, 2:3 v/v). The cell pellets were lysed in 0.5% Triton X-100, 5% TCA was added to precipitate cellular protein and, after centrifugation, the supernatant subjected to dual-label scintillation counting. Galactose uptake was expressed as nmol per mg cell protein with protein being estimated using the method of Bradford [15]. Sugar uptake measured in the presence of phlorizin represented facilitated movement across the basolateral membrane whilst initial unidirectional entry across the brush border was estimated by subtracting data obtained in the presence of phlorizin from that obtained in its absence.

Brush border membrane vesicles

The techniques used have been published previously [16]. In brief, mucosal scrapes from lower intestinal segments were suspended in 50 mM mannitol, 2 mM Hepes (pH 7.1, 25 ml/g tissue), homogenised three times at half speed for 20 s using an Ultra Turrax homogeniser (Janke and Kunkel, Germany). and 1 M $MgCl_2$ was added to achieve a final concentration of 10 mM. After stirring on ice for 20 min, the mixture was centrifuged for 10 min at $3000 \times g$, and the supernatant recentrifuged at $27000 \times g$ for 30 min. The brush border pellet was resuspended in 20 ml resuspension buffer (300 mM mannitol, 20 mM Hepes, 0.1 mM $MgSO_4$ by passing five times through a 21 gauge needle. This suspension was centrifuged for 15 min at $6000 \times g$ and the supernatent recentrifuged at 27000 $\times g$ for a further 30 min. All the above steps were carried out at 4°C.

The pellets consisting of purified brush border membrane vesicles was suspended in resuspension buffer to achieve a protein concentration of 2–4 mg/ml and stored on ice. The concentration of protein and the activities of sucrase and alkaline phosphatase in the initial homogenate and brush border suspension were determined [17,18] in order to calculate enrichment values for these enzymes in the membrane preparation.

Uptake studies were carried out at 20°C on freshly prepared vesicles. For time course experiments, uptake was initiated by mixing equal volumes of vesicle suspension and 200 μM D-[^{3}H]glucose (8.7 Ci/mmol) dissolved in uptake solution consisting of 200 mM NaSCN, 20 mM Hepes and 0.1 mM $MgSO_4$ so that

concentrations of glucose and Na^+ in the uptake mixture were 100 μM and 110 mM, respectively. Aliquots (50 μl) of the solution were removed at intervals of 10 s to 15 min added to 2 ml stop solution consisting of ice-cold NaCl (154 mM) and phlorizin (0.5 mM) and filtered under vacuum using 0.45 μm nitrocellulose filters (Whatman). The filters were washed with an additional 6 ml stop solution and placed in scintillation vials. Filtran scintillation fluid (National Diagnostics) was added and vials counted in a Packard Scintillation Counter (Model 4430). Counts were converted to pmole per mg vesicle protein.

For measurement of initial uptake, 20 μl vesicle suspension and 20 μl glucose-containing buffer were placed in close proximity at the bottom of a polycarbonate tube. Uptake was initiated by vortexing the tube and the process terminated by adding 2 ml stop solution. Filtration and counting was carried out as above. From the results of these experiments a time period of 4 s was chosen in order to derive kinetic parameters for D-glucose uptake over the concentration range 5.6–980 μM. Parallel experiments using L-glucose uptake as a measure of passive transport allowed values for active, Na^+-dependent glucose accumulation to be obtained. Hofstee plots were analysed by the method of least squares and the kinetic parameters of K_T and V_{max} determined for concentration ranges 5.6–31 μM and 49–980 μM glucose.

Statistics

Results are expressed as means ± S.E. Differences between means were evaluated by a Student's t-test and considered significant at $P < 0.05$.

Chemicals

Radioisotopes were obtained from Dupont UK Ltd. Galactose (glucose-free) and phlorizin were obtained from Sigma. All other chemicals were of A.R. Grade and obtained from either Sigma or BDH Ltd.

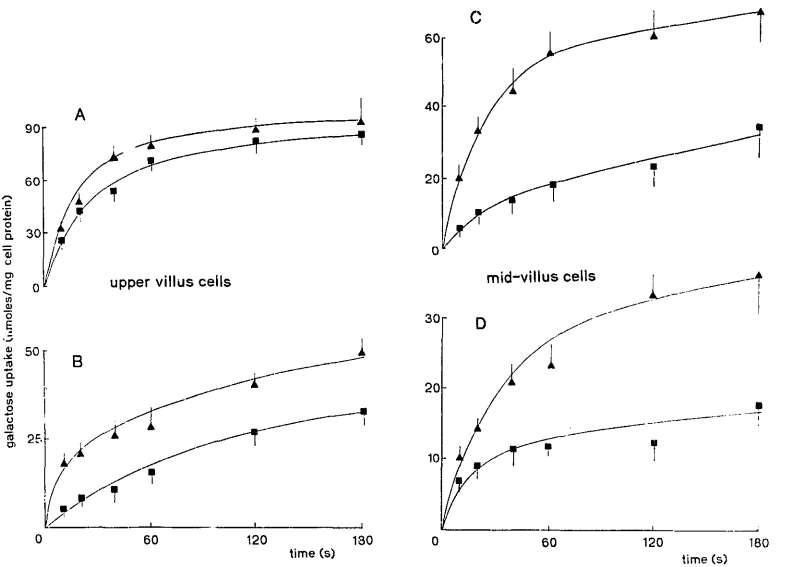

Fig. 1. Phlorizin-sensitive (A and C) and phlorizin-insensitive (B and D) galactose uptake by upper villus and mid-villus enterocytes isolated from ileum of control (■) and diabetic (▲) rats. Galactose was present in the incubation buffer at a concentration of 10 mM. Values are given as means ± S.E. (n = 6–8 observations).

Results

Plasma and luminal glucose concentrations

Animals became glycosuric 1–2 h after streptozotocin treatment. On the day of experimentation, elevated plasma levels of glucose in the animals were noted (control: 10.8 ± 1.3 mM (10), diabetes: 38.2 ± 2.7 mM (11), $P < 0.001$). Glucose levels in the ileal luminal fluid of diabetic rats were also markedly higher compared to respective intestinal regions in control animals (upper ileum: 26.1 ± 4.3 mM (8) vs. 1.3 ± 0.2 mM (7) $P < 0.001$; lower ileum: 6.5 ± 1.3 mM (8) vs. 0.9 ± 0.3 mM (8), $P < 0.001$).

Intestinal histology

Both villus height and enterocyte column size were increased in ileal tissue from diabetic rats (villus height : control 341.2 ± 6.3 μm (10), diabetic 457.3 ± 10.8 μm (10), $P < 0.001$; enterocyte column size: control 62.1 ± 2.6 (10), diabetic 79.4 ± 3.1 (10), $P < 0.01$).

Galactose uptake by isolated enterocytes

Phlorizin-sensitive uptake. Although diabetes was without effect on the phlorizin-sensitive component of galactose uptake using upper villus cells, an enhanced uptake at all time points in cells from the mid-villus was noted (Figs. 1A and C). The initial unidirectional rate of uptake for this latter cell population, measured between 0 and 10 s, was increased by 313% ($P < 0.01$, Table I). As a result of these differential changes in the two cell populations, the large difference in initial uptake between upper and mid-villus cells from control intestine ($P < 0.01$) was abolished by diabetes.

Phlorizin-insensitive uptake. The time course for galactose uptake measured in the presence of phlorizin revealed diabetic-induced increases in both upper and mid-villus cells (Figs. 1B and D). The initial rate of uptake was enhanced by 312% ($P < 0.01$) and 150% ($P < 0.02$), respectively, for these two enterocyte populations (Table I).

TABLE I

Effects of diabetes on initial rate of phlorizin-sensitive and phlorizin-insensitive galactose uptake into isolated upper and mid-villus enterocytes

Values were derived from the data in Fig. 1 between 0 and 10 s and calculated as nmol/mg protein per s. Results are given as means ± S.E. ($n = 6$–8). * $P < 0.01$ compared to upper villus cells. + $P < 0.01$ compared to control values.

Cell origin	Initial rate of galactose uptake	
	control	diabetes
Phlorizin-sensitive:		
upper villus	2.19 ± 0.34	2.50 ± 0.36
mid-villus	0.56 ± 0.13 *	2.31 ± 0.47 +
Phlorizin-insensitive:		
upper villus	0.42 ± 0.13	1.73 ± 0.26 +
mid-villus	0.62 ± 0.16	1.55 ± 0.23 +

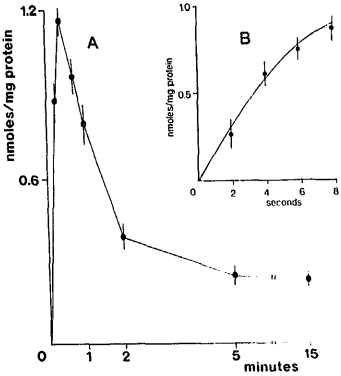

Fig. 2. Time course (A, in minutes; B in seconds) for Na^+-dependent D-glucose uptake by ileal brush border membrane vesicles prepared from control animals. Glucose was present at a concentration of 100 μM in the incubation buffer, together with a 100 mM NaSCN gradient. Values are means ± S.E. ($n = 5$–7 vesicle preparations).

Brush border vesicles

Vesicles from both control and diabetic mucosa were enriched 10–12-fold in alkaline phosphatase and 15–18-fold in sucrase compared to the original homogenate. Na^+-dependent D-glucose uptake displayed the expected overshoot reaching peak levels at 20–40 s (Fig. 2A). Vesicle volumes calculated from equilibrium uptake values at 15 min were found to be 1.52 ± 0.32 μl/mg protein (control, $n = 12$) and 1.41 ± 0.29 μl/mg protein (diabetic, $n = 14$). Uptake was linear with time for the first 6 s of incubation (Fig. 2B). At $t = 4$ s, the time chosen to determine the kinetics of uptake, the rate of L-glucose accumulation was 0.5–2% that of D-glucose, depending on the concentration of glucose used.

Kinetics of glucose uptake

Eadie-Hofstee plots of control and diabetic uptake data were non-linear (Fig. 3) suggesting carrier heterogeneity. The method of least squares was therefore used to derive two lines, the first utilised glucose concentration from 5.6 to 31 μM and the second 49–980 μM (r was > 0.94 in all cases). Kinetic data therefore represented values for a transporter with high-affinity/low-capacity (designated T1) and one with low-affinity/high-capacity (designated T2).

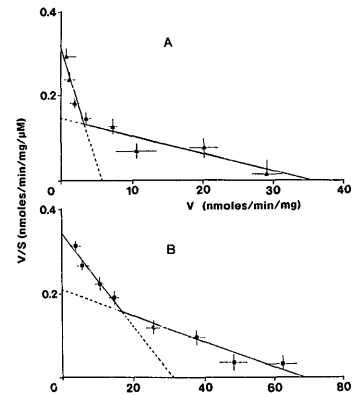

Fig. 3. Kinetics of Na^+-dependent D-glucose uptake by brush border vesicles from control (A) and diabetic (B) rat ileum. Values are given as means ± S.E. ($n = 5$–7). Lines were analysed by the method of least squares.

Analysis of kinetic data revealed that diabetes affected the properties of both transporters. An approximate 5-fold increase in both K_T and V_{max} of T1 was noted together with a 2-fold enhanced V_{max} of T2 (Table II). There was also a 31% increase in K_T for T2 but this was found not to be significant.

Discussion

All studies have recorded an enhanced jejunal sugar transport in the streptozotocin diabetic rat (see review by Karasov and Diamond [1]). The signals responsible for this adaptation are however unclear, Results from previous work using a variety of experimental conditions suggest that the level of sugar in luminal fluid regulates its rate of transport across the jejunal enterocyte, acting via the induction of membrane glucose carriers. Thus, increased luminal glucose appears to be relevant to its enhanced proximal uptake following a high carbohydrate diet [10,19] and a decreased luminal glucose for the reduced capacity for proximal glucose uptake in response to fasting [8,10,20], low carbohydrate diets [21] or total parenteral nutrition [22]. These observations are consistent with the view that, under normal physiological conditions, the rate of glucose uptake in the upper small intestine is very closely matched to the quantity of nutrient entering this region [10,13].

The efficiency of absorption in the jejunum ensures that glucose levels in the ileum of animals fed a normal diet are considerably lower than in the upper small intestine [12,13]. Preliminary experiments however revealed marked increases in the level of glucose in the ileum of streptozotocin diabetic rats. These abnormal levels of glucose undoubtedly reflect the hyperphagic condition of these animals resulting in the increased nutrient load entering the upper small intestine which would allow some glucose to escape the normally almost complete jejunal uptake process. It is also possible that the 4-fold increase in plasma glucose concentration and the increased tendency for secretion of the sugar into the lumen [23,24] may contribute to the high luminal glucose level in diabetes. The consequences of similarly high levels of ileal glucose in human diabetics may contribute to the diarrhoea which is often a clinical feature of this condition.

The rationale behind the present work was that a study of the effect of diabetes on ileal sugar transport might provide evidence, albeit circumstantial, for the importance of luminal sugar in the transport response to diabetes. Uptake results from experiments using brush border membrane vesicles, corrected for diffusion, confirm the existence of at least two Na^+-dependent glucose transporters in rat small intestine [25,26]. However, it is unclear from our own data, whether the non-linear kinetics observed reflect the presence of both mature and immature enterocytes in the original mucosal scrape or if individual cells at any level of the villus are equipped with multiple transporters.

The decreased affinity and enhanced capacity of ileal brush border glucose uptake in diabetes may be a direct response to exposure of enterocytes to abnormally high levels of luminal glucose. It is interesting to note that reduced nutrient levels in the jejunum results in higher affinity for jejunal sugar transport [20,27]. The concept that transported solutes may act as regulators for their uptake is supported by studies on the control of glucose transport in the cultured kidney epithelial cell line LLC-PK_1. Glucose transport across the apical membrane of these cells is Na^+-dependent and interestingly, the glucose concentration in the culture medium regulates the active uptake process [28]. In the ileum of the diabetic rat, the presence of a

TABLE II

Kinetics of glucose uptake by brush border vesicles using Eadie-Hofstee analysis

K_t in μM, V_{max} in nmol/mg protein per min. Means ± S.E. ($n = 5$–7). * $P > 0.05$, ** $P < 0.001$.

	K_{T^1}	K_{T^2}	V_{max^1}	V_{max^2}
Control	19.1 ± 3.0	248.0 ± 38.2	5.9 ± 0.7	35.9 ± 4.9
Diabetic	94.4 ± 5.6 **	324.6 ± 46.7 *	31.5 ± 6.2 **	68.8 ± 3.2 **

similar locally mediated adaptation, together with a reduced intestinal transit [29] would ensure that only minimal amounts of sugar enter the caecum and colon.

Data from experiments using isolated enterocytes reveal important information on both the cellular location of brush border adaptation and changes occurring at the basolateral aspect of the cell. Galactose was chosen to investigate transport since this sugar utilises the same transport pathway as glucose but is not metabolised by enterocytes [30].

The differential effect of diabetes on galactose movement across the brush border of upper and mid-villus cells shows a similar pattern to our recent observations in the jejunum [2] and implies the induction by diabetes of Na^+-dependent transport in normally immature cells of the mid-villus region. This, together with the observation of a greater number of enterocytes on villi in diabetic ileum, would result in a greatly enlarged functional surface area for glucose movement across the brush border membrane.

Measurements of glucose accumulation by brush border vesicles showing a high sensitivity to phlorizin [31] and a very low sensitivity to cytochalasin B [32], specific inhibitors for sugar uptake at the brush border and basolateral membranes respectively, confirm our belief that for isolated enterocytes, the phlorizin-insensitive component of sugar uptake represents sugar movement across the basolateral membrane. Our data imply that sugar transport at this latter site is also influenced by diabetes. In contrast to the brush border however, both populations of cells display an enhanced uptake.

Although there is firm evidence for upregulation of basolateral sugar movement in the jejunum [2,6,19,33, 34], relatively little information is available concerning basolateral regulation of sugar transport by ileal enterocytes. The only previous study reported no effect of acute hyperglycaemia on glucose accumulation by ileal basolateral vesicles despite increased uptake by jejunal membranes [33]. Our study is the first to report basolateral adaptation of ileal enterocytes and, in conjunction with enhanced brush border movement by these cells, would function to increase transcellular movement of the sugar in diabetes. Given that fluctuations in intraluminal glucose concentration in diabetes are able to influence transport at both cellular loci, the obvious question concerns the precise signals which initiate this response In the case of the brush border, luminal glucose per se may control the synthesis of carrier protein. In addition, changes in glucose levels within the enterocyte resulting from hyperglycaemia and/or increased luminal glucose concentration must also be considered. For the basolateral membrane, for the reasons stated above, hyperglycaemia may be of less importance in the response. Recent experiments where dietary glucose was replaced with either metabolised or non-metabolised sugars imply that an altered enterocyte metabolism, presumably in response to changing luminal sugar levels, is a prerequisite for adaptation at the basolateral membrane [19].

Acknowledgements

We are grateful to the MRC and Wellcome Trust for support of this work and to Mr. M. Adams for skilled technical assistance.

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