Biochem. J. (1993) 291, 435–440 (Printed in Great Britain)

Regulation of the ovine intestinal Na^+/glucose co-transporter (SGLT1) is dissociated from mRNA abundance

Leslie LESCALE-MATYS,* Jane DYER,† Dennis SCOTT,‡ Thomas C. FREEMAN,† Ernest M. WRIGHT* and Soraya P. SHIRAZI-BEECHEY†§

*Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90024-1751, U.S.A., †Epithelial Function and Development Group, Department of Biochemistry, University of Wales, Aberystwyth, Dyfed SY23 3DD, U.K. and ‡The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, U.K.

We have investigated the mechanisms of regulation of the Na^+/glucose co-transporter (SGLT1) in a ruminant animal, which is an exceptional model system for studying intestinal glucose transport. Pre-ruminant lambs absorb glucose, produced by hydrolysis of the milk sugar lactose, in the intestine via apical SGLT1 and basolateral facilitative glucose transporters (GLUT2). Weaning coincides with the development of the rumen, and consequently the amount of hexoses reaching the small intestine of the ruminant sheep is undetectable. During development, SGLT1 activity and abundance in intestinal brush-border membranes decreased by over 200-fold, and either maintaining lambs on a milk replacer diet or infusing sheep intestine with D-glucose restored co-transporter activity and expression. We have measured ovine intestinal SGLT1 mRNA levels during development, with changes in diet and after direct infusion of D-glucose or methyl α-D-glucopyranoside into the intestinal lumen, in order to determine the level of regulation. During development, mRNA levels decreased only 4-fold. Lambs maintained on a milk replacer diet showed no change in mRNA levels relative to age-matched controls. Finally, upon infusion of the intestine of the ruminant sheep with sugars, D-glucose infusion increased SGLT1 mRNA, but only by 2-fold, compared with a 60–90-fold increase in co-transporter number and activity. Since the change in Na^+-dependent glucose transport activity is correlated with SGLT1 protein abundance, and since changes in mRNA levels do not account for the dramatic changes in protein abundance, we conclude that the principal level of SGLT1 regulation by luminal sugar is translational or post-translational.

INTRODUCTION

Ruminant animals such as sheep provide an excellent model system for studying the dietary regulation of sugar absorption (Shirazi-Beechey et al., 1989, 1991a,b). The development of the rumen is one of the features of this system. In sheep, weaning coincides with the maturation of the rumen, where ingested carbohydrates are fermented into volatile fatty acids by the rumen microflora. Consequently, the amount of hexoses reaching the small intestine gradually decreases with age and eventually becomes undetectable in the intestines of ruminant animals (Bassett, 1975). The ovine intestinal tract therefore provides a natural and efficient way of limiting the luminal concentration of hexoses having a specific affinity for Na^+-coupled glucose co-transporters (Scharrer et al., 1979; Shirazi-Beechey et al., 1989). Intestinal glucose transport occurs via an apical Na^+-coupled glucose co-transporter (SGLT1) and a basolateral facilitative glucose transporter (GLUT2). SGLT1 is a glycosylated integral brush-border membrane protein which has been cloned, and anti-peptide antibodies have been developed (Hediger et al., 1987; Hirayama et al., 1991; Hirayama and Wright, 1992). Sequence analysis reveals that the protein shows no structural homology to the facilitative glucose carriers (Bell et al., 1990). We have previously shown that, coincident with the developmental change in diet and carbohydrate digestion in sheep, both Na^+-dependent glucose transport activity and SGLT1 protein abundance decrease by approx. 200-fold. The decline in transport activity and protein abundance can be prevented by maintaining lambs on a milk replacer diet, or by introducing D-glucose or methyl α-D-glucopyranoside directly into the small intestine. We proposed that intact luminal sugar increases either transcription or translation of the SGLT1 gene (Shirazi-Beechey et al., 1991b). To determine the level of regulation, we have measured intestinal SGLT1 mRNA abundance during development, and in animals fed on a milk replacer diet or given a direct infusion of sugars into the intestinal lumen. We demonstrate that the dramatic changes in the number of SGLT1 co-transporters is not regulated pre-translationally, and infer that the principal mechanism of regulation is translational or post-translational.

EXPERIMENTAL

Animals and tissue removal

Normally reared sheep aged between 1 week and 3 years were used in this investigation. Some lambs were kept on a milk replacer diet (Lamlac milk replacer, Volac Ltd., Royston, Herts., U.K.) beyond their normal weaning period, which is at 3–8 weeks of age. We refer to lambs as pre-ruminant between birth and 3 weeks, transitional from 3 to 8 weeks and ruminant from 8 weeks onward. The intestines of 2–3-year-old sheep maintained on a conventional roughage diet were infused through duodenal cannulae as previously described (Shirazi-Beechey et al., 1991b). The infusions were carried out for 4 days at a rate of 1.5 litres per day with solutions containing 30 mM D-glucose or methyl α-D-glucopyranoside as described previously (Shirazi-Beechey et al., 1991a).

The animals were anaesthetized and proximal segments of intestine, identified as being 1 m distal to the pyloric sphincter, were removed promptly. The tissues were washed with ice-cold isotonic saline, immediately frozen in liquid nitrogen, and later stored at −80 °C until use for preparation of brush-border membranes or RNA. We have previously reported the levels of Na^+-dependent glucose uptake and SGLT1 protein abundance

§ To whom correspondence should be addressed.

in the brush-border membranes isolated from the intestines of these same animals (Shirazi-Beechey et al., 1991b).

Brush-border membrane vesicle preparation

Brush-border membrane vesicles were prepared from frozen intestinal segments as described in detail previously (Shirazi-Beechey et al., 1988, 1989, 1991a). The method consists of a combination of Mg^{2+} precipitation and differential centrifugation techniques to prepare sealed right-side-out purified brush-border membrane vesicles. The brush-border membrane vesicles were suspended in a solution containing 300 mM mannitol, 0.1 mM magnesium sulphate and 20 mM Hepes/Tris buffer, pH 7.4, and were stored in liquid nitrogen for later determination of Na^+-dependent glucose transport and Western blot analysis.

Assay of Na^+-dependent glucose transport

Na^+-dependent glucose transport into brush-border membrane vesicles was measured at 37 °C by the rapid filtration technique described before (Shirazi et al., 1981; Shirazi-Beechey et al., 1989). Routinely, 3–5 μl of vesicle suspension (approx. 100 μg of protein) was incubated in a solution containing either 100 mM sodium or potassium thiocyanate, 100 mM mannitol, 20 mM Hepes/Tris buffer, pH 7.4, and 0.1 mM D-[^{14}C]glucose. Incubation was stopped 4 s later by addition of 1 ml of an ice-cold buffer containing 250 μM phlorizin. The variation in uptake was less than 3 % in multiple assays using the same preparation.

Immunodetection of SGLT1 protein abundance

Determinations of SGLT1 protein abundance in brush-border membrane vesicles were performed using Western blot analysis with an anti-SGLT1 polyclonal antibody as probe, and quantified as previously described (Hirayama et al., 1991; Shirazi-Beechey et al., 1991b). The antibody was raised in rabbits, based on a procedure described by Lachmann et al. (1986), against a synthetic peptide corresponding to amino acids 402–420 of the rabbit intestinal SGLT1 sequence (Hediger et al., 1987).

SGLT1 mRNA isolation and analysis

Poly(A)$^+$ RNA was isolated from whole intestine using a modification of the method of Chomczynski and Sacchi (1987), followed by oligo(dT)–cellulose chromatography and Northern analysis as described (Chomczynski and Sacchi, 1987; Pajor et al., 1992). The residual ethidium bromide-stained 18 S and 28 S ribosomal species were visualized by illumination with u.v. light and recorded photographically to verify RNA integrity, uniformity of RNA concentration per lane and efficiacy of transfer to the membrane, as described previously (Lescale-Matys et al., 1990). Northern blots were probed using gel-purified cDNA consisting of the coding region of the rabbit intestinal SGLT1 excised with *Eco*RI (Coady et al., 1990). Hybridization and high-stringency wash conditions were as described previously (Pajor et al., 1992). Autoradiography was carried out at −80 °C, and multiple scans of autoradiograms within the linear range of the film were quantified by scanning densitometry.

Statistical analysis

All data are expressed as means ± S.E.M. Differences between groups were examined by Student's t test, and were considered significant when P values were less than 0.05.

RESULTS

Characterization and levels of ovine SGLT1 mRNA during development

Representative Northern blot analyses of polyadenylated RNA isolated from intestinal tissues of pre-ruminant (1 and 3 weeks old), transitional (5 weeks old) and ruminant (10 weeks old) animals are shown in Figure 1(a). In all samples we detected five transcripts, of 6.0, 5.2, 4.1, 3.3 and 2.4 kb. These transcripts are similar in size to those reported from human, rat and rabbit intestine (Coady et al., 1990). Multiple transcripts in sheep may be due to differences in the length of the 3′ or 5′ untranslated regions, as previously determined for human intestine (Hediger et al., 1989) and cultured pig kidney cells (Ohata et al., 1990). Figure 1(b) summarizes the relative changes in SGLT1 mRNA over the time course of development, normalized against the total transcript level in adult (≥ 10 weeks old) tissues. The total transcript mRNA abundance in pre-ruminant animals was 4-fold higher than the adult levels. We have shown previously that the total villus surface area remains unchanged during development and when dietary manipulations are carried out (Shirazi-Beechey et al., 1991a). We have also determined that the amount of total protein and the yield of total RNA per wet weight of tissue remain constant in the intestines of lambs of various ages. Therefore the relative change in SGLT1 mRNA

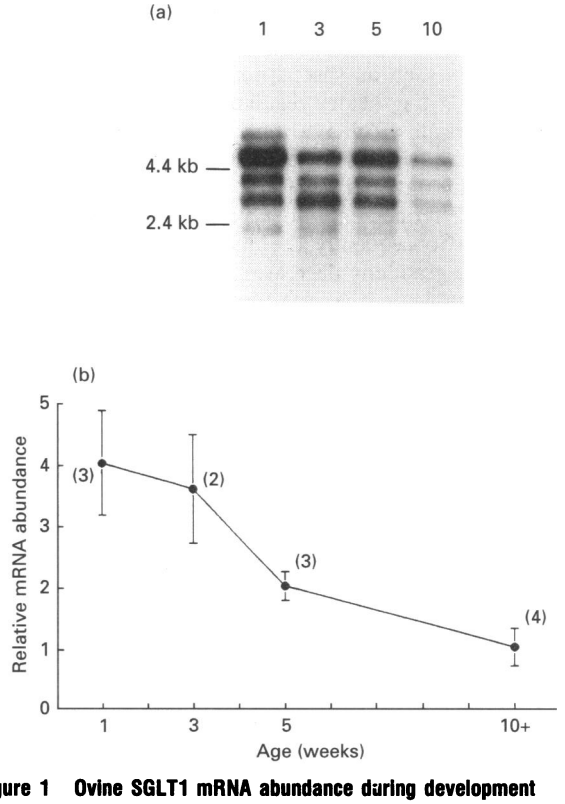

Figure 1 Ovine SGLT1 mRNA abundance during development

(**a**) Representative Northern blot of SGLT1 mRNA. Each lane contains 2.5 μg of poly(A)$^+$ RNA isolated from whole intestinal segments probed with a ^{32}P-labelled 2.2 kb *Eco*RI-excised insert of the rabbit intestinal SGLT1 cDNA. Ages of the animals in weeks are indicated above each lane. Size standards are indicated. (**b**) Relative abundance of the SGTL1 mRNA determined by scanning densitometry of the total transcript area of autoradiograms, as represented in (**a**), normalized to levels in ruminant sheep (≥ 10 weeks old). Values are means ± S.E.M.; n values for each time point are shown in parentheses.

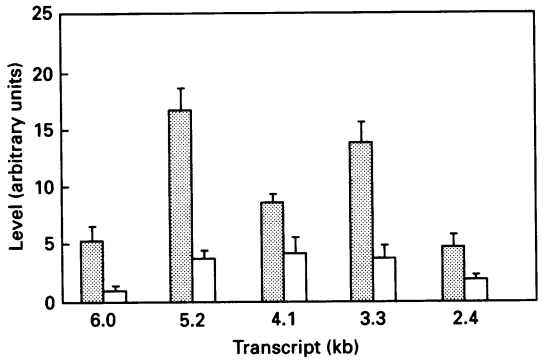

Figure 2 Changes in individual SGLT1 transcripts with development

Individual transcript area was determined by scanning densitometry of autoradiograms for each SGLT1 transcript of intestinal samples prepared from preruminant ▨ ($n = 5$) and ruminant (□) ($n = 4$) sheep. Values are expressed as means ± S.E.M.

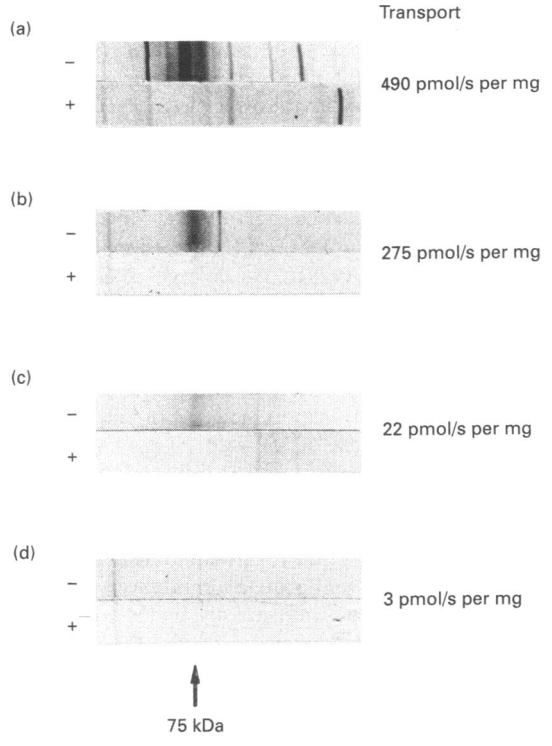

Figure 3 SGLT1 protein abundance and activity during development

SGLT1 protein abundance and corresponding Na^+-dependent glucose uptake was measured in identical intestinal segments, as shown in Figure 1(a), from lamb (**a**, 1 week; **b**, 3 weeks), transition (**c**, 5 weeks) and adult (**d**, 10 weeks) samples. Immunoblots of SGLT1 protein abundance are shown of sheep brush border membranes (30 μg/lane) probed with anti-peptide polyclonal antibody without (−) and with (+) pre-absorption of antibody with specific peptide. The Na^+-dependent glucose uptake (expressed as pmol/s per mg of protein) is indicated beside each sample; this was measured in brush-border membrane vesicles as described in the Experimental section.

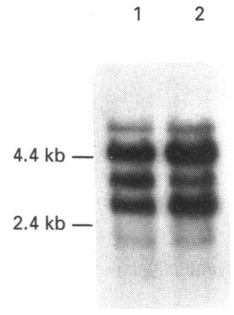

Figure 4 Effect of milk replacer diet on SGLT1 mRNA abundance

Representative Northern analysis is shown of SGLT1 mRNA [2.5 μg of poly(A)$^+$ RNA/lane] prepared either from transition (5-week-old) lambs maintained on milk replacer diet (lane 2) or from weaned, age-matched controls (lane 1). The relative abundances of SGLT1 mRNA quantified by scanning densitometry of the total transcript area of autoradiograms, normalized to controls, were 1.00 ± 0.19 (means ± S.E.M.; $n = 4$) (control) and 1.41 ± 0.49 ($n = 4$) (+ milk), indicating no significant difference in mRNA between the two groups.

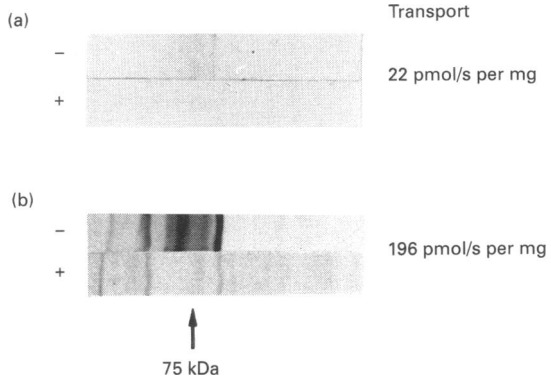

Figure 5 Effect of milk replacer diet on SGLT1 protein abundance and transport activity

Immunoblots are shown of brush border membranes (30 μg/lane) from 5-week-old transition lambs, probed with anti-peptide monoclonal antibody without (−) and with (+) pre-absorption of antibody with specific peptide. The Na^+-dependent gluose uptake (in pmol/s per mg of protein) is indicated beside each sample. (**a**) Normal diet; (**b**) milk replacer diet. Samples used were from identical tissues to those used for Figure 4.

shown in Figure 1(b) is indicative of changes in total mRNA levels.

The magnitude of the changes in the amounts of individual transcripts in pre-ruminant and ruminant animals is shown in Figure 2. The change in SGLT1 mRNA transcript abundance occurred primarily with the 6.0, 5.2 and 3.3 kb transcripts, which decreased 4–5-fold, while the 4.1 and 2.4 kb bands decreased about 2-fold with development.

The corresponding changes in SGLT1 protein abundance and activity were determined in a representative population of intestinal samples. Figure 3 shows Immunoblot analyses of SGLT1 protein abundance along with the Na^+-dependent transport activity determined in brush-border membrane vesicles prepared from the same samples shown in Figure 1(a) (from pre-ruminant, transitional and ruminant animals). The Western blots show the Na^+/glucose co-transporter as a band of immunoreactivity at 75 ± 4 kDa in sheep, which is blocked by pre-absorbing the primary antibody with the immunizing peptide. Both SGLT1 protein abundance and Na^+-dependent glucose transport declined dramatically with development and became barely measurable in the intestinal brush border of the adult ruminant animal. In agreement with our previous results (Shirazi-Beechey et al., 1991b), the transport activity and the abundance of the SGLT1 protein were both over 150-fold greater in the representative intestinal membranes of pre-ruminant animals relative to the ruminant adult. A minor band of 58 kDa, which was also blockable, was detectable in membranes isolated from the intestines of pre-ruminant lambs. The intensity of both this

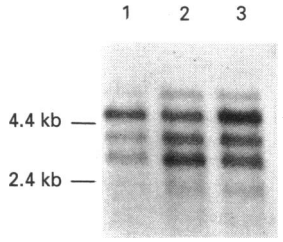

Figure 6 Effect of luminal sugar infusion on SGLT1 mRNA abundance

Northern analysis of SGLT1 mRNA is shown. Each lane contains 2.5 μg of poly(A)$^+$ RNA isolated from the proximal intestine of control sheep (lane 1) or animals whose intestine had been infused with 30 mM of either D-glucose (lane 2) or methyl α-D-glucopyranoside (lane 3) for 4 days. All sheep were $\geqslant$ 10 weeks old. The relative abundances of SGLT1 mRNA determined by scanning denistometry of the autoradiograms, normalized to adult controls, were (**a**) 1.00$\pm$0.30 (means$\pm$S.E.M., $n = 4$), (**b**) 1.87$\pm$0.26 ($n = 4$), and (**c**) 1.24$\pm$0.09 ($n = 2$), indicating a significant 2-fold increase in the level of SGLT1 mRNA in glucose-infused sheep only.

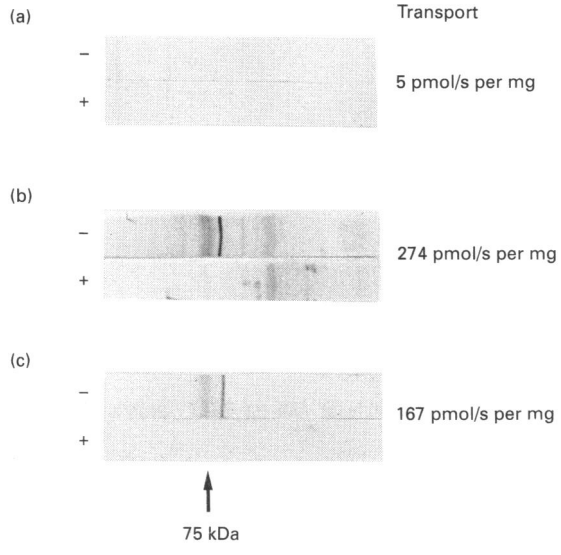

Figure 7 Effect of direct introduction of luminal sugars on SGLT1 protein abundance and transport activity

Immunoblot analysis is shown of adult (3 years old) sheep intestinal brush-border membranes probed with anti-peptide antibody without (−) and with (+) pre-absorption of antibody with specific peptide. The Na^+-dependent glucose uptake (in pmol/s per mg of protein) is indicated beside each sample. (**a**) Control; (**b**) intestine infused with glucose; (**c**) intestine infused with methyl α-D-glucopyranoside. Samples were from identical tissues to those used for Figure 6.

and the 75 kDa band declined with age, and increased on infusion of the intestine with sugars. The significance of this protein is under investigation.

Regulation by luminal sugars

The mRNA levels for SGTL1 in the intestinal tissues of 5-week-old animals maintained on a milk replacer diet and in age-matched controls were determined. A representative Northern blot of the intestinal SGLT1 mRNA of these two groups is shown in Figure 4. The total amount of SGLT1 transcript in animals remaining on a milk replacer diet relative to controls was unchanged. Figure 5 shows SGLT1 protein abundance and activity. With animals on the milk replacer diet, SGLT1 protein levels and transport activity remained as high as the levels present in the pre-ruminant lambs, and were 9-fold higher than in the age-matched controls.

The effects of the direct introduction of D-glucose and methyl α-D-glucopyranoside into the luminal contents on SGLT1 transport activity, abundance and the levels of mRNA were then investigated. A representative Northern blot of SGLT1 mRNA isolated from the intestinal tissues infused with specified sugars is presented in Figure 6. When the intestine of the ruminant animal was infused with glucose, there was a significant, although minor, 2-fold increase in SGLT1 mRNA. Similar infusion with methyl α-D-glucopyranoside, did not significantly alter SGLT1 mRNA levels relative to controls.

The SGLT1 mRNA transcript pattern was unchanged relative to age-matched controls in lambs maintained on a milk replacer diet and in adults with an intestinal infusion with D-glucose. However, on D-glucose infusion, the total amount of SGLT1 transcript increased 2-fold, and this increase was reflected correspondingly in each of the individual transcripts.

SGLT1 protein abundance and activity were determined in the same samples in which we had previously measured SGLT1 mRNA abundance. There was a 60–90-fold enhancement of both Na^+-dependent glucose uptake and SGLT1 protein abundance as a result of direct intestinal infusion with 30 mM D-glucose or methyl α-D-glucopyranoside (Figure 7).

DISCUSSION

Our present study was carried out in order to determine the mechanism of regulation of SGLT1 expression during development and with changes in diet. The changes in Na^+-dependent glucose transport have been shown to be quantitatively correlated with SGLT1 protein abundance in brush-border membranes (Shirazi-Beechey et al., 1991b). In development from the pre-ruminant to the ruminant state, during which Na^+/glucose protein abundance and transport activity in intestinal brush-border membranes decrease > 150 fold, SGLT1 mRNA levels decreased only 4-fold (Figures 1 and 3). Maintaining lambs on a milk replacer diet, which restores SGLT1 transport activity and protein abundance to pre-ruminant levels, did not change mRNA levels (Figures 4 and 5). Finally, in ruminant sheep, in which SGLT1 transport activity and protein abundance were increased 60–90-fold by direct luminal infusion of 30 mM D-glucose for 4 days, there was only a 2-fold increase in SGLT1 mRNA (Figures 6 and 7). In each case, the differences in SGLT1 mRNA abundance cannot account for the dramatic changes in co-transporter protein. Although there are minor 2- and 4-fold increases that are attributable to pre-translational regulation with intestinal glucose infusion and development respectively, we conclude that the regulation of the expression of SGLT1 is dissociated from mRNA abundance, and that the principal step in the regulation of SGLT1 expression is at the translational or post-translational level.

In the intestine, enterocytes are continuously and rapidly renewed with a cellular half-life of a few days, in which they migrate and differentiate along the crypt-to-villus axis. The brush-border Na^+/glucose transport activity has been shown to be maximal at the villus tip (Stirling and Kinter, 1967). Using *in situ* hybridization, we have shown that SGLT1 mRNA abundance in sheep intestine decreases 3–4-fold with development, while the profile of enterocyte expression of SGLT1 mRNA along the vertical (crypt-to-villus) distribution is unchanged (Freeman et al., 1992). This result complements the present study, which establishes that while the co-transporter protein abundance changes over 200-fold, the levels and distribution of SGLT1 mRNA remain relatively unchanged. This is in agreement

with a recent report of intestinal SGLT1 mRNA in chronically diabetic rats (30–60 days of streptozocin treatment) where Miyamoto et al. (1991) determined that SGLT1 mRNA abundance increased 2-fold over control levels, although they did not determine either SGLT1 protein abundance or phlorizin binding in chronically diabetic rats. Previous work by Fedorak et al. (1987, 1989, 1991) has established that in diabetes there is enhanced transmural 3-*O*-methyl-D-glucose flux and 6–10-fold increases in phlorizin binding.

Discrepancies in intestinal brush-border enzyme activity and mRNA levels have been reported previously (Sebastio et al., 1989; Freund et al., 1989). The disparity in the relative changes in mRNA levels and enzyme activities may be due to enhanced biosynthesis, alterations in protein half-life or recruitment of intracellular proteins to their functional site. In the case of aminopeptidase N and sucrase–isomaltase, the disparity is attributed to differences in translational processing (Danielsen et al., 1986; Hoffman and Chang, 1991). Hoffman and Chang (1991) determined that a large proportion of jejunal sucrose–isomaltase mRNA was associated with membrane-bound polyribosomes, which could account for the 3–4-fold difference in enzyme expression along the longitudinal axis of the gut in the absence of any difference in mRNA levels. Translational regulation of other proteins has been demonstrated and is best characterized for ferritin and transferrin synthesis in response to iron availability through unique mRNA–protein interactions. In this case, a 90 kDa regulatory protein regulates translation by interacting with mRNA regulatory sequences (iron-responsive elements) in the 5′ and 3′ untranslated regions of ferritin and transferrin receptor mRNAs respectively (Thiel, 1990). In addition, differences in the 3′ and 5′ untranslated regions are known to have significant effects on translational efficiency by increasing the probability of ribosome re-initiation through interactions between the 3′ and 5′ proximal elements of the same mRNA (Jackson and Standart, 1990). Whether similar mechanisms exist for SGLT1 regulation remains to be determined.

Possible post-translational regulatory mechanisms in intestinal enterocytes include decreasing the half-life of the SGLT1 protein or recruitment of pre-existing intracellular stores. Recruitment appears unlikely, since the observed dramatic increase would require large intracellular stores of SGLT1. Previous histochemical analysis of rabbit (Hwang et al., 1991) and rat (Takata et al., 1992) small intestines demonstrated that most of the SGLT1 protein was present in the brush border of mature enterocytes. However, in the rat intestine a minor amount of SGLT1-positive staining was observed in the supranuclear region, determined to be the Golgi apparatus, in mid-villus enterocytes. This was suggested to represent either a process of SGLT1 synthesis and intracellular transport in differentiating enterocytes or a reservoir of glucose transporter. Our future work on the immunocytochemical localization of SGLT1 protein in the intestinal tissue of lambs of various ages and ruminant sheep, with and without luminal exposure to sugars, should contribute to the understanding of the mechanisms involved in the regulation of the expression of this protein.

The half-life of SGLT1 in enterocytes could be enhanced by increased protein stability in the presence of luminal sugars. Little is known concerning the signals and mechanisms which determine brush-border protein half-life.

The exact signals for the regulation of SGLT1 expression remain unclear, and may involve different signals at different levels of SGLT1 biogenesis. Pre-translationally, there is a modest change in SGLT1 mRNA abundance, resulting from enhanced transcription or stability, with development and on intestinal infusion of D-glucose. Other factors besides glucose could be involved, since the individual transcripts varied with development and were unchanged in animals given a milk replacer diet or by the direct introduction of D-glucose.

Thus the principal mechanism for the regulation of SGLT1 is translational or post-translational. It appears that the mere presence of intact sugars can amplify these processes, since both D-glucose and methyl α-D-glucopyranoside dramatically increased SGLT1 protein abundance and activity (Figure 7). D-Glucose and methyl α-D-glucopyranoside are both substrates of the transporter. In addition, the metabolism of the sugar is not a pre-requisite for the stimulation of SGLT1 synthesis, since methyl α-D-glucopyranoside is not metabolized.

It is clear that the level of regulation of SGLT1 expression is by translational and/or post-translational mechanisms. To determine the definitive molecular mechanisms involved, future experiments are aimed at determining enterocyte SGLT1 protein half-life and mRNA translational efficiency.

We thank Mr. P. J. Barker for raising the antibody. This work was supported by The Agricultural and Food Research Council grant LRG/257, The National Institutes of Health grant NIH DK19567, and by the American Diabetes Association.

REFERENCES

Bassett, J. M. (1975) in Digestion and Metabolism in the Ruminant (McDonald, I. W. and Warner, A. C. I., eds.), pp. 383–398, University of New England Publishing Unit, Armidale, NSW, Australia

Bell, G. I., Kayana, T., Buse, J. B., Burant, C. F., Takeda, J., Lin, D., Fufumoto, H. and Seino, S. (1990) Diabetes Care **13**, 198–208

Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159

Coady, M. J., Pajor, A. M. and Wright, E. M. (1990) Am. J. Physiol. **259**, C605–C610

Danielsen, E. M., Cowell, G. M., Sjostrom, H. and Noren, O. (1986) Biochem. J. **235**, 447–451

Fedorak, R. N., Chang, E. B., Madara, J. L. and Field, M. (1987) J. Clin. Invest. **79**, 1571–1578

Fedorak, R. N., Gershon, M. D. and Field, M. (1989) Gastroenterology **96**, 37–44

Fedorak, R. N., Cheeseman, C. I., Thomson, A. B. R. and Porter, V. M. (1991) Am. J. Physiol. **261**, G585–G591

Freeman, T. C., Heavens, R. P., Dyer, J., Sirinathsinghji, D. J. S. and Shirazi-Beechey, S. P. (1992) Biochem. Soc. Trans. **20**, 186S

Freund, J.-N., Duluc, I. and Raul, F. (1989) FEBS Lett. **248**, 39–42

Hediger, M. A., Coady, M. J., Ikeda, T. S. and Wright, E. M. (1987) Nature (London) **330**, 379–381

Hediger, M. A., Turk, E. and Wright, E. M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 5748–5752

Hirayama, B. A. and Wright, E. M. (1992) Biochim. Biophys. Acta. **1103**, 37–44

Hirayama, B. A., Wong, H. C., Smith, C. D., Hagenbuch, B. A., Hediger, M. A. and Wright, E. M. (1991) Am. J. Physiol. **261**, C296–C304

Hoffman, L. R. and Chang, E. B. (1991) J. Biol. Chem. **266**, 21815–21820

Hwang, E.-S., Hirayama, B. A. and Wright, E. M. (1991) Biochem. Biophys. Res. Commun. **181**, 1208–1217

Jackson, R. J. and Standart, N. (1990) Cell **62**, 15–24

Lachmann, P. J., Strangeways, L., Vyakarnam, A. and Evan, G. I. (1986) Ciba Found. Symp. **119**, 25–40

Lescale-Matys, L., Hensley, C. B., Crnkovic-Markovic, R., Putnam, D. S. and McDonough, A. A. (1990) J. Biol. Chem. **265**, 17935–17940

Miyamoto, K.-I., Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagihira, H. (1991) Biochem. Biophys. Res. Commun. **181**, 1110–1117

Ohata, T., Isselbacher, K. J. and Rhoads, D. B. (1990) Mol. Cell. Biol. **10**, 6491–6499

Pajor, A. M., Hirayama, B. A. and Wright, E. M. (1992) Biochim. Biophys. Acta **1106**, 216–220

Scharrer, E., Liebich, H.-G., Rabb, W. and Promberger, N. (1979) Zentralbl. Veterinarmed. Reihe A **26**, 95–105

Sebastio, G., Villa, M., Sartorio, R., Guzzeta, V., Poggi, V., Auricchio, S., Boll, W., Mantei, N. and Semenza, G. (1989) Am. J. Hum. Genet. **45**, 489–497

Shirazi, S. P., Beechey, R. B. and Butterworth, P. J. (1981) Biochem. J. **194**, 803–809

Shirazi-Beechey, S. P., Gorvel, J. P. and Beechey, R. B. (1988) J. Bioenerg. Biomembr. **20**, 273–288

Shirazi-Beechey, S. P., Kemp, R. B., Dyer, J. and Beechey, B. (1989) Comp. Biochem. Physiol. **94B**, 801–806

Shirazi-Beechey, S. P., Smith, M. W., Wang, Y. and James, P. S. (1991a) J. Physiol. (London) **437**, 691–698
Shirazi-Beechey, S. P., Hirayama, B. A., Wang, Y., Scott, D., Smith, M. W. and Wright, E. M. (1991b) J. Physiol. (London) **437**, 699–708
Stirling, C. E. and Kinter, W. B. (1967) J. Cell Biol. **35**, 585–604
Takata, K., Kasahara, T., Kasahara, M., Ezaki, O. and Hirano, H. (1992) Cell Tissue Res. **267**, 3–9
Thiel, E. C. (1990) J. Biol. Chem. **265**, 4771–4774

Received 1 September 1992/28 October 1992; accepted 10 November 1992