The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane

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We have investigated the mechanism responsible for the diffusive component of intestinal glucose absorption, the major route by which glucose is absorbed. In perfused rat jejunum *in vivo*, absorption was strongly inhibited by phloretin, an inhibitor of GLUT2. The GLUT2 level at the brush-border membrane increased some 2-fold when the luminal glucose concentration was changed from 0 to 100 mM. The phloretin-sensitive or diffusive component of absorption appeared superficially linear and consistent with simple diffusion, but was in fact carrier-mediated and co-operative ($n = 1.6$, $[G_{1/2}] = 56$ mM; where $[G_{1/2}]$ is the glucose concentration at half V_{max}) because of the glucose-induced activation and recruitment of GLUT2 to the brush-border membrane. Diffusive transport by paracellular flow was negligible. The phloretin-insensitive, SGLT1-mediated, component of glucose absorption showed simple saturation kinetics with $[G_{1/2}] = 27$ mM: the activation of protein kinase C (PKC) βII, the isoenzyme of PKC that most probably controls GLUT2 trafficking [Helliwell, Richardson, Affleck and Kellett (2000) Biochem. J. **350**, 149–154], also showed simple saturation kinetics, with $[G_{1/2}] = 21$ mM. We conclude that the principal route for glucose absorption is by GLUT2-mediated facilitated diffusion across the brush-border membrane, which is up to 3-fold greater than that by SGLT1; the magnitude of the diffusive component at any given glucose concentration correlates with the SGLT1-dependent activation of PKC βII. The implications of these findings for the assimilation of sugars immediately after a meal are discussed.

Key words: intestine, protein kinase C, SGLT1, sugar, transport.

INTRODUCTION

In 1960, at a conference in Prague, Crane first proposed that glucose is absorbed across the brush-border membrane into small-intestinal absorptive epithelial cells by the co-transport of glucose and Na^+ [1]; this process of secondary active absorption is powered by coupling to a downhill Na^+ gradient generated by removal of Na^+ from the cell interior by the Na^+/K^+-ATPase of the basolateral membrane. There followed a series of physiological and electrophysiological studies of the mechanism of Na^+/glucose co-transport by many investigators, including Crane [2], Schultz and Curran [3], Semenza et al. [4] and Wright and co-workers [5]. Following cloning of the Na^+/glucose co-transporter [6], SGLT1, the relationship between SGLT1 structure and function was extensively and elegantly investigated by Wright and colleagues in a host of papers (for a review, see [7]). Indeed, the role of SGLT1 has become so well established that it is often presented in the wider biochemical and physiological world simply as the way in which glucose is absorbed across the intestinal brush-border membrane and is described as such in standard texts [8].

Nevertheless, SGLT1-mediated active absorption is not the major mechanism by which glucose gets across the small intestine after a meal. It has been equally well established, in a series of papers stretching back over 40 years and pre-dating the proposal of Na^+/glucose co-transport, that glucose absorption in whole intestine comprises two components: an active (SGLT1-mediated) component with an apparent K_m of 8–23 mM, which starts to saturate around 30–50 mM glucose, and a second component that has been described as 'diffusive', because it is broadly linear in nature, does not appear to saturate even at concentrations over 100 mM and appears, superficially at least, to be consistent with a process of simple diffusion [9–11]. The contribution of the diffusive component to absorption exceeds that of the SGLT1-mediated component at 30–50 mM glucose and continues to increase at higher glucose concentrations to become some 2–3-fold greater than the active component.

In order to understand the physiological importance of the diffusive component, it is necessary to know the concentration of glucose in the jejunum after a meal. Unfortunately, the relevant information is not known with any certainty. Detailed measurements of the average concentration of free glucose over the entire luminal contents yield values for rat jejunum ranging from 16 to 28 mM with a peak of 48 mM immediately after feeding [12]. However, most glucose is delivered to the brush-border membrane in the form of α-limit dextrins and disaccharides, such as maltose and sucrose, in the intestinal chyme. The relevant concentration for absorption is therefore the local concentration of monosaccharides produced from α-limit dextrins and disaccharides by the action of membrane-bound hydrolytic enzymes in the microvilli. Since only a small fraction of the monosaccharides produced escape from the surface of the brush-border membrane, it is clear that the effective concentration of glucose for absorption must be several times the average free luminal concentration. Indirect evidence from the rates of hydrolysis and absorption of maltose suggest that the effective concentration may be as high as 300 mM [13]. The contribution of the diffusive component to glucose absorption will therefore be proportionally higher and so is probably several-fold greater than the SGLT1-mediated active component.

Abbreviations used: PKC, protein kinase C; MAP kinase, mitogen-activated protein kinase.

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The mechanism responsible for the diffusive component of glucose absorption is a matter of some debate. The principal proposal is the theory of paracellular flow, or solvent drag, which is based on the long-standing observation that the diffusive (also termed 'paracellular') component of glucose absorption is associated with high rates of water absorption [9,14]. Pappenheimer and colleagues have proposed that SGLT1-mediated absorption of glucose from the lumen into the intercellular spaces creates an osmotic driving force for solvent flow: concomitant opening of intercellular tight junctions permits bulk absorption of glucose and other nutrients through this paracellular route by solvent drag; that is, the flow of water literally drags glucose through the open tight junctions by non-ideal solvent–solute interaction [14]. In this view, then, the paracellular or diffusive component of glucose absorption is SGLT1-dependent. The paracellular absorption theory is controversial and has provoked significant and sustained criticism [12,15,16], but has been assertively promoted and robustly defended (see [13,17] and references therein).

The alternative view proposed by Diamond, Ferraris and colleagues [12,15] is that adaptation of the capacity of brush-border membrane transporters must be matched to metabolic demands. They argue that paracellular flow is negligible, even in the presence of maltose [16], and maintain that the currently known kinetic properties of SGLT1 are sufficient to account for the observed rates of glucose absorption, seemingly without the necessity of considering a diffusive component of any kind [12,15]. This view has been based largely, although not exclusively, on studies of dietary and developmental adaptation over days or weeks. The applicability of this view to short-term adaptive responses, for example those on a time scale from minutes up to 2 h required during the assimilation of a meal, has not been established. However, recently Cheeseman and Hirsh have reported rapid changes in SGLT1-mediated glucose transport and levels of SGLT1 induced by cholecystokinin (CCK)-8 and glucagon-like peptide (GLP)-2 as a consequence of altered trafficking [18,19]. Such changes in SGLT1 are obviously accommodated within the view that absorption is mediated by membrane transporters, but, as Pappenheimer has noted [13], changes in SGLT1 activity are accommodated equally well within the paracellular flow theory, in which variations in SGLT1 levels and activity serve to induce changes in paracellular (or diffusive) flow. No alternative mechanism has been proposed and no one has advanced, for example, a carrier-mediated mechanism for the diffusive component in which the carrier responsible is concerned solely with the diffusive component.

After absorption across the brush-border membrane, fructose is transported across the basolateral membrane into the circulation by GLUT2, a member of the glucose facilitative transporter family [20,21]. Although GLUT2 has been considered to be solely a basolateral protein, we have previously observed that it is readily detectable in the brush-border membrane of diabetic rat jejunum where it is fully functional and transports fructose [22]. In the previous paper [23], we have shown that the stimulation of fructose absorption by PMA in normal rat jejunum is mediated by the rapid trafficking of GLUT2 to the brush-border membrane and controlled by protein kinase C (PKC), most probably the βII isoenzyme. GLUT2 transports not only fructose, but also glucose [21]. Our observations on GLUT2 trafficking and fructose absorption imply that GLUT2 is responsible for the diffusive component of glucose absorption across the brush-border membrane. The data in this paper show that GLUT2 provides the principal mechanism by which glucose is absorbed across the brush-border membrane during the assimilation of a meal.

EXPERIMENTAL

Animals

Male Wistar rats (240–260 g) were fed *ad libitum* on standard Bantin and Kingman rat and mouse diet with free access to water.

Perfusion of jejunal loops

Rats were anaesthetized by an intraperitoneal injection of a mixture of 1.0 ml of Hypnorm (Janssen Animal Health, High Wycombe, Bucks., U.K.) and 0.4 ml of Hypnovel (Roche Diagnostics, Welwyn Garden City, Herts., U.K.) per kg of body weight. Jejunal loops were perfused *in vivo* with a single-pass of perfusate in which a gas-segmented flow system was used to disrupt the unstirred layer [23]. The system had two perfusate reservoirs to permit a paired comparison between a control and an experimental perfusion period for a single loop. The first reservoir contained glucose at the stated concentration, while the second contained glucose at the same concentration together with phloretin; the concentration of phloretin was 1 mM at 100 mM glucose and was diminished in proportion to glucose concentration to a minimum value of 0.2 mM. Both reservoirs contained [^{3}H]inulin (Amersham Life Science) to permit the determination of water transport; constant osmolarity was maintained between solutions of different glucose concentrations by the addition of mannitol up to a maximum concentration of 100 mM at 0 mM glucose, so as to eliminate the effects of osmolarity and water flow on glucose transport. A jejunal loop (25 cm from a point 5 cm distal to the ligament of Treitz) in an anaesthetized rat was cannulated and perfused with glucose at the stated concentration for a control period of 0–40 min and then switched to perfusion with glucose and phloretin for an experimental period of 40–90 min: the flow rate of perfusate was 0.75 ml·min^{-1} and that of gas was 0.38 ml·min^{-1}. Additional perfusions were performed in which phloretin was not present during the experimental period or in which phloretin was present all the way through the perfusion; after the initial steady-state rate had been achieved in each case, these perfusions showed no change in perfusion rate over the whole time period to 90 min, confirming that the preparation was viable for the whole of the perfusion period. Samples of 0.05 ml were taken from the perfusate effluent as a function of time and triplicate samples were taken from the reservoirs. Glucose was determined with a COBAS MIRA analyser (Roche Diagnostics) using a glucose oxidase-peroxidase GLU kit (Roche Diagnostics): concentrations were corrected for changes in perfusate volume caused by water transport. The rate of glucose transport, termed v and expressed in μmol·min^{-1}·(g of dry weight)$^{-1}$, was calculated from the equation:

$$v = \Delta c.F/w$$

where Δc is the difference in concentration between the eluate and the perfusate reservoir, F is the flow rate and w is the dry weight of the perfused intestinal loop.

HPLC analysis of phloretin

Analysis of samples for phloretin was performed using a Kontron (Bio-Tek Kontron Instruments, Watford, Herts., U.K.) automated HPLC system. The column was a 20-cm × 4.6-mm Hypersil 5 μm ODS C_{18} column (Jones Chromatography, Hengoed, Mid-Glamorgan, Wales, U.K.); the mobile phase was 20 % methanol/80 % 21 mM KH_2PO_4 buffer, pH 4.8, at room temperature with a 1 ml/min flow rate. Detection of phloretin in samples was by absorbance at 210 nm and comparisons were made with

standard solutions of 1 and 10 μM phloretin. The retention time for phloretin was 3.4 min in standard samples and concentrations as low as 0.1 μM could be detected. No contamination by phloretin of the phloridzin batch used in these experiments could be detected, nor could phloretin be detected in undiluted samples of effluent from jejunum perfused with 1 mM phloridzin. These results demonstrated that no conversion of phloridzin by phloridzin hydrolase occurred in jejunum during the relatively short transit time of a single-pass experiment. All other methods were as described previously [22,23].

RESULTS

When rat jejunum was perfused *in vivo* with 75 mM glucose, a steady state was achieved after about 15 min (Figure 1): addition of phloretin after 40 min (Figure 1, arrow) inhibited absorption rapidly, a new steady state being achieved within 15 min. The percentage inhibition decreased as the glucose concentration was decreased from 75 to 30 mM and then to 10 mM. Figure 2 shows the dependence of the total rate and of the rates of the phloretin-sensitive and -insensitive components on glucose concentration over a range from 5 mM to 100 mM.

When the GLUT2-mediated component of glucose transport was inhibited by phloretin, water transport was abolished. From the difference in water and glucose transport, it was therefore possible to calculate that 596 ± 81 mol of water were associated in some way with the transport of 1 mol of glucose mediated by GLUT2 (errors are given as S.E.M. here and throughout the article). This mean value was obtained from 27 perfusions comprising five different glucose concentrations, 100, 75, 50, 30 and 20 mM. The values ranged from 342 ± 22 at 100 mM glucose to 782 ± 80 at 20 mM glucose. The values at higher concentrations are perhaps the most reliable, since the higher rates of water and glucose transport are the most easy to measure in the perfusion system used. At lower concentrations, the combined effects of errors in water and glucose transport were too large to permit accurate determination.

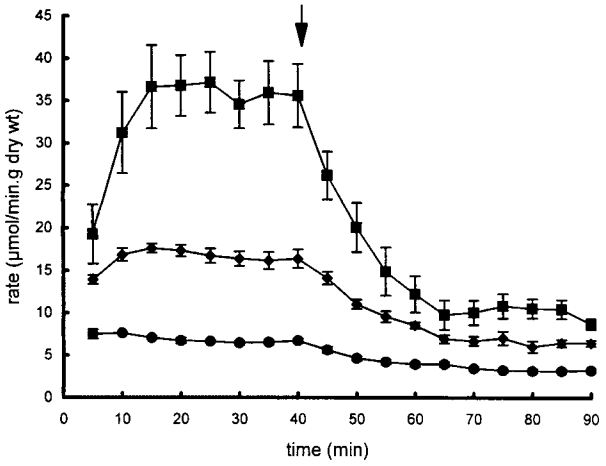

Figure 1 Time course for the inhibition of brush-border glucose absorption *in vivo* by phloretin

Rat jejunum was perfused for 40 min *in vivo* with glucose at the stated concentrations and constant osmolarity was maintained by the addition of mannitol, using a single-pass system as described in the Experimental section. After 40 min (arrow), the perfusate reservoir was switched to one containing glucose at the same concentration and phloretin. The concentrations of glucose and phloretin respectively were: ■, 75 and 1 mM; ◆, 30 and 0.6 mM; and ●, 10 and 0.2 mM. Data are presented as means ± S.E.M.; where error bars are not visible, they are within the dimensions of the symbol: $n = 4$ for each perfusion.

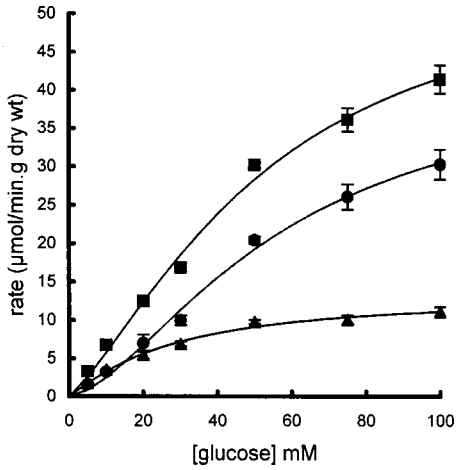

Figure 2 Concentration dependence of brush-border membrane glucose absorption in rat jejunum *in vivo*

Transport components were determined from the mean steady-state rates of perfusion time courses, such as those presented in Figure 1. ■, Total glucose absorption; ●, phloretin-sensitive or GLUT2-mediated component; ▲, phloretin-insensitive or SGLT1-mediated component. Data are presented as means ± S.E.M.; where error bars are not visible, they are within the dimensions of the symbol: $n = 4$–9. The theoretical lines for the diffusive and active components were calculated using the values of the parameters given in Table 2, which were obtained by non-linear-regression analysis (SigmaPlot) according to eqn. (1); the line for the total rate was given by their sum.

In the previous paper [23] we have shown that GLUT2 is present in the brush-border membrane of rat jejunum and that fructose absorption can therefore be inhibited by phloretin. Since GLUT2 is the only member of the facilitative transporter family that transports both fructose and glucose, the phloretin-sensitive component of glucose absorption must be a diffusive component mediated by GLUT2. Support for this conclusion is provided by the fact that, at 50 mM glucose, the amplitude of the cytochalasin B-sensitive component of glucose transport was the same within experimental error as that of the phloretin-sensitive component (Table 1). The remaining, phloretin-insensitive component showed simple saturation kinetics and was assigned to the active component mediated by SGLT1; for confirmation of this analysis, see below. The dependence of the rate, v, of the diffusive component of absorption on glucose concentration, [G], could be readily fitted by linear-regression analysis to a straight line:

$$v = 0.88 + 0.32[\mathrm{G}]$$

with a correlation coefficient of 0.986. In these respects the data are similar to those reported by previous workers [10,11], who used phloridzin to inhibit the active component and ascribed the phloridzin-insensitive component, which also shows a linear dependence on glucose concentration, to simple diffusion.

However, we have now demonstrated that the diffusive component is carrier-mediated. One explanation for the apparent linearity of the data might be that the GLUT2 transporter in the brush-border membrane has a very low affinity for glucose and is operating at concentrations rather less than its $[G_{1/2}]$, that is, the concentration at which half-maximal rate is achieved and which is a measure of the apparent K_m of the transporter for

Table 1 Assignment of absorption components to GLUT2 and SGLT1

These data demonstrate the identity of the fast phase of transport inhibition by Na^+ replacement with the phloretin- and cytochalasin B-insensitive components of glucose absorption, assigning them to SGLT1. Conversely, they demonstrate the identity of the slow phase of Na^+-replacement experiments with the phloretin- and cytochalasin B-sensitive components of glucose absorption, assigning them to GLUT2. n.d., not determined.

Glucose (50 mM)	*n*	Absorption component	Amplitude [μmol · min^{-1} · (g of dry weight)$^{-1}$]	Rate constant (min^{-1})
Phloretin (1.0 mM)	9	Sensitive (GLUT2)	20.4 ± 0.3	n.d.
		Insensitive (SGLT1)	9.8 ± 0.4	n.d.
Cytochalasin B (0.2 mM)	3	Sensitive (GLUT2)	21.3 ± 0.6	n.d.
		Insensitive (SGLT1)	9.1 ± 0.5	n.d.
Na^+ replacement with choline	3	Fast phase (SGLT1)	9.2 ± 1.0	0.24 ± 0.06
		Slow phase (GLUT2)	21.0 ± 0.7	(9.0 ± 0.7) × 10^{-3}

Table 2 Kinetic analysis of transport data

Data for the phloretin-sensitive (GLUT2) and -insensitive (SGLT1) components of glucose absorption were fitted to eqn. (1) by non-linear-regression analysis using SigmaPlot. $v/[GLUT2_{rel}]$ is the rate of the phloretin-sensitive component divided by the level of GLUT2 at a given glucose concentration expressed relative to that at 0 mM glucose (100 mM mannitol); the normalized rate data were analysed by eqn. (2). The PKC βII data were analysed also according to the Michaelis–Menten function (eqn. 1 with *h* fixed at 1). For full details, see the text.

	GLUT2 component (phloretin-sensitive)	SGLT1 component (phloretin-insensitive)	GLUT2 intrinsic activity ($v/[GLUT2_{rel}]$)	Activation of PKC βII
V_{max} [μmol · min^{-1} · (g of dry weight)$^{-1}$]	42.7 ± 7.3	13.6 ± 1.5	19.4 ± 4.5	0.73 ± 0.24*
$[G_{1/2}]$ (mM)	56.4 ± 14.0	26.9 ± 6.5	31.4 ± 11.8	21.0 ± 16.2
h	1.6 ± 0.24	1.15 ± 0.18	1.71 ± 0.74	(1.0)

* The maximal increase in the level of the 49-kDa active form of PKC βII expressed relative to that at 0 mM glucose.

glucose. Attempts to fit the GLUT2-mediated component to a single, hyperbolic Michaelis–Menten function nevertheless revealed very quickly that rate dependence is in fact sigmoidal or co-operative with respect to glucose concentration.

In order to define the co-operativity, the dependence of the rate of the diffusive component on glucose concentration was fitted by non-linear-regression analysis to a Hill-type equation:

$$v = (V_{max} \cdot [G]^h)/(K + [G]^h) \quad (1)$$

where V_{max} is the maximal rate of transport, $K = [G_{1/2}]^h$, $[G_{1/2}]$ is the glucose concentration at half V_{max}, and *h* is the Hill coefficient. Table 2 shows the values of the parameters obtained for the diffusive component. Of particular note, the value of n is 1.60 ± 0.24, confirming that the curve is indeed co-operative in nature. When the data for the rate dependence of the SGLT1-mediated component were analysed, *h* was much lower at 1.15 ± 0.18 and was not significantly different from 1, that is, simple Michaelis–Menten, non-co-operative behaviour (Table 2). Figure 2 shows the excellent fit of the non-linear-regression curves for the GLUT2- and SGLT1-mediated transport components and their sum to the experimental data. From the equations describing each set of data, the two components are equal in magnitude at 15 mM glucose.

The co-operativity of the diffusive component implies that the activity of the GLUT2 transporter must be increasing with increasing glucose concentration, either through an increase in its intrinsic activity (that is, the activity per molecule) or through an increase in its level at the brush-border membrane. In order to distinguish between these possibilities, brush-border membrane vesicles were prepared after luminal perfusion for 60 min with a range of glucose concentrations from 0 to 100 mM; constant osmolarity was maintained by the addition of mannitol as appropriate. Western blotting showed that the level of GLUT2 increased 2.2 ± 0.2 fold ($P < 0.001$, $n = 3$) from 0 to 100 mM glucose, whereas there was no significant change in the level of SGLT1 (Figure 3). This finding demonstrates that glucose-induced recruitment of GLUT2 to the brush-border membrane is an important factor in co-operativity, but still leaves open the question of whether there is a change in GLUT2 intrinsic activity.

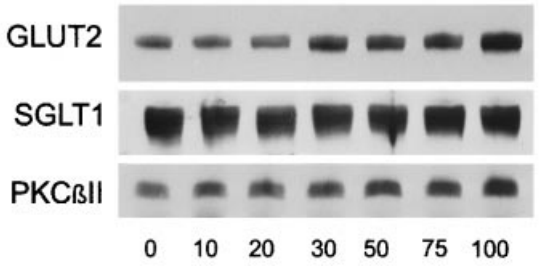

Figure 3 Dependence of GLUT2, SGLT1 and PKC βII on glucose concentration

Brush-border membrane vesicles were prepared from normal rat jejunum perfused for 40 min with the stated concentration of glucose as described in Figure 1. The figure beneath each band corresponds to the concentration of glucose in mM with which jejunum was perfused. For full details, see the Experimental section. Vesicle protein (20 μg) was then separated by SDS/PAGE (10% gels), transblotted on to nitrocellulose and Western blotted for GLUT2, SGLT1 and PKC βII.

We therefore also re-analysed the phloretin-sensitive, GLUT2-mediated transport data to take into account the glucose-induced recruitment of GLUT2 to the rapid trafficking compartment at the brush-border membrane. This allowed us to assess how the

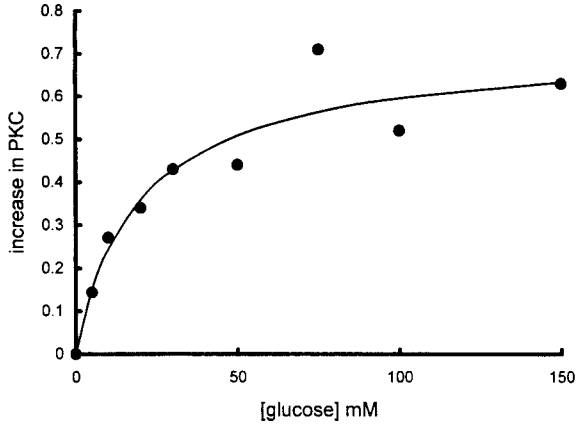

Figure 4 The amount of PKC βII in its active form is SGLT1-dependent

The amount of PKC βII at a given concentration of glucose concentration was determined as described in the legend to Figure 3 and expressed relative to that at 0 mM glucose (100 mM mannitol). Since the relative increase showed a simple saturation response with respect to glucose concentration, the data were fitted by non-linear-regression analysis to the Michaelis–Menten equation to give $[G_{1/2}] = 21.0 \pm 16.2$ mM. This compares with the $[G_{1/2}]$ for SGLT1 of 26.9 ± 6.5 mM.

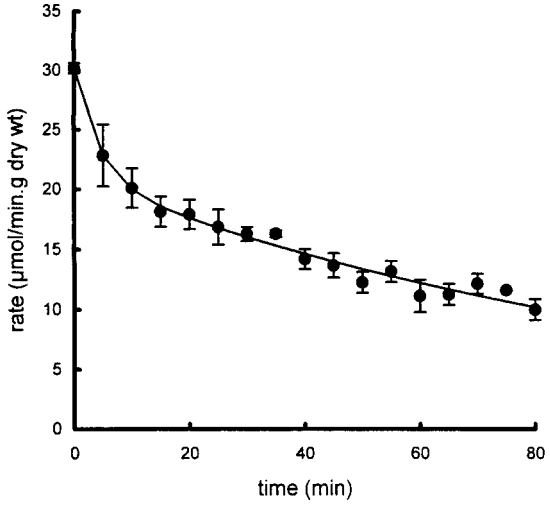

Figure 5 Effect of Na^+ replacement on glucose transport

Rat jejunum was perfused *in vivo* with 50 mM glucose in modified Krebs–Henseleit buffer for 40 min, using a single-pass system as described in the Experimental section: after 40 min, the perfusate reservoir was switched to one containing glucose at the same concentration but in which all NaCl was replaced with choline chloride and Na^+ in the buffer salts was replaced by K^+. The time course of glucose transport (with the time at replacement shown as 0 min) was fitted to the sum of two exponentials, as described in the text, to return the parameters given in Table 1. Data are presented as means ± S.E.M.; $n = 3$.

intrinsic activity ($v/[GLUT2_{rel}]$) depended on glucose concentration. Eqn (1) was recast in the form:

$$v/[GLUT2_{rel}] = (V_{max,rel} \cdot [G]^h)/(K + [G]^h) \quad (2)$$

where $[GLUT2_{rel}]$ is the level of GLUT2 at a given concentration, [G], relative to that at 0 mM glucose (100 mM mannitol); $V_{max,rel}$ is the corresponding V_{max}. The values of the parameters returned from this analysis are given in Table 2. Comparison of the value of V_{max} from eqn. (1) with $V_{max,rel}$ from eqn. (2) reveals that at V_{max} the relative level of GLUT2 is 2.2-fold that at 0 mM glucose. However, even though allowance had been made for changes in GLUT2 recruitment, the value of h was not significantly changed at 1.71 ± 0.74. The error in h is unsurprisingly large given the nature of this particular data set, which combines both rate and GLUT2-level data. Nevertheless, attempts to fit the data to a simple, non-co-operative Michaelis–Menten function did not give sensible answers. We therefore conclude that the intrinsic activity of GLUT2 also increased with glucose concentration. The fact that the SGLT1-mediated component is hyperbolic and that SGLT1 levels do not change means that the intrinsic activity of SGLT1 is independent of glucose concentration.

In the previous paper [23], we have reported that trafficking of GLUT2 to the brush-border membrane is likely to be controlled by the βII isoenzyme of PKC associated with the brush-border membrane. The Western blot in Figure 3 shows that the brush-border level of PKC βII increases with increasing glucose concentration. When the level is expressed relative to that at 0 mM glucose, the dependence follows simple Michaelis–Menten saturation kinetics (theoretical curve in Figure 4 determined by non-linear-regression analysis) with a $[G_{1/2}]$ of 21.0 ± 16.2 mM, which compares with that of 26.9 ± 6.5 mM for SGLT1 (Table 2). The closeness of the mean values of $[G_{1/2}]$ and especially the saturation response demonstrate that activation of PKC βII is dependent on the rate of glucose transport mediated by SGLT1. It seems, however, that activation of PKC βII has to achieve a certain threshold level before GLUT2 levels increase, since increase in GLUT2 was not detectable until the concentration reached 30–50 mM glucose and increased further at 75–100 mM (Figure 3).

If this view of events is correct, then blocking SGLT1 should inhibit PKC βII and diminish GLUT2 levels; as a consequence, it should also inhibit glucose absorption by the sum of the SGLT1 component and that part of the GLUT2 component that is inhibited as a result of GLUT2 loss from the brush-border membrane. We therefore perfused jejunum with 50 mM glucose with perfusate buffer in which all the NaCl had been replaced with choline chloride and the Na^+ of the buffer salts with K^+. Figure 5 shows that total Na^+ replacement resulted in a biphasic inhibition of glucose transport, comprising a fast initial phase of SGLT1 inhibition and a much slower second phase attributable to the GLUT2 diffusive component. The progress curve of rate, v, against time, t, was therefore fitted by non-linear-regression analysis to the sum of two exponentials plus a constant, a, according to eqn. (3):

$$v = v_1 e^{-k_1 t} + v_2 e^{-k_2 t} + a \quad (3)$$

where v_1 and v_2 are the initial amplitudes and k_1 and k_2 are the rate constants of phases one and two respectively. At 50 mM glucose, the value of the constant, a, returned by the analysis was about 1.0 compared with a total amplitude of 31 and was not significantly different from zero, showing that there were only two phases. The data were therefore analysed with the constant fixed at 0 to give the values shown in Table 1. The fast and slow phases of transport inhibition caused by replacement of Na^+ with choline had $t_{1/2}$ values of 2.9 ± 0.8 and 77.3 ± 6.4 min respectively. Crucially, the amplitude of the fast phase was the same within experimental error as that of the phloretin- and cytochalasin B-insensitive components (Table 1), which showed simple saturation kinetics. This agreement therefore confirmed the initial assignment of these two components to SGLT1. Conversely, the amplitude of the slow phase of Na^+ replacement with choline was identical within experimental error to that of the phloretin- and cytochalasin B-sensitive components (Table 1), confirming their assignment to GLUT2.

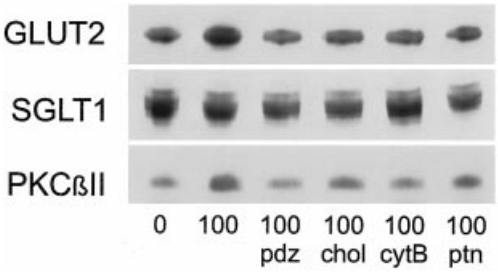

Figure 6 Effect of inhibitors of glucose transport on the levels of GLUT2, SGLT1 and PKC βII

Normal rat jejunum was perfused for 40 min with 100 mM glucose. It was then perfused for a further 40 min with 100 mM glucose, alone (100) or in the presence of either 1 mM phloridzin (100 pdz), 1 mM phloretin (100 ptn) or 0.2 mM cytochalasin B (100 cytB). Alternatively it was perfused for a further 60 min with 100 mM glucose and perfusate buffer in which all the Na^+ had been replaced by choline (100 chol) as described in Figure 5. For the perfusion without glucose and without inhibitors, jejunum was perfused for two consecutive periods of 40 min with 100 mM mannitol (0). After perfusion, brush-border membrane vesicles were prepared; vesicle protein (20 μg) was then separated by SDS/PAGE (10% gels), transblotted on to nitrocellulose and Western blotted for GLUT2, SGLT1 and PKC βII. For full details, see the Experimental section.

Since choline has no direct effect on the transport of glucose by GLUT2, the slow phase of Na^+ replacement is likely to represent the trafficking of GLUT2 away from the brush-border membrane under conditions *in vivo*. In order to confirm this, Na^+-replacement experiments were performed at 100 mM glucose where there is a greater change in GLUT2 levels. Although the fast and slow phases were not sufficiently well resolved to permit accurate determination of their amplitudes and rate constants at 100 mM glucose, glucose absorption was inhibited 71% under conditions where the GLUT2 level was diminished to 51% of that for glucose alone; this level is similar to that for perfusion with 100 mM mannitol (Figure 6). The level of SGLT1 was unchanged. Assuming that choline inhibits glucose absorption by the sum of the SGLT1 component and that part of the GLUT2 component that is lost, we calculate the expected inhibition of absorption to be 63%, in good agreement with the observed value. As anticipated, replacement of Na^+ with choline inhibited PKC βII (Figure 6).

Blocking SGLT1 with phloridzin had similar results to replacement of Na^+ with choline. Phloridzin (1 mM) inhibited the transport of 100 mM glucose by 71% against a calculated value of 67% expected from the loss of GLUT2 and blocking of SGLT1 (Figure 6). HPLC analysis of the perfusate effluent excluded the possibility that conversion of phloridzin into phloretin by the action of phloridzin hydrolase in the jejunum could contribute to this result in any way (see the Experimental section). Phloretin (1 mM) and cytochalasin B (0.2 mM) at 100 mM glucose both diminished GLUT2 to the same level as seen for mannitol perfusions, and inhibited PKC βII. However, whereas phloridzin and Na^+ replacement both diminished GLUT2 level and GLUT2-mediated transport, phloretin and cytochalasin B had no effect on SGLT1 level or SGLT1-mediated transport.

DISCUSSION

SGLT1-dependent activation of PKC controls GLUT2 recruitment to the brush-border membrane

We have shown that glucose transport in rat jejunum *in vivo* comprises two components, an active component mediated by SGLT1 and a facilitated diffusion component mediated by GLUT2. The active component is detected either as a phloretin- and cytochalasin B-insensitive component, or as the fast phase of transport inhibition caused by replacement of Na^+ with choline, whereas the diffusive component is detected as a phloretin- and cytochalasin B-sensitive component or as the slow phase in Na^+-replacement experiments (Table 1). As far as we are aware, it has not been reported previously that glucose absorption *in vivo* is inhibited by phloretin.

The $[G_{1/2}]$ for the GLUT2-mediated component is 56 ± 14 mM. Although this value is comparable with the published value for GLUT2 in basolateral membrane vesicles of 48 ± 5 mM [24], at least four factors contribute to this value. These are: the K_m of GLUT2 that reflects intrinsic activity in its basal state; the activation of GLUT2 intrinsic activity with increasing glucose concentration; the way in which GLUT2 recruitment to the brush-border membrane correlates with and is therefore probably controlled by the activation of PKC βII; and how PKC βII activation is controlled by the transport properties of SGLT1. This complexity contrasts sharply with the simple behaviour of SGLT1, where there is no change in intrinsic activity or brush-border level over the range of glucose concentrations studied. The value of $[G_{1/2}]$, 26.9 ± 6.5 mM, for the SGLT1-mediated component is not significantly different from that of 22.6 ± 1.3 mM for the K_m reported 25 years ago by Debnam and Levin [10] for the phloridzin-sensitive component of transport *in vivo*.

That GLUT2 at the brush border is controlled by the transport of glucose by SGLT1 is shown in several ways: brush-border GLUT2 and PKC βII levels increase with increasing glucose concentration; the dependence of PKC βII activation with respect to glucose concentration shows a saturation response and has a K_a similar to that of SGLT1, and inhibition of SGLT1 at high glucose concentrations, either by total replacement of Na^+ with choline and K^+ or by phloridzin, diminishes brush-border GLUT2 levels and proportionally inhibits GLUT2-mediated transport. The fact that phloretin and cytochalasin B also diminish GLUT2 levels is less clear, but may very well reflect the co-ordinated nature of the regulatory mechanism. This is perhaps best seen in the context of the working model now proposed.

Assimilation of sugars after a meal

Our current working hypothesis concerning the respective roles of SGLT1 and GLUT2 in the absorption of sugars across the brush-border membrane after a meal envisages the following sequence of events. When the lumen is empty before a meal, the level and intrinsic activity of GLUT2 at the brush-border membrane are low, minimizing escape of glucose from the mucosa. After a meal, the action of membrane-bound hydrolytic enzymes in the microvilli on sugars such as maltose, sucrose and α-limit dextrins results in a high local concentration of glucose. Initially, because both the brush-border level of GLUT2 and its intrinsic activity are low, transport will occur predominantly through SGLT1. Saxon et al. (Figures 4C and 7B of [25]) have reported that PKC βII is not located in the microvilli but in the terminal web region of cells in the top part of the villus, which are the mature cells principally responsible for glucose absorption. Somehow, PKC βII is activated by glucose, a phenomenon previously reported for PKCα in isolated pancreatic cells [26]. One possible mechanism is that concentrative Na^+-coupled transport may increase the enterocyte volume as a result of the rise in osmolarity and trigger the entry of extracellular Ca^{2+} [27], which would activate PKC βII; such a mechanism would provide a ready explanation of why amino acids and other Na^+-dependent nutrients stimulate the diffusive component [14].

Activation of GLUT2 already at the brush-border membrane, as well as trafficking of additional GLUT2 to the membrane, would then occur as a probable consequence of PKC βII activation. Trafficking is very rapid: steady-state levels of glucose absorption are achieved within a maximum of 15 min and often more rapidly, putting the $t_{1/2}$ at no more than about 5 min, while trafficking of GLUT2 away from the membrane occurs equally quickly when jejunum is excised and PKC βII is inactivated. The trafficking pathway for GLUT2 is different from that of SGLT1 and GLUT5, whose levels are relatively unchanged in response to different stimuli, including glucose, PMA [23] and effectors of mitogen-activated protein kinase (MAP kinase) signalling pathways [28]. At the same time access of luminal glucose to GLUT2 would be increased. Thus Madara and Pappenheimer [29] have reported that when glucose is absorbed the apical surface of absorptive cells are 'subtly rounded'; the effect would be to splay out the microvilli compared with the flattened surface in the absence of glucose, so that the microvilli are further apart near their tips than at their bases. Such rounding, which is caused by contraction of the peri-junctional actomyosin ring in response to SGLT1-mediated transport, would enhance the access of complex sugars to the hydrolases and transporters of the brush-border membrane, including GLUT2. All these features require a high degree of co-ordination in the signalling mechanisms and structural changes associated with the regulation of the diffusive component. Such co-ordination might explain why, when brush-border GLUT2 levels are high, blocking GLUT2-mediated absorption with phloretin or cytochalasin B diminishes GLUT2 levels and inhibits PKC βII without any effect on SGLT1 level or transport; the sequence of signalling events is reversed.

The theory of paracellular solvent drag through tight junctions

In their theory of paracellular solvent drag, Pappenheimer and Reiss [14] proposed that 'Na^+-coupled transport of organic solutes from lumen to intercellular spaces provides the principal osmotic force for fluid absorption and triggers widening of intercellular junctions, thus promoting bulk absorption of nutrients by solvent drag'. In terms of glucose absorption then, the diffusive component is paracellular and SGLT1-dependent. Their purpose was to explain the fact, documented for over 40 years, that glucose absorption was observed to increase almost linearly with concentration, up to ~ 246 mM [9], when the only known carrier, the Na^+/glucose co-transporter, had a K_m of 8–23 mM and started to saturate at concentrations in the range 30–50 mM. Glucose and fructose data typical of those they wished to explain were reproduced from the literature in Figure 7 of their paper [14]. Pappenheimer and Reiss did not refer to the possibility that the existence of a high-K_m, high-capacity, transporter might provide an explanation for the relatively linear nature of the dependence of the rate of the diffusive component on sugar concentration. However, at the time of their proposal, no brush-border transporter for fructose was known and only SGLT1 was thought to transport glucose across the brush border. Now it is known that GLUT5 transports fructose and GLUT2 transports both glucose and fructose across the brush-border membrane.

We acknowledge that Pappenheimer has emphasized the importance of the diffusive component of glucose absorption in recent years almost single-handedly; we clearly accept the importance of that component. Nevertheless, given that we now find that the diffusive component is mediated by GLUT2, that the linear nature of the concentration dependence can be explained by glucose-induced activation and recruitment of GLUT2 to the brush border and that the SGLT1- and GLUT2-mediated components account within experimental error for the total rate of glucose absorption, we conclude that there is no longer any need to consider paracellular flow as an explanation for glucose absorption at high concentrations.

Pappenheimer also proposed that the diffusive component is SGLT1-dependent and, with Madara, documented the underlying structural and morphological changes in intestine [29]. Our demonstration that activation of PKC βII has the same kinetics as SGLT1-mediated glucose transport, and that the level of brush-border GLUT2 correlates with activation of PKC βII, provides clear support for this view. Diamond and colleagues have recently reported direct measurements showing that paracellular flow is negligible even at the high local concentrations of glucose produced by the hydrolysis of maltose [16]. Accordingly, they have argued that this provides support for their proposal, based on extensive reviews of the recent literature on long-term dietary studies, that the kinetics of intestinal glucose absorption *in vivo* can be explained solely in terms of SGLT1 [12,15]. However, given that activation and recruitment of GLUT2 to the brush border are ultimately SGLT1-dependent, we now see that much of the literature in which adaptive responses were attributed solely to SGLT1 must be reassessed. The glucose-induced activation and recruitment of GLUT2 to the brush border provides a very clear example of the general proposal of Ferraris and Diamond that intestinal absorptive capacity is matched to dietary intake with little excess capacity [12,15], but our data illustrate the importance of the diffusive component.

The most accurate determinations of water transport show that 342 ± 22 molecules of water are associated in some way with the transport of 1 molecule of glucose by GLUT2. If water were to be transported by GLUT2 directly, as proposed for SGLT1 [30] and GLUT2 [31] in oocytes, it would explain the feature of the diffusive component emphasized by Pappenheimer and Reiss [14], namely that water transport is proportional to absorption at high glucose concentrations.

Conclusion

As reported in previous papers [22,23], fructose absorption can undergo regulation via GLUT2, although GLUT5, like SGLT1, is located constitutively in the microvilli. In the following paper [28] we show that, in addition to a PKC-dependent pathway, fructose absorption is rapidly modulated by several major intracellular signalling pathways associated with growth or stress, namely the extracellular signal-regulated kinase MAP kinase-, phosphatidylinositol 3-kinase- and p38 MAP kinase-dependent pathways [28]. We therefore expect that trafficking of GLUT2 and the resulting sugar absorption will be regulated by a range of endocrine and local hormones that control these pathways, including insulin, growth factors and cytokines. In some cases nutrients themselves will provide the initial stimulus, as described above. The pattern of regulation we have seen for GLUT2 is that a protein which is constitutively located in the basolateral membrane is capable of rapid trafficking to the brush-border membrane to mediate the diffusive component of absorption. We expect that, in due course, studies of the regulation of the brush-border absorption of other nutrients, such as peptides and amino acids, are likely to reveal a similar pattern of regulation controlled by similar intracellular signalling pathways. Investigation of these possibilities will reveal new insights at the molecular level into the short-term control of nutrient absorption and tell us how the absorption of nutrients is regulated during the assimilation of a meal.

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