Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2

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A possible role for GLUT2 transiently expressed in the rat jejunal brush-border membrane (BBM) under the influence of glucagon-like peptide 2 (GLP-2) was investigated using *in vivo* perfusion of the intestinal lumen as well as isolation of membrane proteins and immunohistochemistry. A 1 h vascular infusion of GLP-2 *in vivo* doubled the rate of fructose absorption and this increase could be blocked by luminal phloretin. Immunohistochemistry of frozen sections of rat jejunum showed the expression of GLUT2 in both the basolateral and BBMs of mature enterocytes. Perfusion of the intestinal lumen with 50 mM D-glucose or vascular infusion of 800 pM GLP-2 for 1 h increased the expression of GLUT2 in the BBM. Quantification of these changes using Western blotting of biotinylated surface-exposed protein showed a doubling of the expression of GLUT2 in the BBM, but the effects of glucose and GLP-2 were not additive. These results indicate that vascular GLP-2 can promote the insertion of GLUT2 into the rat jejunal BBM providing a low-affinity/high-capacity route of entry for dietary hexoses.

Key words: carbohydrate absorption, glucose transporter 2, hexose transport, intestinal transport.

INTRODUCTION

The current model of hexose absorption in the small intestine proposes that there are two routes of entry for hexoses into the brush-border membrane (BBM) of the epithelial cells (enterocytes). There is a sodium-dependent transporter, SGLT-1, which mediates the uptake of glucose and galactose [1] and a facilitated carrier, GLUT5, which is the route of entry for fructose [2,3]. SGLT-1 can be inhibited by phloridzin but GLUT5 is unaffected by this inhibitor. In addition, phloretin and cytochalasin B, both of which can block the activity of the other GLUTs, have no effect on GLUT5 [4,5]. Characterization of these transport proteins when expressed in *Xenopus* oocytes indicates that the affinity of SGLT-1 for fructose is minimal, while that of GLUT5 for glucose is very low, but may vary depending upon the species [6,7]. Therefore, within the physiological concentrations of these hexoses in the intestinal lumen it appears that fructose has only one major route for uptake and that is GLUT5. There are, however, some observations which cannot be explained by this model. First, *in vivo* measurements of glucose uptake in the small intestine fail to show saturation even at luminal concentrations as high as 100 mM, yet SGLT1 has a K_m in the range of 200 μM. Even allowing for unstirred water layers adjacent to the epithelial cell surface, which would shift the apparent K_m for the uptake kinetics, saturation should occur at much lower concentrations than those observed. Also, when fructose uptake is measured in isolated BBM vesicles the fluxes are not saturable even at high concentrations and have a profile more like diffusion [8] even though, when GLUT5 is expressed in oocytes, it has a K_m of between 6 and 12 mM [9]. Recently, Kellett's group published a series of papers [10–12] indicating that there could be a second pathway for the uptake of fructose (and concomitantly glucose) in the intestine that was related to the rapidly inducible expression of GLUT2-like immunoreactivity in the BBM. Exposure of the small intestine *in vivo* to PMA resulted in the appearance of a phloretin-inhibitable component for fructose transport which correlated with the appearance of GLUT2-like immunoreactivity in the BBM [10–12]. The only reports of GLUT2 expression in the apical surface of enterocytes had up until then been in animals with diabetes and had been interpreted as possibly a pathological event [13]. The indication that GLUT2 could be rapidly inserted into or removed from the BBM raises a number of interesting challenges to the conventional model for hexose transport. This hexose transporter has a low affinity for its substrates, glucose, galactose and fructose, with a K_m in the range of 16–20 mM. Thus, at lower concentrations the relationship between rate of transport and substrate concentration is relatively linear and could be confused with diffusion. Also, this protein would offer a second route for the uptake for fructose in addition to that via GLUT5. Similarly, SGLT-1 would then not be the only point of entry for glucose and GLUT2 could provide a low-affinity/high-capacity pathway which at high luminal substrate concentrations would dominate.

It has long been assumed that SGLT-1 expression only adapts in response to altered dietary intake over a period of days. Recently, we showed that several enteric peptide hormones, including gastric inhibitory peptide and glucagon-like peptide 2 (GLP-2), could induce a rapid increase in the expression of SGLT-1 in the BBM within 30–60 min [14]. The release of these hormones is promoted by the presence of hexoses in the intestinal lumen and Kellett et al. [10] have shown that high luminal glucose concentrations will induce the phloretin-inhibitable fructose fluxes. Further, we have shown that the activity of GLUT2 in the basolateral membrane (BLM) is significantly increased by the infusion of GLP-2 [15,16]. This indicates that the transport capacity for hexoses is increased at both poles of the enterocytes in parallel to promote absorption. It is possible that GLP-2 could also mediate the hexose-induced insertion of GLUT2 into the

Abbreviations used: BBM, brush-border membrane; BLM, basolateral membrane; GLP-2, glucagon-like peptide 2; NHS, *N*-hydroxysuccinimido.

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intestinal BBM as part of this response. To test this hypothesis we have looked at the effects of acute vascular infusion of GLP-2 on the transport of fructose *in vivo* and the expression of GLUT2 and GLUT5 in the rat jejunal BBM.

MATERIALS AND METHODS

Perfusion of the small intestine *in vivo*

Male rats of 200–250 g body weight were anaesthetized with Somnotol® and the intestine exposed by a laparotomy. A 30 cm segment just below the ligament of Trietz was cannulated, flushed free of nutrients and perfused with a single-pass system at a flow rate of 0.5 ml/min with a modified Krebs bicarbonate saline (118 mM NaCl, 4.74 mM KCl, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$ and 24.8 mM $NaHCO_3$ [11]) segmented with 95 % O_2/5 % CO_2. The effluent was collected every 5 min using a Gilson FC203B fraction collector (Mandel Scientific). The jugular vein was cannulated with a silastic tube and infused with saline at a flow rate of 3 ml/h. Radiolabelled substrate was added to the perfusate and radiolabelled poly(ethylene glycol) 4000 was included as a non-absorbable marker to allow for the correction of fluid movements. At the end of the perfusion period the intestine was removed, weighed wet and then dried overnight before reweighing. For the addition of phloretin the luminal solution required the use of modified Krebs bicarbonate saline (see above) to keep the inhibitor in solution. Phloretin was initially made up as a stock solution in DMSO and 200 μl of stock then added to 150 ml of perfusate to give a final concentration of 1 mM. Final DMSO concentration was therefore 0.13 % (v/v), which we have demonstrated previously to have no effect on tissue permeability (results not shown). Phloretin stayed in solution for the length of the experiment.

Vascular perfusion of jejunum *in situ*

The technique employed in these experiments has been described previously [17]. Male Sprague–Dawley rats (200–250 g) were fed *ad libitum* on a standard chow diet (Purina PMI Rodent Food). Before the start of the experiment food was withdrawn overnight to minimize luminal contents during surgery. All rats were anaesthetized using sodium pentobarbital prior to surgery with an intraperitoneal injection (60 mg/kg of body weight) and placed on a heated (37 °C) surgical table. After performing a laparotomy, the blood supply to the spleen, pancreas, stomach, rectum and ileum were ligated before removal. The vasculature to the duodenum was also tied off, but it was left in place. A 35 cm segment of jejunum starting 5 cm distal from the ligament of Trietz was isolated, cleaned by gently flushing with 20 ml of warm saline (0.9 %), and cannulated at both ends. The lumen was perfused with modified Krebs bicarbonate saline (see above) maintained at 37 °C and gassed with 95 % O_2/5 % CO_2. The single-pass luminal circuit was perfused using a Gilson Minipuls 2 pump (Mandel Scientific) at a flow rate of 1.6 ml/min. The solution was segmented by 95 % O_2/5 % CO_2 gas bubbles. The aorta, proximal to the superior mesenteric artery, was ligated just prior to insertion of a cannula into the superior mesenteric artery. The single-pass vascular circuit was perfused at a rate of 1.6 ml/min with Krebs bicarbonate saline solution containing 5 mM D-glucose, 0.034 mM streptomycin sulphate, 5 mM L-glutamine, 1120 USP (U.S. Pharmacopeia) units of heparin and 10 % (w/v) Ficoll 70 as a plasma expander, which was maintained at 37 °C and gassed with 95 % O_2/5 % CO_2. Once the vascular circuit was established, the rat was killed, and the vascular perfusate was collected via a cannula placed in the hepatic portal vein and was continuously collected using a Gilson fraction collector. The radioactive labelled substrates were added to the luminal solution immediately after the portal vein was cannulated.

Preparation of BBMs

BBMs were prepared from mucosal scrapings using a standard procedure [18]. Briefly, the mucosa was scraped off with a microscope slide and placed in 65 ml of ice-cold mannitol/Tris buffer (300 mM mannitol, 5 mM EGTA, 12 mM Tris/HCl, pH 7.4, and 0.1 mM PMSF). The tissue was homogenized in a Polytron homogenizer for 2 min before addition of $MgCl_2$ to a final concentration of 12 mM. After stirring the solution on ice for 15 min the solution was centrifuged at 1600 ***g*** for 15 min to remove debris. The supernatant was further centrifuged at 20000 ***g*** for 30 min and the pellet homogenized in half-strength mannitol/Tris buffer (150 mM mannitol, 2.5 mM EGTA, 6 mM Tris/HCl, pH 7.4, and 0.05 mM PMSF) with a glass homogenizer before further addition of $MgCl_2$. After stirring on ice the centrifugation was repeated as before, and the pellet was then washed (300 mM mannitol/5 mM Tris/HCl, pH 7.4) before repelleting at 20000 ***g***. This vesicle preparation was diluted to a protein concentration of 8 mg/ml.

Western blotting

Membranes (15 μg of protein) were solubilized in Laemmli sample buffer and run on a SDS/polyacrylamide gel (10 % gel) using a Mini-PROTEAN II cell (Bio-Rad). The proteins were blotted on to nitrocellulose membrane (Bio-Rad) by electrotransfer for 75 min at 4 °C using the Mini Trans-Blot Cell (Bio-Rad). The membranes were stained for total protein with Ponceau S to ascertain that equivalent amounts of protein were loaded and transferred from each lane. Blocking of the membrane was carried out in 3 % non-fat milk in PBST (0.05 % Tween 20/PBS, pH 7.4) for 1 h and then incubated with 1:1000 rabbit polyclonal antibody to rat GLUT2 in 3 % non-fat dry milk in PBST overnight at 4 °C. The membrane was washed three times in 3 % non-fat dry milk/PBST for 15 min, 1 h and 15 min respectively. The nitrocellulose membrane was then incubated with a secondary antibody, anti-rabbit IgG coupled to horseradish peroxidase diluted 1:2000 in 3 % non-fat dry milk/PBST for 1 h. Three subsequent washes followed as described above. Finally, the membrane was treated with the ECL detection solution (Amersham Biosciences) before autoradiography for 30 s using Kodak XAR-5 film with an intensifying screen. The specificity of the antibody was determined by running parallel lanes of isolated BLMs and probing them with the native antibody or with antiserum which had been preincubated at room temperature for 1 h with the peptide used to raise the antibody. Two antibodies were employed: one that recognized an epitope in the large extracellular loop between transmembrane segments 1 and 2, amino acids 40–55 (SHYRHVLGVPLDDRRA; Biogenesis, Poole, Dorset, U.K.) and a second which recognized a portion of the C-terminal sequence of rat GLUT2 (CVQMEFLGSSETV; Research Diagnostics, Flanders, NJ, U.S.A.).

Biotinylation of surface proteins

Proteins expressed on the apical surface of jejunal enterocytes were labelled with *N*-hydroxysuccinimido (NHS)-SS-biotin introduced into the intestinal lumen. At the end of the *in vivo* perfusion the intestine was maintained *in situ*, but cooled on ice. The luminal solution containing NHS-SS-biotin, 1.5 mg/ml in 10 mM triethanolamine/2.5 mM $CaCl_2$/250 mM sucrose buffer (pH 9.0), was introduced into the lumen and left for 30 min. The

lumen was then flushed with a PBS/100 mM glycine buffer to quench the free biotin before two final washings with PBS. Mucosal scrapings were then used to make protein extracts as described below.

Isolation of biotinylated proteins

Proteins were extracted from the homogenate for 1 h at 4 °C using the following buffer: 1.0 % Triton X-100, 150 mM NaCl, 5 mM EDTA and 50 mM Tris, pH 7.5. After centrifugation at 14000 ***g*** for 10 min the supernatant was collected and incubated overnight with streptavidin beads. After washing twice with the Triton X-100 buffer to remove non-linked protein, the beads were washed with a high-salt buffer (500 mM NaCl) and finally with a no-salt buffer (10 mM Tris, pH 7.5). The isolated biotinylated proteins were then solubilized in SDS sample buffer to be run on SDS/PAGE for Western blotting. Comparisons of total cell GLUT2 with apical GLUT2 were made by running samples of the supernatant after spinning down the streptavidin-coated beads on the same Western blots as samples of recovered biotinylated protein.

Immunohistochemistry

The jejunum of rat small intestine was flushed *in situ* with ice-cold PBS containing PMSF, everted, and cut into 1 cm-wide rings to aid subsequent orientation. These rings were embedded in OCT embedding medium (Shandon) and flash-frozen in liquid nitrogen. Sections of 12 μm thickness were cut on a cryostat, placed on slides and stored at -20 °C.

On the day of use the sections were brought to room temperature and fixed with methanol for 90 s. Following a 5 min wash with PBS the sections were treated with 0.1 % SDS for 5 min to increase antigen exposure, and again thoroughly washed in PBS (three times for 5 min each). The sections were then treated with 10 % goat serum (0.1 % Tween 20) to decrease non-specific binding of the secondary antibody, followed by a quick rinse with PBS and primary antibody treatment. The primary anti-GLUT2 antibody was diluted 1:1000 in a 25 % milk solution (0.05 % Tween 20) and placed on slides for 1 h at room temperature, followed by washes with high-salt PBS and then PBS. Control slides were treated with only the milk solution or with preabsorbed primary antibody, which was exposed to its peptide antigen for an hour at room temperature prior to application. The slides were then incubated with biotinylated goat anti-rabbit secondary antibody (1 mg/ml), diluted by 1:200 in PBS, for 1 h at room temperature. The sections were washed in high-salt PBS and PBS and then treated with streptavidin-conjugated FITC for 30 min in the dark. After three 5 min washes with PBS in the dark, the slides were mounted and sealed with nail polish. They were then viewed and photographed under a confocal microscope.

RESULTS

Effect of GLP-2 on fructose transport *in vivo*

Fructose absorption was studied *in vivo* under control conditions when saline alone was infused into the jugular vein. The uptake of 5 mM D-fructose was reduced in the presence of 1 mM phloretin in the lumen by 24 % from 428.3 ± 107.3 to 325.2 ± 58.6 μmol/g of dry weight per h, respectively. The vascular infusion of 800 pM GLP-2 at a flow rate of 3 ml/h caused a 53 % increase in the uptake of fructose (656.4 ± 79.4 μmol/g of dry weight per h). This response was highly significant ($P < 0.01$). The addition of 1 mM phloretin to the lumen abolished this stimulation and reduced uptake to a rate that was only 63 % of the control (269.2 ± 73.4 μmol/g dry weight per h; Figure 1).

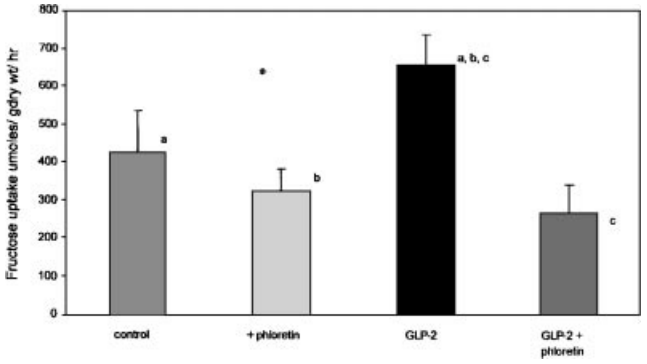

Figure 1 Uptake of D-fructose *in vivo* from rat jejunum

Radiolabelled fructose was perfused through the rat jejunum for 1 h with poly(ethylene glycol) as an extracellular marker. Saline with or without 800 pM GLP-2 was infused into the jugular vein for 1 h prior to and during the luminal perfusion. Where indicated 1 mM phloretin was included in the luminal perfusate. Each bar represents the mean $\pm$ S.E.M. of the steady-state uptake of fructose from a minimum of five animals. Bars sharing the same letter are significantly different ($P < 0.05$).

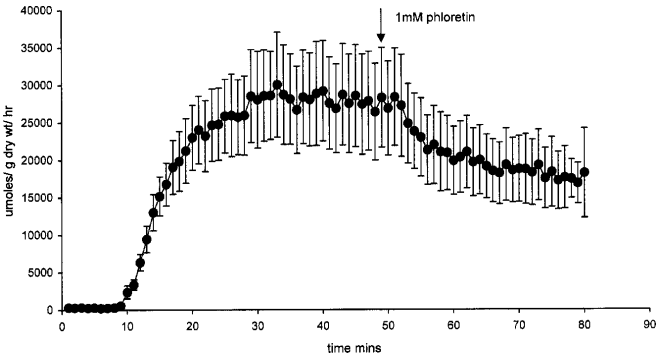

Figure 2 Glucose transport across the vascularly perfused isolated rat jejunum

Values are the average rate of appearance of glucose in the vascular bed from five experiments in which radiolabelled 50 mM D-glucose was perfused through the intestinal lumen. At the time indicated by the vertical arrow 1 mM phloretin was introduced into the luminal perfusate. Error bars represent S.E.M.

Pathways for glucose absorption

When 50 mM D-glucose was perfused through the lumen of the vascularly perfused isolated jejunum a plateau transport rate was attained within 45–50 min and this was highly reproducible between preparations. The introduction of 1 mM phloretin, an inhibitor of some GLUT proteins including GLUT2, into the luminal perfusate reduced the net absorption of glucose by 39 % (Figure 2).

Detection of surface GLUT2

The apparent abundance of GLUT2 close to the terminal web of the enterocytes indicated that cryptic protein might be co-purified with that actually in the apical membrane when preparing

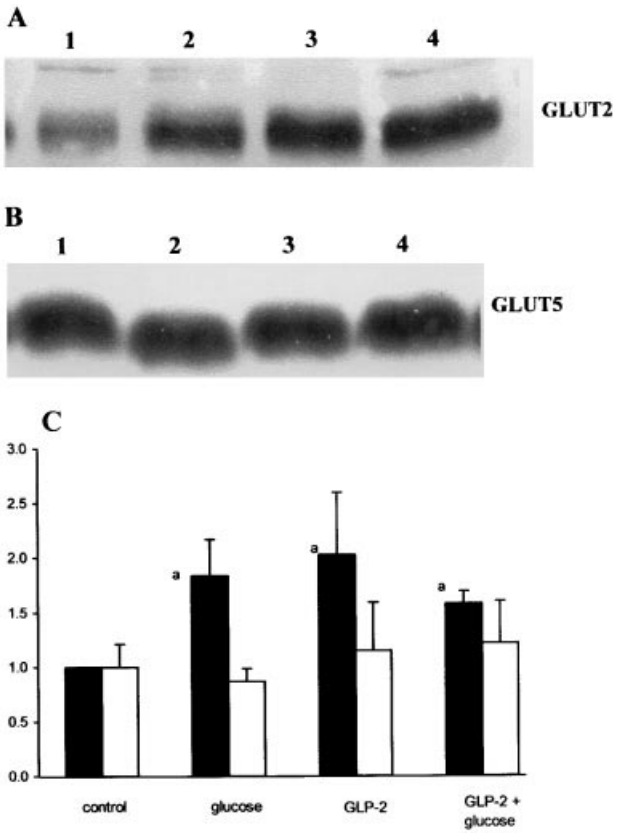

Figure 3 Quantification of surface-expressed GLUT2 in the BBM

After luminal perfusion of the jejunum and/or vascular infusion of GLP-2 (800 pM) the intestine was cooled to 4 °C *in situ* and the lumen filled with a biotinylation solution for 30 min. The free biotin was then quenched with glycine and mucosal scrapings prepared. Biotinylated proteins were extracted using streptavidin-coupled beads and run on SDS/PAGE before probing with a primary antibody raised against a C-terminal sequence of the GLUT2 protein. (**A**) A typical series of lanes for: 50 mM mannitol-perfused control (lane 1), 50 mM D-glucose-perfused (lane 2), 800 pM GLP-2-infused (lane 3) and 50 mM glucose-perfused + plus 800 pM GLP-2-infused (lane 4). (**B**) A representative Western blot of BBMs prepared under the same conditions probed for GLUT5. (**C**) The collated data from nine separate experiments where the absorbance of the bands corresponding to GLUT2 (black bars) and GLUT5 (white bars). Each lane was scanned and expressed in (**C**) as a ratio of the control (mannitol-perfused) band. Error bars represent S.E.M. Significance was determined by unpaired Student's *t* test; bars found to be significantly different from the control are indicated by the adjacent letters ($P < 0.05$).

isolated membrane vesicles. We therefore employed a biotinylation technique to assay only GLUT2 that was exposed to the lumen. After treating the tissue *in vivo* with luminal mannitol or glucose, or with vascular GLP-2, the jejunum was cooled to 4 °C and then the luminal surface exposed to the non-permeant NHS-SS-biotin. Biotinylated proteins were then isolated and Western blots used to quantify the abundance of GLUT2. The antibody to the C-terminal epitope was used in these assays because it was found that biotinylation interfered with the ability of the extracellular loop antibody to detect the protein. Under these conditions significant alterations in surface GLUT2 were observed. Perfusion of 50 mM glucose through the lumen induced a 2-fold increase in GLUT2 abundance as did the infusion of GLP-2. However, the combination of luminal glucose and GLP-2 did not result in any further increase in GLUT2 expression (Figure 3). Comparison of the amount of non-biotinylated GLUT2 in the supernatant compared with the protein bound to the streptavidin beads indicated that the proportion of GLUT2 exposed at the apical surface represented 6–11 % of the total GLUT2 protein in the cells. This estimate assumes complete biotinylation of luminally exposed GLUT2 and 100 % recovery of biotinylated protein from the beads.

Immunolocalization of GLUT2 in rat jejunum

We have recently shown that GLUT2 protein can be detected in the intestinal BBM when an antibody detecting the extracellular loop of the protein is employed (G. Kellett, C. I. Cheeseman and E. Brot-Laroche, unpublished work). Figure 4 shows images obtained when frozen sections of rat jejunum were probed with this antibody. Under control conditions there is some GLUT2 expressed in the microvilli of the BBM, but closer inspection clearly shows a high concentration of the protein lying just below the apical membrane. The cryptic GLUT2 appears to form a line which might correspond with the terminal web, a cytoskeletal structure to which are attached the microfilaments forming the core of each microvillus (Figure 4E). Exposure of the tissue to either luminal glucose or vascular GLP-2 resulted in a significant increase in the expression of GLUT2 immunoreactivity in the BBM (Figures 4B and 4C). The specificity of the reaction is clear from the image obtained when the primary antibody was first preabsorbed with the antigenic peptide (Figure 4D).

Fructose transport in isolated BBM vesicles

If, indeed, there is increased expression of GLUT2 in the BBM after exposure to luminal glucose or vascular GLP-2 then it should be possible to see changes in fructose transport capacity in isolated membrane vesicles. Fructose transport was measured over a wide range of substrate concentrations in membrane vesicles prepared from jejuna exposed to all four sets of conditions. Incubations of 3 s were employed to approximate initial rate conditions. Figure 5 shows the averaged fructose-uptake data corrected for diffusion from four sets of experiments under each condition. Diffusion into the vesicles was measured using a range of concentrations of L-glucose and was unaffected by the perfusion conditions prior to vesicle preparation (results not shown). The k_d for L-glucose uptake was then used to correct the total fructose uptake for each experiment to give the carrier-mediated fructose uptake. The k_d for the uptake of L-glucose was not significantly different between the different conditions and so the data were pooled to give a k_d of 3.28 ± 0.31 pmol/mg of protein per s. Mannitol perfusion produced the lowest rate of carrier-mediated fructose uptake, whereas luminal glucose provided some stimulation. Vascular GLP-2 gave an even greater rate of transport ($P = 0.03$; using ANOVA) and finally a combination of luminal glucose and vascular GLP-2 induced the highest rate of uptake ($P = 0.007$, using ANOVA). The data over the entire concentration range of substrates were then analysed for kinetic constants using non-linear regression analysis. It was not possible, however, to assign meaningful values from these analyses due to the apparently linear transport data.

DISCUSSION

The possibility of significant expression of a facilitated hexose transporter (GLUT) other than GLUT5 in the BBM of the small intestine at first seems unlikely. The intestinal epithelium is designed to reduce the luminal hexose concentration almost to zero and move dietary hexoses into a bloodstream containing at least 5 mM glucose. However, several long-standing observations are not fully explained by the currently accepted model of

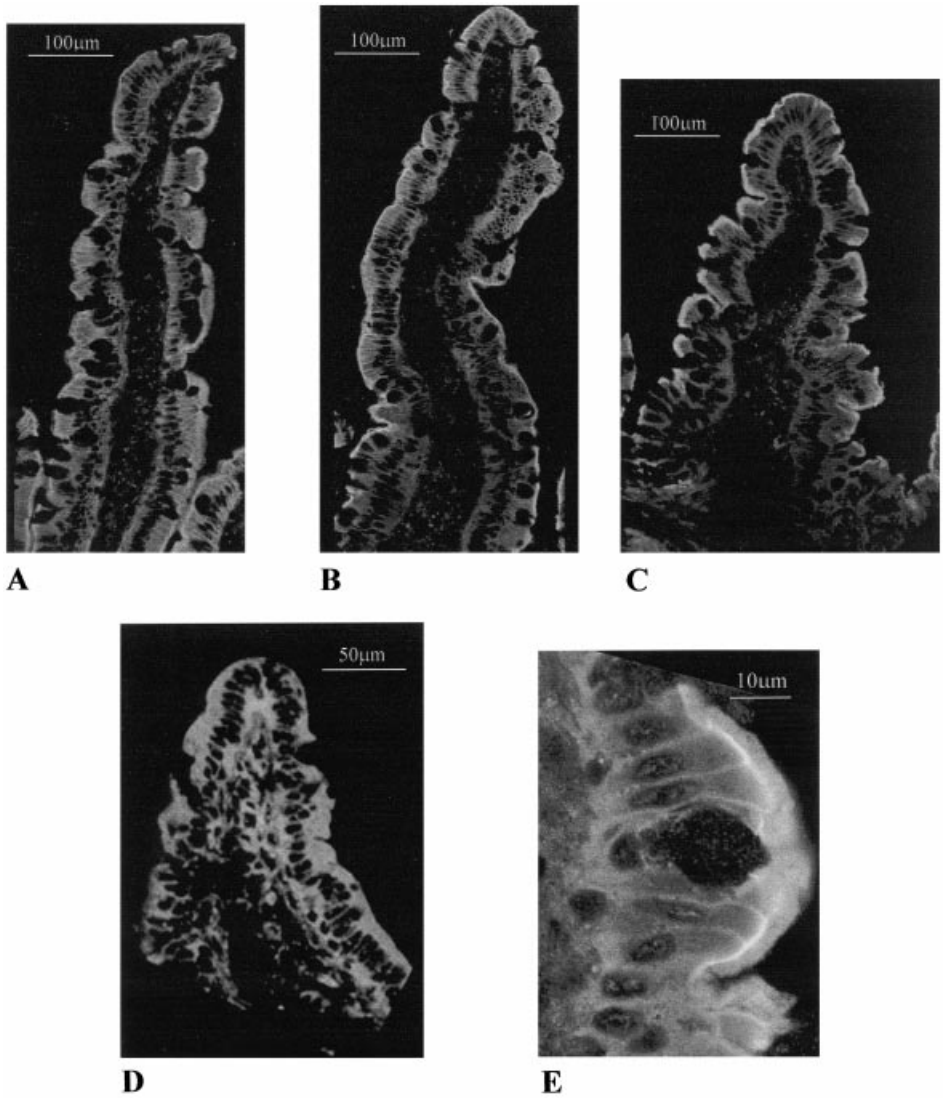

Figure 4 Immunohistochemistry of the rat jejunum showing expression of the hexose transporter GLUT2 in both the BLM and BBM

Frozen sections of rat jejunum were prepared after perfusion *in vivo* of the lumen with 50 mM mannitol (**A**) or D-glucose (**B**) or vascular infusion of 800 pM GLP-2 (**C**). Sections of 11 μm were probed with anti-GLUT2 antibody recognizing an epitope in the extracellular loop of the GLUT2 protein. Binding was visualized with a secondary antibody coupled to biotin followed by incubation with streptavidin-coupled FITC. Images were captured using a confocal microscope. **A**–**C** are shown at the same magnification. (**E**) A higher-power image from an animal perfused with GLP2. Controls to detect non-specific binding were performed with primary antibody preabsorbed with the antigenic peptide (**D**) shown at an intermediate magnification. This villus is from a glucose-perfused tissue corresponding to the conditions for (**B**).

absorption, i.e. a single sodium-coupled transporter (SGLT-1) that can absorb glucose and galactose and a facilitated pathway for fructose, GLUT5. The major difficulty is that at high luminal concentrations of hexoses, which are present during the early part of a meal, the rate of hexose absorption is very fast and increases almost linearly with the luminal concentration. This has previously been interpreted to represent some kind of diffusional event either through the epithelial cells or between them. Clearly, SGLT-1 cannot be responsible for such a linear increase in transport because of its very low K_m [6]. Pappenheimer and Madera [19,20] have suggested that SGLT-1 might have a signalling role by which the entry of luminal glucose results in the opening up of the intercellular junctions in the epithelium to allow rapid diffusion of substrate between the cells. The major objection to such a hypothesis is the apparent non-specific nature of such a route which could allow many different organic solutes to move between the epithelial cells. Very recently, Kellett [21] has proposed an alternate possibility in which the presence of luminal glucose signals through SGLT-1 the rapid insertion of GLUT2 into the BBM. Kellett et al. [11] have used Western blotting to detect the presence of GLUT2-like immunoreactivity in the apical membrane and shown that the trafficking of this protein is very rapid and influenced by protein kinase C βII. If GLUT2 can indeed be inserted into the BBM it would provide a low-affinity/high-capacity route for the entry of glucose, galactose and fructose into the enterocytes and could offer an alternative explanation for the large absorptive capacity observed at high luminal concentrations of carbohydrate.

The data presented here both confirm Kellett and co-worker's original observations [10] and extend them substantially. We can

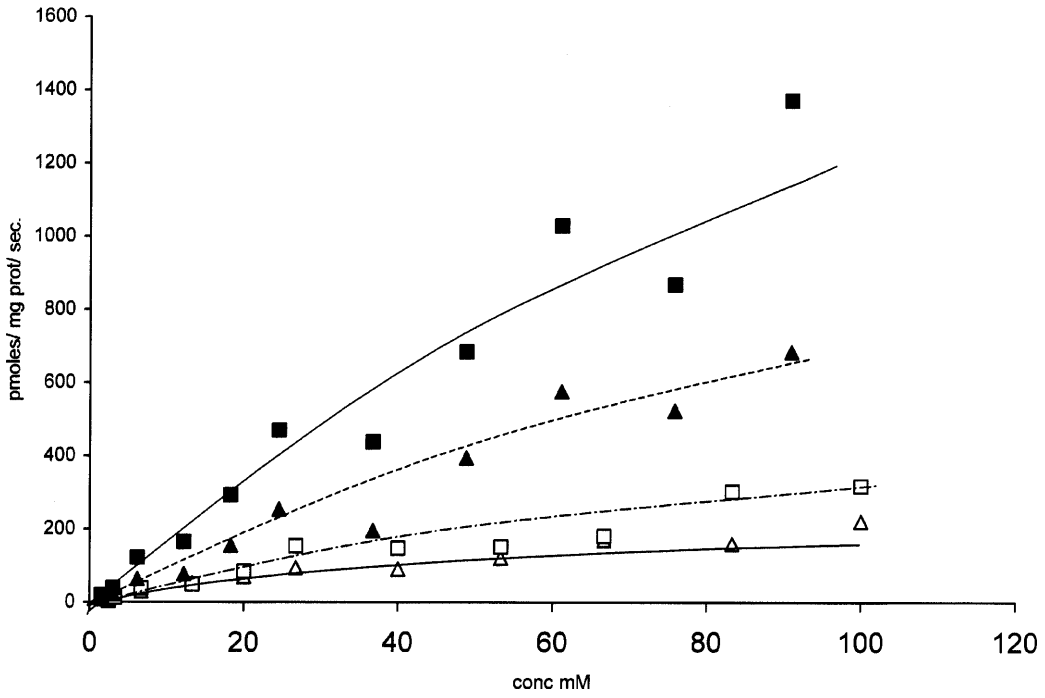

Figure 5 Effect of luminal glucose and/or vascular GLP-2 pretreatment on fructose uptake across isolated jejunal BBM vesicles

Uptake data were obtained using rapid filtration and 3 s uptakes. Vesicles were prepared from mucosal scrapings of rat jejuna which had been perfused *in vivo* through the lumen with 50 mM mannitol (△, ▲) or glucose (□, ■) while the jugular vein was infused with saline (△, □) or 800 pM GLP-2 (▲, ■). Fructose concentration is shown on the *x* axis. Data points represent the means of four experiments for each condition corrected for passive uptake measured using L-glucose. S.E.M. ranged from 6 to 21% of the mean values.

detect GLUT2 protein in the BBM of the jejunum and GLUT2-like functional activity when either glucose is present in the lumen or GLP-2 is infused *in vivo*. The Western blotting reported here employed only the antibody which recognized the C-terminal epitope because biotinylation interfered with the reaction of the antibody recognizing the extracellular loop domain of GLUT2. Given that the lysine residue that is likely to be the site of biotinylation is found in the same loop as the antigenic epitope it is not surprising that the ability of this antibody was reduced by the biotinylation. Kellett et al. [10] originally employed an antibody for the C-terminal epitope for Western blotting and we have confirmed Kellett's observation of GLUT2 in the BBM. When we employ the antibody recognizing the extracellular loop between transmembrane domains 1 and 2 it also produces a band with BBMs (results not shown). However, as stated above we could not use this with the biotinylation for accurate quantification of changes in abundance. The fact that antibodies recognizing two different epitopes can detect GLUT2 in the BBM strongly supports the argument that the protein really is GLUT2.

Immunohistochemistry has previously been used to show that GLUT2 is expressed only in the BLM domain of enterocytes. However, Thorens et al. [22] used a C-terminal antibody to probe for GLUT2 expression. When we employ an antibody which recognizes part of the extracellular loop between transmembrane domains 1 and 2 we are able to show extensive expression of GLUT2 in or adjacent to the BBM as well as in the BLM. Luminal glucose or vascular GLP-2 both stimulated the expression of GLUT2 at the luminal surface after 1 h, showing that both can signal the trafficking of this protein. Careful examination of the distribution of GLUT2 under control conditions indicates that there is a substantial amount of the protein lying beneath the microvilli in the region of the terminal web. This raises the possibility that this cytoskeletal structure may be involved in the trafficking of the protein to the surface.

We also have functional evidence indicating that there is GLUT2 activity in the rat jejunal BBM. The absorption of 50 mM D-glucose across the vascularly perfused intestine could be inhibited 40% by luminal phloretin. This inhibitor has a very low affinity for SGLT-1 [1] and is known to be relatively specific for some GLUTs [23]. So, this observation would suggest that nearly half of the glucose influx across the BBM is mediated by GLUT2 when the substrate concentration is 50 mM. However, this is even more convincing when combined with the observation that under control conditions (no luminal glucose, no vascular GLP-2) fructose absorption *in vivo* is only reduced by 30% by luminal phloretin. However, when stimulated by GLP-2, all of the additional fructose absorption could be blocked by phloretin. GLUT5 is insensitive to phloretin [24] so these observations can be interpreted such that GLUT5 activity is not regulated by GLP-2 and represents a constitutive pathway for fructose absorption. There is some evidence that rat GLUT5 may be able to transport glucose as well as fructose but this has only been observed when the protein is expressed in oocytes and the affinity for glucose was much lower than for fructose. The combination of the phloretin inhibition data and the lack of change of GLUT5 abundance strongly supports the hypothesis that the increased fructose flux is mediated by GLUT2 and not GLUT5. Thus the above observations indicate that the increased fructose uptake induced by GLP-2 is mediated by GLUT2 newly inserted into the BBM.

The stimulation of fructose transport into isolated BBM vesicles prepared from jejuna which were prepared from tissue previously perfused with glucose and/or exposed to GLP-2

supports the view that the increase in absorption of fructose is in part mediated by changes in transport capacity in the apical membrane. The difficulty in fitting meaningful kinetic constants to these transport data is of interest. Previous reports have also indicated an almost linear relationship between uptake rates and substrate concentration for fructose in BBM vesicles [25].

It is noteworthy that the transport data for fructose under these conditions indicate that a combination of luminal glucose and vascular GLP-2 gave a larger increase in transport than was indicated from the changes in BBM GLUT2 abundance determined by Western blotting. This would suggest that there may be two components to the regulation of apical GLUT2 transport. In addition to the insertion of new protein into the membrane, it is likely that the activity of GLUT2 already at the surface can be modulated. Under control conditions, we observed the presence of GLUT2 protein in the BBM when there was very little phloretin-sensitive fructose uptake. Kellett [21] also demonstrated that the relationship of GLUT2-associated transport capacity in the BBM and GLUT2 protein abundance was sigmoidal, and he argued that the activity of the protein in the apical membrane must be regulated.

The remaining question relates to whether there is sufficient GLUT2 inserted into the BBM to provide the transport capacity for the proposed additional hexose entry into the enterocytes. This is a very difficult estimate to make for several reasons. Currently there are no published rates of substrate turnover for GLUT2 and our measurements of surface-expressed protein are only relative. However, the observation that a minimum of 6–11 % of the total GLUT2 in the cells is present at the apical surface would support the view that this is a significant proportion of the total GLUT2 expressed in the enterocytes. These values are very likely to be an underestimate given that we have no evidence of 100 % efficiency of surface labelling of GLUT2 by biotin nor recovery of the biotinylated protein from the streptavidin beads.

Signalling pathways for GLUT2 trafficking

Kellett and Helliwell [12] have proposed that luminal glucose acts as a signal for the rapid insertion of GLUT2 into the BBM when it is transported into the cell. This would explain why phloridzin, which is a specific inhibitor of SGLT-1, can block all glucose uptake because it will interfere with the signalling pathway for GLUT2 insertion. Similarly in patients with glucose and galactose malabsorption, where they have defective SGLT-1 [26], the signalling pathway would also be missing. We have now provided evidence that GLP-2 can also promote the apical surface expression of GLUT2 which raises the possibility that this represents part of the control loop or a parallel pathway. The release of GLPs from 'L' cells in the ileum is promoted by gastric inhibitory peptide [7,8], the release of which from 'K' cells is in turn promoted by luminal glucose in the duodenum and jejunum [7,27]. So the release of GLP-2 is already coupled to the presence of glucose in the intestinal lumen. Our data, obtained with the vascularly perfused jejunum, showing the inhibition of glucose uptake by phloretin would support the view that glucose can directly stimulate GLUT2 insertion. In a single-pass perfusion experiment there is no possibility for GLP-2 release from the ileum to influence the isolated jejunum. However, our fructose-transport experiments in the isolated BBM vesicles show that the effect of GLP-2 can add to that of luminal glucose. So it is possible that both pathways can act together to maximize the response. The relationship between these two signals merits further investigation.

Summary

We have detected GLUT2 in the BBM of the rat jejunum using both molecular and functional techniques. It appears that the protein is stored adjacent to the apical plasma membrane and can be rapidly inserted in response to vascular GLP-2 and/or luminal glucose. This transport protein could provide a low-affinity/high-capacity route of entry into the enterocytes for glucose, galactose and fructose and may explain the apparent 'diffusional' pathway for hexose absorption across the small intestine when luminal concentrations of substrate are high.

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