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Streptozotocin diabetes and the expression of GLUT1 at the brush border and basolateral membranes of intestinal enterocytes

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Abstract Changes in membrane expression of sodium-dependent glucose transporter (SGLT1) and glucose transporter isoform (GLUT2) protein have been implicated in the increased intestinal glucose transport in streptozotocin-diabetes. The possible involvement of GLUT1 in the transport response, however, has not previously been studied. Using confocal microscopy on tissue sections and Western blotting of purified brush border membrane (BBM) and basolateral membrane (BLM), we have examined enterocyte expression of GLUT1 in untreated and in 1 and 21 day streptozotocin diabetic rats. In control enterocytes, GLUT1 was absent at the BBM and detected at low levels at the BLM. Diabetes resulted in a 4- to 5-fold increased expression of GLUT1 at the BLM and the protein could also be readily detected at the BBM. Insulin treatment of diabetic rats increased GLUT1 level at the BBM but was without effect on expression of the protein at the BLM.

Key words: Sugar transport; GLUT1 transporter; Diabetes mellitus; Intestinal glucose uptake; Brush border membrane; Basolateral membrane; Insulin

1. Introduction

Experimental diabetes mellitus is associated with increased intestinal sugar transport and both the BBM and BLM have been implicated in this transport upregulation [1–3]. Recent studies have found raised enterocyte levels of SGLT1 [4,5] and the facilitated transporter GLUT2 [5] in streptozotocin (STZ)-treated rats and increased expression of these proteins are now known to be involved in the enhanced glucose transport at the BBM and BLM, respectively.

GLUT1 was the first facilitated glucose transporter to be identified and characterised. It is expressed in a wide variety of cell types and is important in mediating glucose transport across erythrocyte and endothelial membranes. Although the protein is found in certain transporting cells e.g. renal proximal tubule and Caco-2 cells [6,7], there is, to date, no evidence for its expression in normal small intestinal epithelium [8]. In the present study we have used confocal immunofluorescence microscopy of rat jejunum, together with Western blotting of BBM and BLM prepared from intestinal mucosa, in order to examine the normal enterocyte distribution of GLUT1 and to determine the effects of early (1 day) and longer term (21 day) streptozotocin-induced diabetes on expression of the protein. Insulin-treatment or fasting of diabetic rats was also carried out in order to assess the importance of changes in plasma glucose concentration on expression of GLUT1.

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Abbreviations: BBM, brush border membrane; BLM, basolateral membrane; SGLT1, sodium-dependent glucose transporter; GLUT, glucose transporter isoform; PBS, phosphate-buffered saline; PMSF, phenylmethyl sulphonyl floride; STZ, streptozotocin; BSA, bovine serum albumin

2. Materials and methods

2.1. Animals

Diabetes was induced by a single tail vein injection of streptozotocin (65 mg/kg, dissolved in pH 4.5 citrate buffer) into male Sprague-Dawley rats (initial weight 220–250 g) with the animal under light ether anaesthesia. Animals were allowed free access to a standard maintenance diet prior to their use 1 or 21 days later. Some 1 day diabetic animals were given Actrapid insulin (8 U subcutaneously, Novo Industries, Denmark) 2 h before use whilst some 21 day diabetics were fasted 24 h before use but had free access to water. For removal of tissue, rats were anaesthetised with pentobarbitone sodium (Sagatal, May and Baker, 90 mg/kg i.p) and 5 cm of jejunum (beginning 10 cm from the ligament of Trietz) was removed for confocal microscopy and a further 20 cm for preparation of enterocyte membranes. Blood samples were then obtained by cardiac puncture and a glucose oxidase method was utilised for assay of the sugar in plasma.

2.2. Preparation of membrane vesicles

Brush border and basolateral membranes were prepared using a previously described method with some modifications [9]. Jejunal mucosal scrapings were suspended in hypotonic buffer (in mM: 50 mannitol, 2 HEPES, pH 7.1) and homogenised for 2×30 s using an Ultra Turrax homogeniser (Janke and Kunkel, Germany) at full speed. This homogenate was centrifuged at $2500\times g$ for 15 min and the supernatant centrifuged further (20 min at $20\,500\times g$). This step produces a double precipitate consisting of a white fluffy upper pellet and a hard brown lower pellet. The upper layer was used to purify BLM and could be easily removed by gentle swirling following the addition of 5 ml resuspension buffer containing (mM) 300 mannitol, 20 HEPES, 0.1 $MgSO_4$, 0.5 PMSF and 100 U/ml aprotinin. The pellet was resuspended with 6 strokes in a hand-held homogeniser and was layered onto a 30%/40% sorbitol density gradient. Following centrifugation at $70\,000\times g$ for 1 h the cloudy band at the 30%/40% interface was removed, diluted with resuspension buffer, and centrifuged for a further 20 min at $100\,000\times g$ to obtain the basolateral pellet.

For preparation of BBM, the hard brown pellet obtained as described above was resuspended in the resuspension buffer using a hand-held homogeniser. The homogenate was diluted to a final volume of 60 ml with the same buffer and $MgCl_2$ (final concentration 10 mM) and stirred on ice for 20 min. Following centrifugation at $3000\times g$ for 10 min, the resulting supernatant was centrifuged for a further 30 min at $27\,000\times g$ to obtain the brush border pellet. All the above steps were performed at 4°C.

BBM and BLM preparations were resuspended in the resuspension buffer described above to a protein concentration of 3–6 mg/ml. The activities of sucrase and alkaline phosphatase in the initial homogenate and BBM suspensions were determined [10,11] together with Na^+,K^+-ATPase activity [12] for the BLM preparation in order to calculate enrichment values for these enzymes in the membrane preparations.

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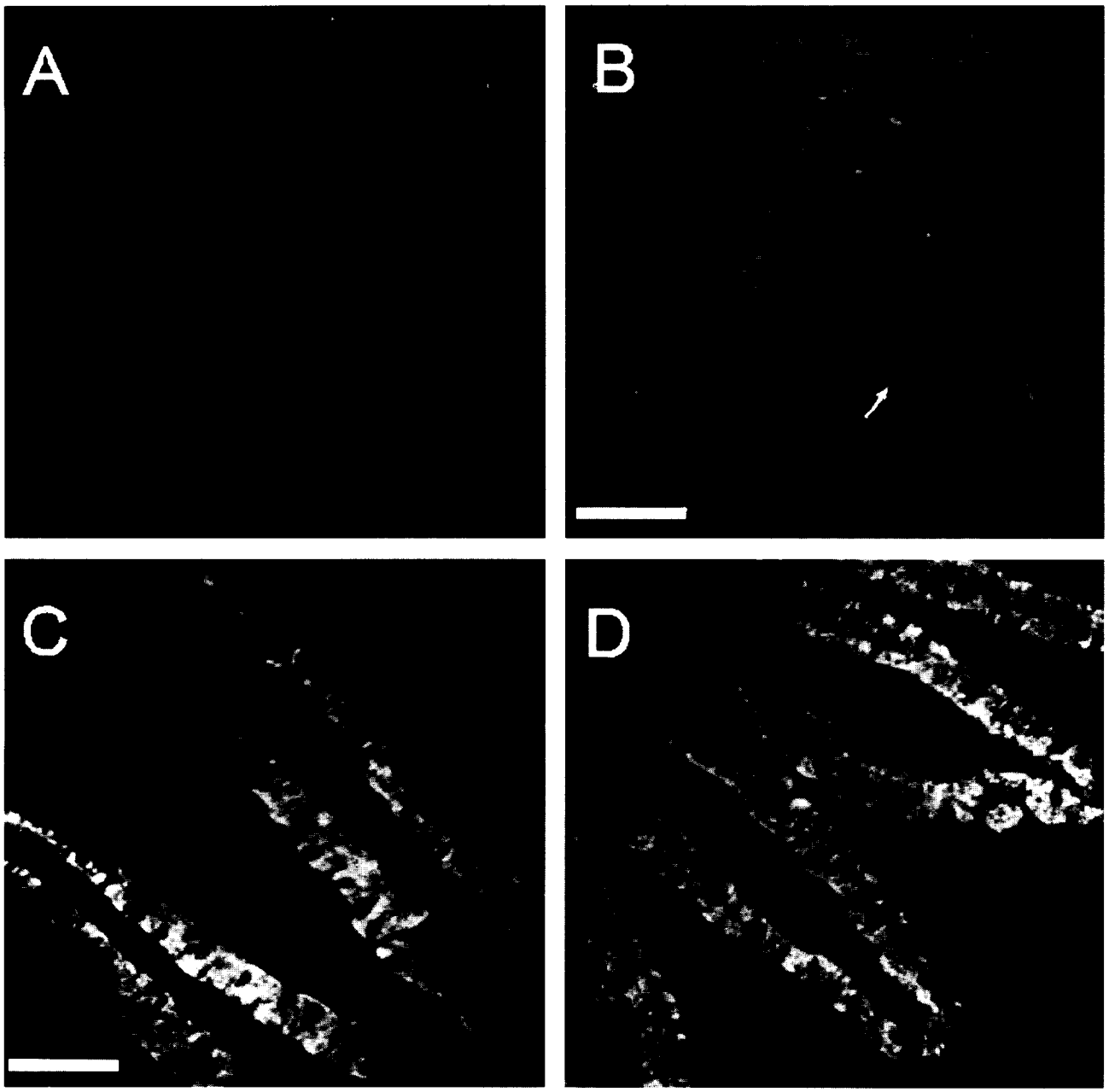

Fig. 1. Confocal immunofluorescence images showing the localisation of GLUT1 in non-diabetic and diabetic jejunum. (A) Control section incubated with non-specific rabbit IgG in place of anti-GLUT1 antibodies. (B) GLUT1 in non-diabetic jejunum. Low magnification image showing immunostaining over the entire villus length. Note the widespread intracellular distribution of GLUT1 and the lack of Goblet cell staining (arrow). (C,D) 1 and 21 day diabetic jejunum, respectively, showing increased expression of GLUT1. Scale bars=100 μm

2.3. Western blotting

Antiserum against GLUT1 was produced by immunising a rabbit with a synthetic peptide corresponding to residues 477–492 of rat GLUT1, conjugated via an additional N-terminal cysteine residue to ovalbumin. Peptide-specific antibodies were affinity purified by passage of the antiserum through a column of peptide immobilised on Sulfolink gel (Pierce), followed by elution in 50 mM diethylamine-HCl and dialysis into PBS. For Western blotting, membranes from control animals (20 μg) or diabetics (10 μg) were solubilised in Laemmli sample buffer [13] containing 1% SDS and electrophoresed on 10% SDS polyacrylamide gel. Proteins were then transferred from the gel to nitrocellulose filters by electrophoretic blotting for 1 h at a constant voltage of 100 V using a Bio-Rad Mini Trans-Blot Cell. Non-specific protein-binding sites on the filters were blocked by incubation for 2 h at room temperature with TTBS buffer (pH 7.5) containing (mM) 20 Tris, 500 NaCl, 0.2% Tween 20, with the addition of 5% fat-free milk powder. Filters were next incubated overnight at room temperature with 1 μg/ml affinity purified anti-GLUT1 antibodies, in TTBS containing 1% fat-free milk powder. They were then washed (3×15 min) in TTBS, incubated for 2 h at room temperature with sheep anti-rabbit IgG conjugated to horseradish peroxidase, and finally washed again with TTBS (3×30 min). Bound antibodies were then detected using a chemiluminescence detection procedure (Boehringer Mannheim, Germany) according to the manufacturer's instructions.

For quantitation of the relative amounts of GLUT1 protein, X-ray films were scanned using a Scanmaster III Densitometer (Howtek, USA) and images were analysed using Quantity One v4.0 software. Preliminary work using known levels of GLUT1 protein on blots revealed a linear relationship between the intensity of chemiluminescence and the amount of GLUT1 present (data not shown).

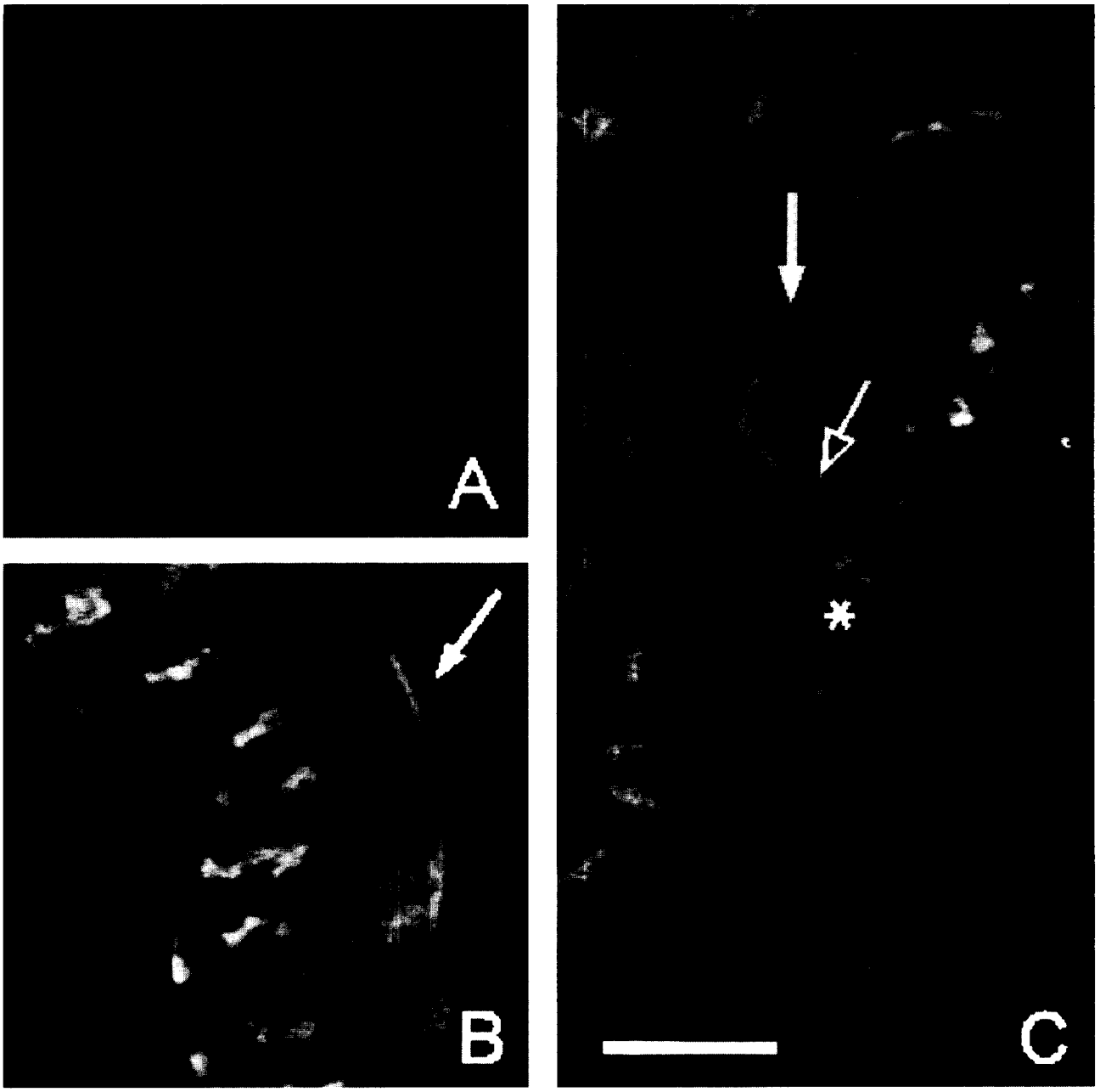

Fig. 2. Higher magnification confocal immunofluorescence images showing localisation of GLUT1 in non-diabetic (A) and 1 and 21 day diabetic jejunum (B and C, respectively). Note faint basolateral membrane staining of enterocytes in (A) in addition to intracellular staining. (B,C) Show increased intracellular expression of GLUT1, together with clear brush border (solid arrows) and basolateral membrane staining (open arrows). Also evident is dense staining in the peri-nuclear regions of enterocytes (*). Scale bar = 20 μm

2.4. Immunofluorescence microscopy

Jejunal segments were flushed through with PBS and cut into portions 0.5 cm in length. These were fixed overnight at 4°C in PBS containing 4% paraformaldehyde and then transferred to PBS containing 7% sucrose and 0.1% sodium azide. Tissue was equilibrated in this solution overnight at 4°C and then embedded in OCT and frozen in liquid nitrogen cooled isopentane. Cryostat-cut sections (8 μm thick) were placed on Vectabond coated glass slides and stored at 4°C in PBS.

For immunostaining, slides were incubated for 90 mins in PBS containing 3% BSA and then overnight at 4°C with the affinity purified anti-GLUT1 antibody (20 μg/ml in PBS with 1% BSA). Sections were then washed (3×10 min) in PBS and incubated for 1 h at room temperature with a 1:100 dilution (in PBS with 1% BSA) of FITC-conjugated goat anti-rabbit IgG (Sigma). Sections were finally washed (3×10 min) in PBS, mounted in Vectashield and finally visualised using a Leica Confocal Laser Scanning Microscope.

2.5. Statistics

Results are given as mean ± S.E.M. Differences between means were evaluated by a Student's *t*-test and considered significant at $p < 0.05$.

3. Results

Streptozotocin-treated animals were hyperglycaemic (plasma glucose concentration, in mM: control, 10.8 ± 0.2; 1 day STZ, 34.4 ± 2.9; 21 day STZ, 41.9 ± 3.0). Insulin treatment for 2 h reduced glucose level in 1 day diabetic animals to 8.6 ± 0.5

mM whilst an overnight fast lowered plasma glucose concentration in 21 day diabetics to 11.2 ± 1.3 mM.

Confocal immunofluorescence microscopy of non-diabetic rat jejunum revealed a widespread intracellular distribution of GLUT1 (Fig. 1B). Staining was greatest in the lower/middle two thirds of the villi with the upper villus showing reduced expression of the protein. Staining was absent from the goblet cells (arrow). Staining was not seen using non-specific rabbit IgG instead of the anti-GLUT1 antibodies (Fig. 1A). Increased expression of GLUT1 was evident in jejunal sections from 1 and 21 day STZ-treated rats (Fig. 1C and D, respectively). As with non-diabetic villi, the diabetic villi show highest expression of GLUT1 in the lower two thirds of the villi, with lower expression in the tips of the villi. The specificity of GLUT1 staining of non-diabetic and diabetic jejunum was assessed by prior absorption of the antibody with the GLUT1 immunising peptide, which completely eliminated the signal seen (data not shown).

Higher magnification confocal immunofluorescent images of non-diabetic rat jejunum revealed low, though detectable levels of GLUT1 at the BLM, in addition to intracellular GLUT1. It was possible to discern increased basolateral (open arrow) and brush border membrane (solid arrow) GLUT1 expression 1 and 21 days after treatment with STZ (Fig. 2B and C, respectively), as would be expected from the Western blot results described below. In addition, to the generally increased GLUT1 staining throughout the cytoplasm, STZ treatment also specifically induced the expression of GLUT1 in the vicinity of the nuclei (*) (Fig. 2B,C).

BBM preparations used for Western blotting were enriched 16–18- and 10–13-fold for sucrase and alkaline phosphatase, respectively, compared to the homogenate, but showed no enrichment of Na^+,K^+-ATPase. BLM vesicles were enriched 9–14-fold in Na^+,K^+-ATPase but not for the two BBM enzymes. Diabetes was without effect on enzyme enrichments.

Western blotting of membranes for GLUT1 revealed clearly defined bands at an M_r of 54 000 (Fig. 3) in accordance with the apparent M_r of this protein in other tissues [14]. The bands migrated with the same mobility as that of purified human erythrocyte GLUT1. Specificity of the antibody was shown by complete elimination of the bands on Western blots by prior absorption of the antibody with the immunising GLUT1 peptide (results not shown). Quantitation of the bands revealed that BLM levels of GLUT1 were 78.6 and 387.1% higher at 1 and 21 days, respectively, after STZ (ranges: control, 0.86–8.34; 1 day, 1.30–12.79; 21 day, 3.43–30.0 a.u.), although the increase only reached statistical significance after 21 days ($p < 0.02$ compared to control) (Fig. 4). These results confirm the diabetic pattern of GLUT1 expression detected by immunofluorescence. GLUT1 expression at the BBM was increased by 200.8% ($p < 0.02$) and 810.2% ($p < 0.005$) after 1 and 21 days treatment with streptozotocin respectively (ranges: control, 0.49–0.83; 1 day, 0.90–4.61; 21 day, 4.81–13.94 a.u.). Insulin treatment of 1 day diabetics markedly enhanced BBM and BLM levels of GLUT1 whilst overnight fasting of 21 day diabetics was without significant effect on expression of the transporter at the BBM but reduced by 53%, GLUT1 expression at the BLM.

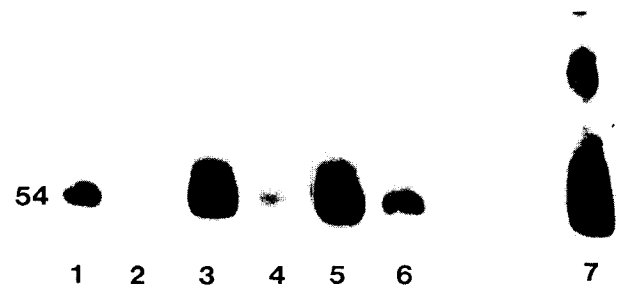

Fig. 3. Western blot detection of GLUT1 in basolateral membranes (lanes 1,3,5) and brush border membranes (lanes 2,4,6) prepared from control rats (lanes 1,2) and 1 day (lanes 3,4), and 21 day (lanes 5,6) diabetic rats. 20 μg protein was applied per lane. Lane 7: 10 μg of purified human erythrocyte membrane.

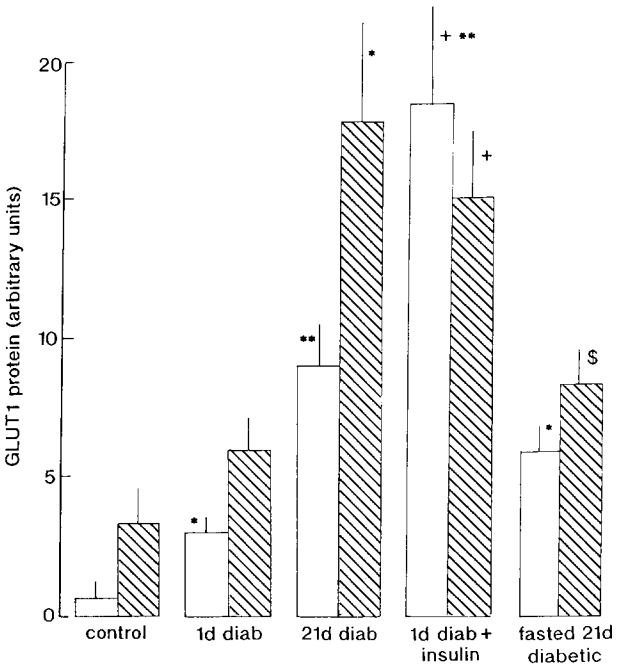

Fig. 4. Quantification of GLUT1 in BBM (open bars) and BLM (striped bars) prepared from jejunal mucosa of control, 1 d and 21 d diabetic rats, 2 h insulin treated 1 d diabetic rats and overnight fasted 21 d diabetic rats. Results were obtained by densitometry of Western blots and are means (arbitrary units) ± SEM for 4–10 membrane preparations. *$p < 0.02$, **$p < 0.005$ compared to control; $^+p < 0.005$ compared to 1 d diabetic; $p < 0.02$ compared to 21 d diabetic.

4. Discussion

Upregulation of intestinal glucose transport during experimental diabetes has been widely reported and the response involves enhanced epithelial expression of the sodium-dependent transporter, SGLT1, at the BBM and the facilitated transporter GLUT2 at the BLM [1–3]. The effects of diabetes on enterocyte expression of GLUT1 have not previously been studied, presumably because of the reported absence of this protein in enterocytes from non-diabetic animals [8]. GLUT1 is, however, expressed in Caco-2 cells, a colonic cell line which displays enterocyte-like properties [7].

Results from the present study show that the villus distribution of GLUT1 was similar to that reported for the other two enterocyte transporters, GLUT2 and SGLT1 [5]. In all cases, the progressive maturation of cells during their migration along the villus results in maximal expression of transport protein in the mid-villus region. Transporter levels at the vil-

lus tip are generally lower than the mid-villus because of enterocyte senescence and desquamation.

The most important feature of our study is the finding that diabetes results in expression of GLUT1 at the brush border whilst, at the same time increasing levels of the protein at the BLM. It is likely that the very low density of GLUT1 detected on Western blots of non-diabetic BBM were a consequence of slight contamination with BLM, which is inevitable, even in purified membrane preparations. However, such contamination cannot explain the large elevation in GLUT1 levels seen in BBM from diabetic animals, since diabetes was without effect on the purity of either BBM or BLM membrane fractions as assessed using marker enzymes. Moreover, the results of confocal microscopy sections were unequivocal (Fig. 2). The finding that GLUT1-mRNA is below the level of detection in enterocytes from non-diabetic rats [15] suggests transcriptional control of GLUT1 expression in diabetes. However, studies of mRNA levels in diabetic rats will be required to confirm this point. Although there is a growing body of evidence that diabetes alters the membrane distribution of GLUT2, another facilitated glucose transporter found at the enterocyte BLM, [16,17], our present work presents the first immunocytochemical evidence for the targeting of glucose transporters to the BBM in this condition.

The widespread cytoplasmic distribution of GLUT1 is not seen with other enterocyte facilitated glucose transporters. However, a cytoplasmic localisation for a plasma membrane protein that cycles through the endocytic compartment is to be expected. Indeed, intense intracellular vesicular staining for GLUT1 in BHK cells, particularly in the perinuclear region, has been reported and the transporters move from this compartment to the cell surface in response to a variety of stimuli including metabolic stress [18].

The precise role of enterocyte GLUT1 in diabetes and its function in relation to other glucose transporters present at the BBM and BLM are unknown. However, enhanced membrane expression of GLUT1 in diabetes will result in greater facilitated glucose transport at the BLM and increased phlorizin-insensitive (non SGLT1-mediated) glucose uptake at the brush border, both of which are a feature of the disease [1,2,19,20]. As such, increased enterocyte GLUT1 expression in diabetes will contribute to postprandial hyperglycaemia.

The signals which influence GLUT1 expression and its subcellular localisation are unknown. Although it has been shown that insulin rapidly stimulates translocation of GLUT1 to the plasma membrane in other cell types [21] and, in the longer term, enhances expression of the protein [22], the differential effects of insulin treatment and fasting of diabetic animals, both of which normalise plasma glucose levels, imply that alterations in blood levels of neither insulin nor glucose are responsible for the changes in expression of GLUT1 at the BBM and BLM. A possible humoral mediator is pancreatic glucagon which is known to increase BBM and BLM sugar transport [9] and which is secreted in increased amounts during diabetes. The hormone may act by increasing the production of cAMP within enterocytes and in this context it is of interest that experimentally raised cytosolic levels of cAMP promote enterocyte sugar transport [23]. cAMP has also been implicated in the regulation of glucose transporter expression in other cell types [24]. Gaining an understanding of the extracellular factors which regulate the expression of GLUT1 will clearly be of major importance in elucidating the mechanisms by which enterocyte glucose transport is altered in diabetes.

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