Appearance of phloridzin-sensitive glucose transport is not controlled at mRNA level in rabbit jejunal enterocytes

Experimental Physiology (1992), **77**, 525–528
Printed in Great Britain

APPEARANCE OF PHLORIDZIN-SENSITIVE GLUCOSE TRANSPORT IS NOT CONTROLLED AT mRNA LEVEL IN RABBIT JEJUNAL ENTEROCYTES

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(MANUSCRIPT RECEIVED 17 FEBRUARY 1992, ACCEPTED 4 MARCH 1992)

SUMMARY

Glucose uptake by rabbit intestine has been shown to be confined to the upper two-thirds of jejunal villi. Inhibition by phloridzin shows most of this transport to take place through the Na^+–glucose linked transporter (SGLT1). Parallel measurements on the other hand show SGLT1 mRNA expression to increase rapidly as enterocytes reach the crypt–villus junction. Levels of SGLT1 mRNA then remain elevated in all villus enterocytes. These results provide direct evidence that SGLT1-mediated glucose transport is subject to post-transcriptional control.

INTRODUCTION

The fact that enterocytes differentiate digestive and absorptive functions sequentially is now fully accepted. How enterocytes control gene expression throughout this process is, however, only now becoming amenable to study. Previous findings show that mRNA species coding for a variety of gene products increase rapidly as enterocytes emerge from crypts (see Darmoul, Rouyer-Fessard, Blais, Voisin, Sapin, Baricault, Cibert, Geraud, Couvineau, Laburthe & Trugnan, 1991). Quantitative analysis of this early event has been carried out recently for a specific mRNA coding for calbindin expression in chicken enterocytes (Kiyama, Wu, Smith, Lawson & Emson, 1991). Increase in mRNA coding for the SGLT1 transporter has, however, been reported to take place more slowly during enterocyte development, maximal levels occurring near the villus tip (Hwang, Hirayama & Wright, 1991). Present work tests this finding further, and relates the results obtained to separate measurements of SGLT1-mediated glucose transport.

METHODS

Jejunal tissue was taken from six 10-week-old female New Zealand rabbits killed by intravenous injection of sodium pentobarbitone (3 ml; 20% w/v). Tissue was either frozen immediately in liquid N_2-cooled isopentane for subsequent mRNA determination or incubated for 1 min at 37 °C in a rapid-uptake apparatus in medium containing 4 mM-D-[^{3}H]glucose (400 μCi/ml; Amersham International) to measure glucose uptake in the presence and absence of 1 mM-phloridzin (Sigma Chemical Company). Tissue taken after incubation was rapidly frozen in liquid N_2-cooled isopentane before being cut into 2–3 mm pieces for freeze drying. Dried samples were fixed in osmium tetroxide vapour, embedded in Araldite and sectioned for autoradiography. Sections were then subjected to microdensitometric analysis of silver grain densities. Further details of these methods are described by King, Sepúlveda & Smith (1981).

The method of *in situ* hybridization has already been described by Sirinathsinghji, Morris, Wisden, Northrop, Hunt & Dunnett (1990). Briefly, it involved initial fixation of thaw-mounted cryostat sections

(10 μm) in paraformaldehyde for later overnight incubation at 42 °C with complementary 'sense' and 'antisense' oligodeoxyribonucleotide probes corresponding to nucleotides 1521–1561 of the rabbit SGLT1 sequence (Hediger, Coady, Ikeda & Wright, 1987). Probes were labelled with deoxy-[^{35}S]ATP (NEN Research Products, Stevenage) using terminal transferase. Washed sections were later prepared for autoradiography and microdensitometric analysis of silver grain densities.

RESULTS

Autoradiographs showing the typical localization of SGLT1 mRNA and glucose transport in rabbit jejunal villi are shown in Fig. 1. The 'sense' probe for SGLT1 mRNA did not hybridize to any part of the tissue section (Fig. 1*A*). 'Antisense' probe applied to the same tissue under identical conditions hybridized only to sectioned villus enterocytes (Fig. 1*B*). Glucose uptake into upper villus enterocytes (Fig. 1*D*) was strongly inhibited by phloridzin (Fig. 1*C*). Sequential measurement of silver grain densities carried out along the crypt–villus axis of sectioned tissue produces developmental profiles for SGLT1 mRNA expression and SGLT1-mediated glucose transport by enterocytes (Fig. 2).

Phloridzin-sensitive glucose uptake, mediated by the SGLT1 transporter, is detected in enterocytes only after they have migrated about 200 μm from the crypt–villus junction. Previous work suggests that these cells will be about 50 h old by the time they reach this position on the villus (Smith, Paterson & Peacock, 1984). SGLT1 mRNA levels are already increasing rapidly in crypt enterocytes which are only about 10 h old. There is then no further significant change in mean SGLT1 mRNA levels as enterocytes continue to migrate along villi.

DISCUSSION

The present results showing SGLT1-mediated glucose transport to be confined to the upper regions of intestinal villi confirm earlier findings obtained using a variety of

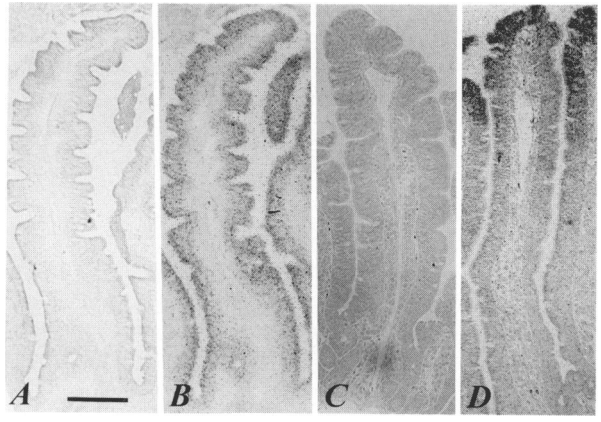

Fig. 1. Autoradiographs showing the location of SGLT1 mRNA and glucose transport activity along the crypt–villus axis of rabbit jejunal villi. Tissue sections were incubated with 'sense' (*A*) or 'antisense' (*B*) probes to detect SGLT1 mRNA as described in the text. Glucose uptake was also measured in the presence (*C*) or absence (*D*) of 1 mM-phloridzin. Scale bar: 100 μm.

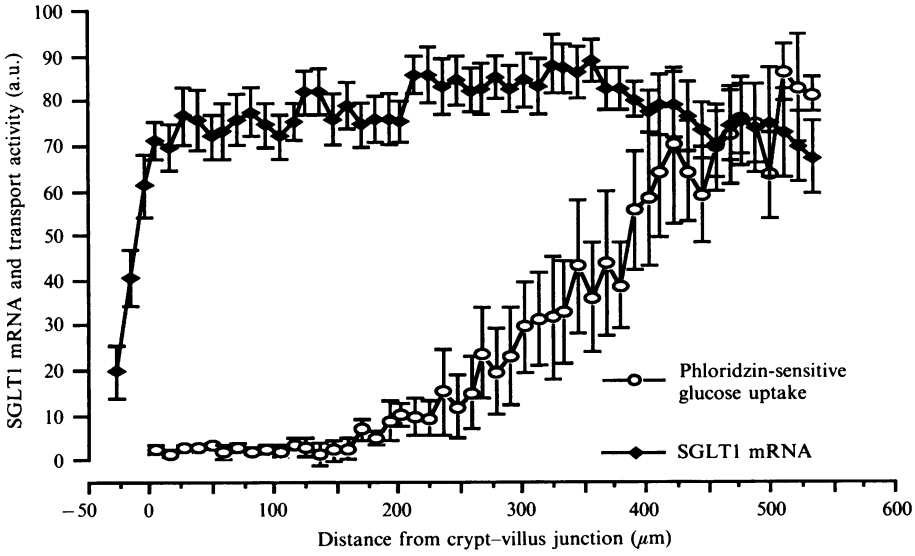

Fig. 2. Relation between enterocyte expression of SGLT1 mRNA and phloridzin-sensitive SGLT1 glucose uptake. SGLT1-mediated glucose uptake is compared directly with SGLT1 mRNA levels measured as described in the text. Each profile shows means±S.E.M. (six rabbits); a.u., arbitrary units.

experimental techniques (Kinter & Wilson, 1965; Stewart & Turnberg, 1987; Debnam, Ebrahim & Swaine, 1990). Use of quantitative autoradiography now allows the study of this aspect of enterocyte development at the cellular level in an animal model already used extensively to characterize similar gradients in amino acid transport (King *et al.* 1981). Glucose and amino acid transport can now be measured in the same tissue to investigate how these two processes might adapt selectively to controlled changes in diet.

In the case of glucose transport, however, it has also been possible to measure the mRNA species coding for the SGLT1 transporter by *in situ* hybridization and show that this is present in enterocytes long before these cells begin to transport glucose across the brush-border membrane. Previously it has been reported that both SGLT1 mRNA and protein expression increase steadily as enterocytes migrate to the villus tip; from this it was inferred that the increase in glucose transport towards the villus tip was due to the simultaneous production of SGLT1 mRNA and functionally active protein (Hwang *et al.* 1991). However, other workers have already shown SGLT1 protein to be uniformly distributed in all villus enterocytes (Haase, Heitmann, Friese, Ollig & Koepsell, 1990; Takata, Kasahara, Kasahara, Ezaki & Hirano, 1992), a result which supports the finding of the present study. Whatever the processes controlling the production of active SGLT1 may be, the fact remains that overall levels of SGLT1 mRNA give no true indication of the ability of enterocytes to transport glucose. Further work aimed at identifying the reason for this disjunction is now being carried out.

T. C. Freeman is funded by an Agricultural and Food Research Council Link grant.

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