Transcriptional and translational control over sodium-glucose-linked transporter (SGLT1) gene expression in adult rat small intestine

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Abstract We have measured SGLT1 mRNA content and SGLT1-mediated glucose transport at different positions along the small intestine of control and streptozotocin diabetic rats and shown both parameters to be similar but higher in jejunal compared with ileal tissue. No such correlation was seen when comparing measurements of SGLT1 mRNA along jejunal villi with previous estimates of SGLT1 protein and SGLT1-mediated glucose transport [Debnam et al., Eur. J. Physiol. 430 (1995) 151–159]. This is the first time it has been possible to directly relate these three aspects of SGLT1 gene expression in a single species. Results are discussed in terms of a possible time rather than positional control over translation of SGLT1 mRNA.

Key words: Sodium-dependent glucose transport; SGLT1; Regulation; Small intestine

1. Introduction

Enterocytes emerging as the predominant cell line from stem cells dividing in intestinal crypts begin to express both digestive and absorptive functions during subsequent migration to the tips of villi. This process of differentiation, involving the synthesis of various digestive enzymes and transporter proteins, can itself be modified by many factors acting at the same or different sites along the small intestine. These observed effects have been used recently to identify molecular mechanisms controlling expression of lactase phlorizin hydrolase (LPH) and sucrase-isomaltase (SI) genes [1–4]. Both of these proteins perform their digestive functions as ectoenzymes present in the enterocyte brush border membrane. The aim of the present work was to test whether control over expression of a sodium-glucose-linked transporter (SGLT1), also present in enterocyte brush border membranes, would be similar or different from that reported previously for LPH and SI.

Identifying different levels of control over gene expression depends initially on making quantitative comparisons between mRNA and protein levels in situations where gene expression can be shown to vary. Measurement of pre-RNA levels and the ability of gene products to exhibit biological activities then gives further information about transcriptional and post-translational processing. Gene expression can, however, also change in unpredictable ways during enterocyte migration along the crypt-villus axis [5,6] and this possibility should also be considered when describing any overall process of gene regulation in the small intestine [7].

The present experiments use a recently obtained rat SGLT1 cDNA [8] to make a quantitative analysis of SGLT1 mRNA levels in fractionated and villus-attached enterocytes as well as in whole tissue extracts prepared from jejunal and ileal tissue. The results show an expected correlation between SGLT1 mRNA and SGLT1-mediated glucose transport in jejunal and ileal tissues. The villus position of enterocytes expressing SGLT1 mRNA is, however, different from that found previously when measuring SGLT1 protein and SGLT1-mediated glucose transport [9]. Taken together these results suggest a transcriptional control over SGLT1 expression along the intestine with an additional translational control along villi.

2. Materials and methods

2.1. Animals and preparation of intestinal tissue

Male Sprague-Dawley rats (230–250 g) were either maintained as controls or given a single tail injection of streptozotocin (60 mg/kg) to induce diabetes 17 days before starting the experiment. During this time rats (five per group) were allowed free access to water and maintenance diet under controlled conditions of heating and lighting. Tissues removed 10 cm distal to the ligament of Trietz (jejunum) or 10 cm proximal to the ileo-caecal junction (ileum), from animals anaesthetised by pentobarbitone sodium (90 mg/kg, i.p.), were washed thoroughly in ice-cold isotonic saline before being processed to determine SGLT1 mRNA and glucose transport. Estimates of SGLT1 mRNA levels in whole tissue extracts and villus-attached enterocytes were carried out on samples frozen immediately in liquid N_2. Estimates of SGLT1 mRNA in isolated enterocytes involved a preliminary incubation of intestinal sacs at 37°C to collect enterocytes which were then frozen in liquid N_2. Rapid uptake of glucose into whole tissue was measured within 3 min of its removal from the animal.

2.2. Experimental techniques

Upper and lower villus enterocytes were isolated as separate fractions from control jejunal tissue by first incubating a ligated intestinal sac filled with phosphate buffer containing sodium citrate, β-hydroxybutyric acid and bovine serum albumin for 15 min at 37°C. This solution was then discarded and replaced by a similar solution but with dithiothreitol added and substitution of EDTA for sodium citrate. Upper villus enterocytes were collected from this solution after a further 8 min incubation at 37°C. This procedure was then repeated for 6 min to obtain mid-villus enterocytes which were discarded and again for 6 min to obtain basal villus enterocytes. Further details of this procedure, including morphological evidence of villus structure establishing the origin of enterocytes collected in these two fractions, can be obtained from the original publication [10]. All solutions were made up in diethyl pyrocarbonate-treated distilled water containing 1 U/ml of RNAase inhibitor (Sigma Chemical Co., St. Louis, MO, US).

SGLT1-mediated glucose entry into rat jejunal and ileal tissue was determined using a rapid uptake apparatus as described previously [11]. Tissue samples mounted mucosal face upwards were first equilibrated for 2 min at 37°C in bicarbonate buffer gassed with 95% O_2/5% CO_2. This solution was then replaced by an identical one containing 4 mM [^{3}H]glucose with or without 1 mM phlorizin. The buffer also contained [^{14}C]carboxyl inulin for subsequent correction for extracel-

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Abbreviations: LPH, lactase-phlorizin hydrolase; SGLT1, sodium-dependent glucose transporter; SI, sucrase-isomaltase

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lular [^{3}H]glucose accumulation. Tissues removed after 1 min incubation were disrupted in 0.1 N HNO_3 and the amount of radioactivity determined by dual isotope scintillation spectrometry. The phlorizin-sensitive glucose uptake was then taken to represent that mediated through the SGLT1 transporter.

2.3. RNA extraction and Northern blot analysis

Total RNA was extracted from frozen pellets by homogenisation in 4 M guanidinium isothiocyanate followed by density-gradient centrifugation through 5.7 M CsCl. RNA concentrations were estimated by OD_{260} readings before denaturing in formaldehyde-containing buffer for electrophoresis in 1% agarose/2.2 M formaldehyde gels (10 μg/lane). These gels were then stained briefly with ethidium bromide to confirm RNA integrity and size distribution before being transferred overnight to nylon filters (Hybond-N, Amersham, UK) to be fixed by UV cross-linking. Hybridisation was performed at 42°C in 50% formamide/4×SCC, 5×Denhardt's solution/0.1% SDS/10 mg ml^{-1} herring sperm DNA. Blots were washed three times in 2×SCC 0.1% SDS at room temperature followed by two washes in 0.1% SCC/0.1% SDS at 65°C. This method is similar to that described previously [8].

2.4. In situ hybridisation

Cryostat sections of tissue samples (10 μm) fixed in 4% paraformaldehyde (15 min at 4°C) and acetylated in 0.1 M triethanolamine containing 0.33% acetic anhydride were incubated in hybridisation buffer containing [^{35}S]UTP-labelled sense or antisense cRNA probes for SGLT1 mRNA. Hybridisation involved initial overnight incubation of sections at 50°C in Denhardt's solution containing labelled probe, 10% dextran, yeast RNA and 50% formamide. Slides were then washed thoroughly, first with 1×SCC (60 min, 50°C), and then twice with 1×SCC (60 min at room temperature). Further details of this method can be obtained from a previous publication [12].

2.5. Quantification of Northern blot and villus-attached enterocyte SGLT1 mRNA

Northern blots exposed to film for 1 day at −70°C were developed and autoradiographic images captured using a low light CCD camera (Photonics Science, UK). These images were then processed on a KS 400 image analyser. Integrated grey values obtained within areas traced around bands of interest gave values of relative amounts of mRNA present.

Relative amounts of SGLT1 mRNA present in villus-attached enterocytes were measured directly by scanning microdensitometry (M85, Vickers, UK). Autoradiographs of sectioned tissue were scanned sequentially from base to tip of villi at a final magnification of ×1000 at a light wavelength of 490 nm. Each measurement integrated readings within a 12×6 μm area of enterocyte cytoplasm. Further details of conditions used to ensure these measurements were linearly related to mRNA content are given in an earlier publication describing vitamin D effects on calbindin mRNA expression [5]. Only antisense SGLT1 mRNA probes gave detectable readings of optical density.

3. Results and discussion

The relationship between jejunal and ileal SGLT1 mRNA and the effect diabetes has upon these values is shown by Northern blot analysis in Fig. 1a. Blots from jejunal tissue had a greater density than those using ileal tissue and diabetes increased the amount of SGLT1 mRNA in both jejunum and ileum. Values for SGLT1 mRNA abundance measured in arbitrary units (curved brackets) are compared with measurements of SGLT1-mediated glucose transport measured in nmol·cm^{-2}·min^{-1} [square brackets] as follows: diabetic jejunum (31.4) [36.7], control jejunum (17.6) [18.1], diabetic ileum (13.2) [5.6] and control ileum (7.1) [3.2]. The close agreement seen above between values of mRNA abundance and glucose transport, obtained from pooled tissues taken from three control and three diabetic rats, strongly suggests that the SGLT1 gene is regulated by the accumulation of mRNA along the small intestine.

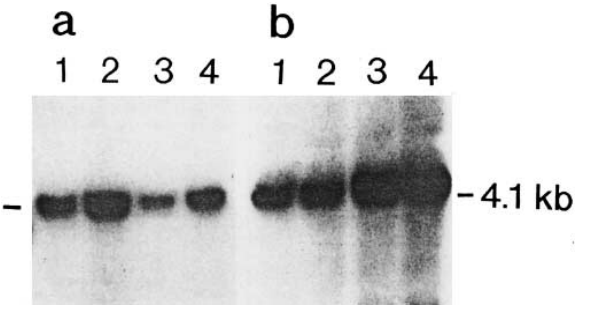

Fig. 1. High stringency Northern blot analysis of rat intestinal SGLT1. Blots were probed with ^{32}P-labelled rat SGLT1 cDNA at 43°C. Subsequent high stringency washing was as described in the text. a: Lanes 1, 2, 3, and 4 show blots prepared from diabetic ileum, diabetic jejunum, control ileum and control jejunum respectively. b: Blots obtained from villus tip (lanes 1 and 2) and basal villus (lanes 3 and 4) enterocytes harvested from the jejunum of normal rats.

The above correlation gives no information on the cellular origin of the differences observed. This is because jejunal villi are considerably longer than ileal villi and diabetes further increases the length, and therefore the surface area, of both jejunal and ileal villi. Extracting RNA from fractionated enterocytes reveals an additional possible complication with the amount of SGLT1 mRNA in basal villus enterocytes being twice that found in villus tips (mean values of 213 and 117 arbitrary units respectively; Fig. 1b). For these reasons it was decided to use in situ hybridisation to investigate these differences in more detail at the cellular level. Initial observation of in situ autoradiography confirmed previous findings that mRNA abundance was highest in basal villus enterocytes but that some SGLT1 mRNA was present in the crypts [8].

Further quantitative analysis shows profiles for SGLT1 mRNA expression from base to tip of jejunal and ileal villi in tissues taken from control and diabetic rats (Fig. 2). The relative amounts of SGLT1 mRNA fall steadily along the villus in all cases. The amount of SGLT1 mRNA is, however, approximately twice as high in jejunal compared with ileal villi. This difference reflects a change in the programming of cell differentiation in the intestinal crypt. Diabetes was without effect on jejunal levels of SGLT1 mRNA a result not predicted from analysis of Northern blots. However, diabetes increased SGLT1 mRNA levels in the basal 50 μm of ileal villi. The present results agree with recent findings showing that diabetes has no effect on expression of SGLT1 protein or glucose transport into villus-attached enterocytes [9]. We conclude from this that diabetes affects different aspects of SGLT1 gene expression as a result of structural adaptation increasing the number and age of villus enterocytes able to transport glucose.

Further insight into the way SGLT1 mRNA translation might be controlled can be gained by plotting control and diabetic data for jejunal SGLT1 mRNA alongside previously published profiles describing SGLT1 protein and SGLT1-mediated glucose transport into villus-attached enterocytes [9]. In this case it was also decided to plot results against enterocyte age instead of position on the villus using a previously published value of 9 μm h^{-1} for enterocyte migration rate [13]. The results obtained from carrying out this transformation are shown in Fig. 3.

Little or no SGLT1 protein or glucose transport occurs in

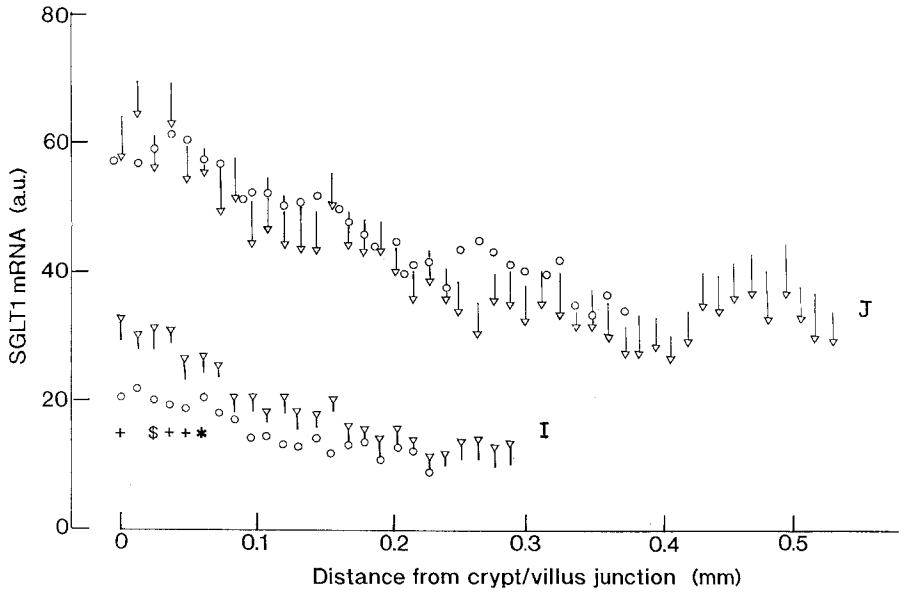

Fig. 2. Positional dependence of SGLT1 mRNA appearance in rat villus-attached enterocytes. Estimates of SGLT1 mRNA in control (○) and diabetic (▽) jejunal (J) and ileal (I) enterocytes give means obtained from analysing villi from 5 rats (±S.E.M. for diabetic animals). For clarity, standard error bars of control data have not been included. There were however no significant differences between values obtained from diabetic and control jejunal villi. For the ileum, *, + and $ indicate significance at $P<0.05$, $P<0.02$ and $P<0.005$ respectively. Sense probes for SGLT1 mRNA gave no detectable signal.

5–10 h old upper crypt enterocytes containing large amounts of SGLT1 mRNA. There is then a slow appearance of both SGLT1 protein and transport function in 10–25 h old enterocytes associated with a fall in enterocyte SGLT1 mRNA in the basal half of the villus. The rate of appearance of SGLT1 then increases markedly in 25–50 h old enterocytes at a time when the amounts of SGLT1 mRNA are further reduced. Some evidence that it is enterocyte age rather than villus position which initiates this change in translational efficiency can be seen when working with human jejunal tissue where both SGLT1 protein and glucose transport function increase linearly from base to tip of jejunal villi [7,14]. This difference from rat could reflect the fact that human enterocytes are already about 25h old when they begin to migrate onto villi [15]. In

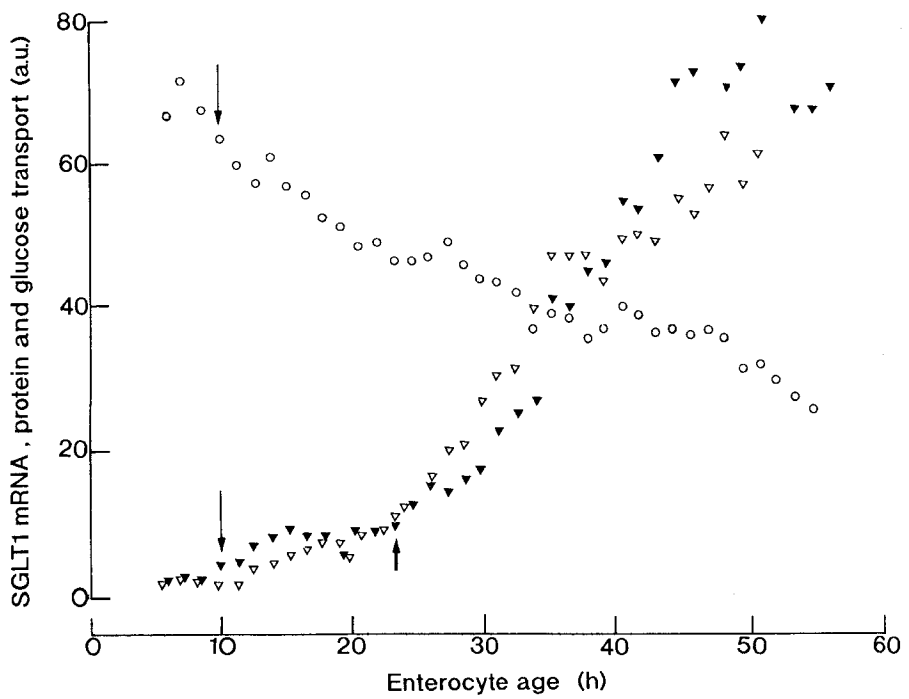

Fig. 3. Temporal aspects of SGLT1 gene expression in rat jejunal villus-attached enterocytes. Values for SGLT1 mRNA (○), SGLT1 protein (▽) and SGLT1-mediated glucose transport (▼), obtained from present (Fig. 2) and previous work [9], are related to enterocyte age assuming an enterocyte migration rate of 9 μm h^{-1} [13]. All values give pooled means using tissue taken from 5 control and 5 diabetic rats. ↓, crypt-villus junction. ↑, approximate age when enterocytes begin to translate SGLT1 mRNA efficiently.

order to test this hypothesis further it will be necessary to measure SGLT1 mRNA directly in human villus-attached enterocytes and possibly determine how induced changes in enterocyte migration rate affect the position or time when SGLT1 mRNA first becomes efficiently translated.

Previous work with LPH and SI shows the early appearance of enzyme activities in basal and mid villus enterocytes to correlate closely with that for their respective mRNAs [16–18] and there is therefore no need to postulate a major role for translational control over gene expression. Present results do, however, show clearly that villus regulation over SGLT1 expression is different from that found for LPH and SI.

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