Preparation and characterization of basolateral membrane vesicles from pig and human colonocytes: the mechanism of glucose transport

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Membrane vesicles were isolated from the basolateral domains of pig and normal human colonocytes. The activity of the ouabain-sensitive K^+ -activated phosphatase, the basolateral membrane marker, was enriched 13-fold in these membrane vesicles over the original homogenate. The membranes displayed cross-reactions with antibodies to the $(Na^+/K^+)ATPase$ and the RLA class I major histocompatibility antigen, both known indicators of the basolateral membrane. There was negligible contamination by other organelles and the luminal membrane, as revealed by marker-enzyme analysis and Western blotting, using

an antibody to villin. The vesicles transported D-glucose in a cytochalasin B-inhibitable Na^+ -independent manner, with a K_m of 28.1 ± 0.8 mM and V_{max} of 3.1 ± 0.4 nmol/s per mg of protein. The transport was inhibited by 2-deoxy-D-glucose and 3-O-methyl-D-glucose, but not by L-glucose or methyl- α -D-glucose. Probing the colonocyte basolateral membranes with an antibody against the C-terminus of the human liver GLUT 2 produced a cross-reaction at 52 kDa. These properties indicate the presence of a GLUT 2 isoform on the basolateral membranes of human and pig colonocytes.

INTRODUCTION

The uptake of glucose into mammalian cells is of great physiological interest, due to the importance of glucose as an energy source. Two classes of glucose carriers have been described in mammalian cells. The Na^+ -dependent D-glucose transporter absorbs glucose at the luminal surface of absorptive epithelial cells, whereas glucose is transported across the basolateral region through facilitated glucose carriers (Baly and Harouk, 1987). In adults of many species, including humans, there is no mechanism to transport D-glucose across the luminal membranes of colonocytes (Buddington and Diamond, 1989; S. P. Shirazi-Beechey and S. M. Gribble, unpublished work). This is in contrast with the foetal colon and the small-intestinal luminal membranes, which possess a Na^+ -dependent D-glucose transporter (Shirazi-Beechey et al., 1990, 1991). The mechanism(s) by which D-glucose is transported across the basolateral membrane in colonocytes are unknown. The mammalian colon is lined with a moderately tight epithelium, consisting of absorptive cells, colonocytes and mucus-secreting goblet cells (Binder and Sandle, 1987). The luminal membrane of colonocytes in adults of many species is known to absorb Na^+ , Cl^- and water, but not nutrients (Binder and Sandle, 1987; Buddington and Diamond, 1989). Much work has focused on the transcellular flux of electrolytes in the intact colonocyte and the transport of these ions across the luminal membrane. The transport functions of the basolateral membrane of these cells have received little attention.

Membrane vesicles isolated from luminal and basolateral domains of polarized cells provide appropriate systems to study the nature and mechanisms of membrane transport processes. They have been used successfully to characterize the transport functions present in the plasma membranes of several epithelia, including small intestine, renal tubule and parotid gland (Lash and Jones, 1984; Dyer et al., 1990; Vayro et al., 1992; Mullins et al., 1992). There have been some reports describing procedures for the isolation of basolateral membrane vesicles from colo-

cytes (Biber et al., 1983; Brasitus and Keresztes, 1983; Wiener et al., 1989). However, very few functional studies have been conducted with these membranes. Moreover, there are no data available on the isolation, characterization and functional properties of human colonocyte basolateral membrane.

In this work we describe the isolation and characterization of membrane vesicles from pig and human colonocytes. Using independent approaches we have shown that these membranes originate from the basolateral domain of the colonocytes and are free from contamination by other cellular organelles and membranes. Utilizing various criteria, we have demonstrated that these membrane vesicles can transport D-glucose in a Na^+ -independent cytochalasin B-inhibitable manner with the characteristics of a GLUT 2 isoform. Our ultimate goal is to study the regulation of the expression of this isoform in the human colon during the transition from normality to malignancy.

METHODS

Materials

D-[U- ^{14}C]Glucose (10.7 GBq/mmol) was purchased from Amersham International (Amersham, Bucks., U.K.). L[1- $^3H(n)$]-Glucose (740 GBq/mmol) was purchased from New England Nuclear Corp. (Boston, MA, U.S.A.).

Monoclonal antibody against purified chicken villin was purchased from Immunotech S.A. (Marseilles, France). Monoclonal antibody (TK3B5) against purified pig renal $(Na^+/K^+)ATPase$ was a gift from Dr. A. J. Kenny (Leeds). Polyclonal antibodies against the C-terminus peptide residues 513–522 and the exofacial loop (residues 47–60) of the human liver GLUT 2 isoform and the C-terminus of human liver GLUT 2 protein (Orci et al., 1990) were gifts from Dr. G. W. Gould (Glasgow). Polyclonal antibody against the RLA class I major histocompatibility antigen was a gift from Dr. J.-P. Gorvel (Marseilles).

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Removal and storage of tissue

Pig colonic tissue was obtained from the abattoir within 10 min of slaughter. Histological examinations indicated that the tissue was undamaged and the integrity of the cells was maintained. A 20 cm section of tissue was removed from the descending colon. It was everted and washed in ice-cold 0.9% (w/v) NaCl (pH 7). The surface epithelium was removed by scraping with glass microscope slides. The scrapings were then wrapped in aluminium foil, frozen in liquid nitrogen and subsequently stored at -80°C .

The colon from human donors were obtained at the time of organ retrieval. The tissue was removed and flushed with 0.9% (w/v) NaCl, wrapped in aluminium foil and dropped into liquid nitrogen. They were then stored at -80°C . Before use, human tissue was thawed in Hanks buffered physiological saline (Dawson et al., 1986) containing 1 mM dithiothreitol and 0.2 mM phenylmethanesulphonyl fluoride. The tissue was opened longitudinally and wiped with paper towels to remove excess mucus. The surface epithelium was then scraped with glass microscope slides. The epithelial scrapings were then treated the same as for pig scrapings. None of the donors had a history of intestinal disease. For the work reported in this paper, the approval of the Ethical Committee was obtained.

Histological examination revealed that the scrapings obtained both from pig and human colon originate from the area of epithelium termed the free-surface area. This area contains predominantly mature colonocytes (Kaye et al., 1973).

Preparation of basolateral membrane vesicles

The colonocyte basolateral membranes were isolated by a method based on that described by Wiener et al. (1989), with modifications. The tissue scrapings were thawed in a solution which contained 250 mM sucrose, 25 mM choline chloride, 10 mM Tris/Hepes, pH 8.3, 1 mM dithiothreitol and 0.2 mM phenylmethanesulphonyl fluoride (1 g of scrapings/10 ml of buffer). When defrosted, the scrapings were homogenized in a Polytron at speed setting 4 for 1 min. The homogenate (H; Table 1) was filtered through nylon gauze to remove mucus. The filtrate was centrifuged at 500 *g* for 15 min to remove cellular debris. The supernatant (*S*₁; Table 1) was further centrifuged at 100 000 *g* for 30 min to produce a crude membrane fraction (*P*₂; Table 1). This fraction was resuspended by 100 strokes in a Dounce tissue disperser with a tight-fitting pestle in 4 ml of buffer containing 250 mM sucrose and 10 mM Hepes/Tris, pH 7.4. Approx. 0.7 ml was loaded on a continuous linear sucrose gradient [25–55% (w/v) sucrose in 10 mM Hepes/Tris, pH 7.4]. The gradient was centrifuged at 100 000 *g*_{av} for 60 min. A discrete band near the top of the gradient was collected. The sample was resuspended in a suspension buffer containing 300 mM mannitol, 0.1 mM MgSO₄ and 20 mM Hepes/Tris, pH 7.4, and centrifuged at 100 000 *g* for 30 min, to remove traces of sucrose. The pellet was resuspended in suspension buffer by repeated passage through a 27-gauge needle. Subsequently they were stored as 50–100 μl portions (0.5–1 mg of protein) in liquid nitrogen. Storage of the vesicles under these conditions over several months had no effect on their ability to transport D-glucose.

Protein assay

Protein was assayed by its capacity to bind Coomassie Blue, according to the Bio-Rad technique. Bovine γ -globulin was used as the standard. The protein values obtained by this assay were the same as those obtained after treatment of the membranes with 0.02% (v/v) Triton-X-100 before assay, indicating that

there is complete solubilization of the membrane-bound proteins in the assay reagent.

Assay of marker enzymes

All enzymes were assayed at 37°C . Ouabain-sensitive K⁺-activated phosphatase was assayed by the method of Colas and Maroux (1980). The substrate was 4-nitrophenyl phosphate and the assay time was 10 min. Succinate dehydrogenase was assayed by the method of Pennington (1961), Tris-resistant α -glucosidase by the method of Peters (1976), and α -mannosidase by the method of Tulsiani et al. (1977). Alkaline phosphatase was measured in the presence of 5 mM MgCl₂ as described by Shirazi et al. (1981).

Glucose uptake in basolateral membrane vesicles

The uptake of D- or L-glucose was measured by the rapid-filtration stop method described by Shirazi-Beechey et al. (1988). The uptake was started by addition of 100 μl of incubation medium consisting of 300 mM mannitol, 20 mM Hepes/Tris, pH 7.4, 0.1 mM MgSO₄ and 1 mM of D-[U-¹⁴C]- or L-[1-³H(n)]glucose, approx. 35 000 c.p.m., to 5 μl of vesicles (50–100 μg of protein). At the appropriate time, 1 ml of ice-cold stop buffer (incubation medium without glucose) was added. A 0.9 ml portion of the quenched reaction mixture was removed and filtered under vacuum through a 0.22 μm -pore cellulose acetate/nitrate filter (GSWP02500; Millipore). The filter was then washed with 5 $\times$ 1 ml of stop buffer. The radioactivity remaining on the filter was determined by scintillation counting. Uptake at zero time was determined by addition of the ice-cold stop buffer before addition of the incubation medium. Initial rates given are the values for a 5 s uptake period. Uptake of D-glucose was shown to be linear over this time period.

For the kinetic analysis of D-glucose uptake, the D-glucose concentration was varied from 0.5 to 50 mM by addition of unlabelled D-glucose. Carrier-mediated uptake was corrected for by subtraction of L-[1-³H(n)]glucose uptake values from the uptake in the presence of D-glucose, at each assay point.

For the inhibition studies, the glucose analogues, L-glucose, 2-deoxy-D-glucose, 3-O-methyl-D-glucose and methyl- α -D-glucose, were added to a final concentration of 50 mM. The osmolarity was kept constant by adjusting the concentration of mannitol.

Inhibition of D-glucose uptake by cytochalasin B was determined at concentrations of 5 and 50 mM D-glucose and 0.1 and 1 μM cytochalasin B. Cytochalasin B was stored as a 1 mM stock in ethanol. Final ethanol concentration during uptake was less than 1%, it was shown that this concentration had no effect on D-glucose uptake.

Gel electrophoresis and Western blotting

Electrophoretic resolution of proteins was performed on either 7.5 or 10% (w/v) polyacrylamide mini-gels, containing 0.1% (w/v) SDS, with a 4.5% (w/v) polyacrylamide stacking gel. Protein was transferred to nitrocellulose sheets by using the Bio-Rad Mini-Transblot apparatus. The excess protein-binding capacity of the nitrocellulose sheets was blocked by incubating for 1 h in PBS (140 mM NaCl, 7 mM Na₂HPO₄, 3 mM KCl, 1.5 mM KH₂PO₄) containing 5% (w/v) non-fat dried milk, 0.1 mM EDTA and 0.1% Triton X-100. The nitrocellulose sheets were incubated at room temperature with primary antibody diluted in PBS containing 0.1 mM EDTA and 0.1% Triton X-100, RLA class I rabbit antibody (1:500), mouse antibody to villin (1:2000), mouse antibody to (Na⁺/K⁺)ATPase (1:500) and rabbit antibody

Table 1 Distribution of ouabain-sensitive K⁺-activated phosphatase activity in the preparation of pig colonocyte basolateral membrane vesicles

Colonocytes from the pig descending colon were fractionated as described in the Methods section. The specific activity and recovery of activity for ouabain-sensitive K⁺-activated phosphatase was determined at each stage of the preparation. Specific activity is expressed as nmol of 4-nitrophenol phosphate hydrolysed/min per mg of protein. Enrichment is the ratio of the specific activity of a fraction to that of the original homogenate. Recovery is the total activity of a fraction as a percentage of the total activity in the original homogenate. Results are shown for a typical preparation.

Fraction	Specific activity	Enrichment	Recovery of activity (%)	
			In the specific fraction	Total contained in all fractions
Homogenate (H)	2.8	—	100	100
Supernatant (S ₁) after centrifuging homogenate at 500 <i>g</i> for 15 min	5.0	1.8	151	170
Pellet (P ₁) after low speed centrifugation	3.5	1.3	S ₁ 19	S ₁ + P ₁
Supernatant (S ₂) after centrifuging S ₁ at 100 000 <i>g</i> for 35 min	3.6	1.3	69	163
Pellet (P ₂) after centrifuging S ₁ for 100 000 <i>g</i> for 35 min	14.0	5.0	S ₂ 75	P ₁ + S ₂ + P ₂
Final membrane fraction obtained after density-gradient centrifugation	29.5	10.5	P ₂ 13	138 P ₁ + S ₂ + other fractions from sucrose gradient

to GLUT 2 (1:10). For the RLA class I, anti-villin and anti-(Na⁺/K⁺)-ATPase antibodies the incubation was for 1 h; the anti-GLUT 2 antibody was incubated overnight. Sheets were then rinsed for 30 min in wash buffer (PBS containing 1 % non-fat dried milk, 0.1 mM EDTA and 0.1 % Triton X-100). Affinity-purified peroxidase-linked secondary antibody, raised to appropriate species (Dako AC, Denmark; The Binding Site, U.K.), was diluted in wash buffer and incubated for 1 h at room temperature. Sheets were rinsed for 30 min in wash buffer. Cross-reactions were detected with PBS containing 0.06 % (w/v) 3,3'-diaminobenzidine, 0.2 % (w/v) CoCl₂ and 0.1 % (v/v) of a 30 % (v/v) H₂O₂ solution.

RESULTS AND DISCUSSION

Isolation of basolateral membranes from pig colonocytes

The procedure described in the Methods section was the resultant of experiments that were designed to identify a fraction of the homogenized colonocytes that contained (1) a significant proportion of the ouabain-sensitivity K⁺-phosphatase activity with a high specific activity, and (2) low specific activities of enzymes that were characteristic of other subcellular membranes. The results are presented in Table 1, showing that the ouabain-sensitive K⁺-phosphatase activity present in the final membrane preparation was enriched 10-fold over that of the original homogenate. There was 13 % of the original activity present in this fraction. Eight experiments were performed by this procedure. The mean enrichment was 13.7 ± 3.7 (S.D.)-fold and the mean recovery was 13.2 ± 4.7 %. A consistent feature of this procedure was the 70 % increase in the total activity of the ouabain-sensitive K⁺-phosphatase that followed the centrifugation of the homogenate (see Table 1). We presume that an endogenous inhibitor was removed at this stage. There was no further activation, nor apparent inhibition, at any other stage of the fractionation procedure.

Isolation of basolateral membranes from human colon

Colonocytes from human descending colon were fractionated in a similar manner to that described for pig colonocytes. In the final membrane fraction the mean enrichment of the ouabain-

sensitive K⁺-phosphatase was 12.8 ± 2.3 -fold and the mean recovery was 29 ± 5.8 %.

Enzyme studies

The activities of marker enzymes characteristic of other subcellular structures were determined in the membrane vesicles isolated from pig colon. The data are shown in Table 2. Enzymes associated with organelles, such as succinate dehydrogenase (mitochondria), α -mannosidase (Golgi apparatus) and Tris-resistant α -glucosidase (endoplasmic reticulum), all show negligible enrichments (0.5–2.1-fold) and recovery of activity (0.7–2.7 %). These results are in marked contrast with the enhancement of the specific activity of the ouabain-sensitive K⁺-activated phosphatase. On the basis of these results we conclude that the final fraction obtained in the cell-fractionation procedure used here is mainly composed of basolateral membranes from the colonocytes.

Distribution of alkaline phosphatase activity

The values for enrichment and recovery of alkaline phosphatase activity in the basolateral-membrane preparation were 3.5-fold and 4.5 % respectively. This slight enhancement of the alkaline phosphatase activity might be interpreted as indicating the presence of a small amount of the luminal membrane in the final preparation. However, investigations into the possibility of alkaline phosphatase being a luminal membrane marker have led to conflicting results (Helander, 1975; Vangesa and Hopfer, 1979; Steiger et al., 1986). We performed histochemical studies to determine the localization of alkaline phosphatase in pig colonocytes (Pearse, 1968). In the colonocytes there appeared to be no alkaline phosphatase activity associated with any specific structure within the cells, and none with the luminal or basolateral membrane. Steiger et al. (1986), using other approaches, have also concluded that alkaline phosphatase is not a marker of the rat colonocyte luminal membrane. Pig small intestine was used as a positive control. The results showed that there was a high concentration of alkaline phosphatase on the luminal membrane of the enterocytes; in this tissue alkaline phosphatase has been shown to be located on the luminal membrane (Eichholz and Crane, 1965; Shirazi et al., 1981).

Table 2 Enrichment and recovery of marker enzymes in the basolateral-membrane fractions

Basolateral membrane vesicles were prepared from pig and human colonocytes and assayed for marker enzyme activities as described in the Methods section. Enrichment is the ratio of the specific activity of the basolateral membrane fraction to that of the original homogenate. Recovery is the total activity of the basolateral membrane fraction as a percentage of the total activity in the original homogenate. Values are means $\pm$ S.D. for the number of preparations in parentheses.

Tissue source	Enzyme	Enrichment	Recovery (%)
Pig	K ⁺ -activated phosphatase	13.68 $\pm$ 3.68 (8)	13.16 $\pm$ 4.72
	α -Mannosidase	1.34 $\pm$ 0.29 (6)	1.63 $\pm$ 0.41
	α -Glucosidase (Tris-resistant)	2.34 $\pm$ 0.83 (7)	2.65 $\pm$ 1.09
	Succinate dehydrogenase	0.48 $\pm$ 0.12 (7)	0.67 $\pm$ 0.27
	Alkaline phosphatase	3.47 $\pm$ 1.15 (7)	4.49 $\pm$ 1.75
Human	K ⁺ -activated phosphatase	12.83 $\pm$ 2.34 (3)	29.01 $\pm$ 5.82

Immunological studies

Antibodies to various proteins characteristic of the luminal and basolateral membranes of colonocytes were used to provide an independent assessment of the presence of any luminal membrane contamination in the final preparation of basolateral membranes and an unequivocal recognition of the final membrane preparation being basolateral in origin.

The putative basolateral membranes from pig and human tissue were dissolved in Laemmli (1970) sample buffer, reducing conditions, or in non-reducing sample buffer and electrophoresed under either reducing or non-reducing conditions. The proteins were transferred to nitrocellulose sheets and probed with polyclonal antibodies to the major histocompatibility antigen, anti-RLA class I (Feracci et al., 1986; Massey et al., 1987). This has been shown to be uniquely localized to the basolateral membranes of intestinal cells (Ahnen et al., 1982; Moktari et al., 1986). It has a molecular mass of 43 kDa. Antibodies raised to this antigen were used to probe the basolateral-membrane fractions from human and pig colonocytes. There was a cross-reaction at a molecular mass of 43.6 kDa, confirming the presence of this protein in the final product of the fractionation of the colonocytes, indicating the presence of membranes which originated from the basolateral domain of the colonocytes.

The ouabain-sensitive K⁺-phosphatase activity is a partial reaction of the (Na⁺/K⁺)ATPase. The enzyme assays presented in Table 1 show clearly that this activity is concentrated in this final fraction. The presence of the (Na⁺/K⁺)ATPase protein in the membranes from pig colon was confirmed by using a monoclonal antibody (TK3B5) raised against pig kidney (Na⁺/K⁺)ATPase (Bourne et al., 1989). Basolateral-membrane fractions from pig colonocytes and purified pig kidney (Na⁺/K⁺)ATPase (used as a control) were electrophoresed under non-reducing conditions in a 7.5%-polyacrylamide gel. The separated proteins were subsequently transferred to nitrocellulose sheets and probed with the monoclonal antibody TK3B5. Figure 1 shows that the antibody reacts with proteins of the same electrophoretic mobility in both samples.

Presence of the luminal membrane in the final fraction

Villin has been shown to be a reliable marker for the colonocyte luminal membrane (Robine et al., 1985). The homogenate and basolateral-membrane preparations from human colonocytes

Figure 1 Detection of (Na⁺/K⁺)ATPase in the colonocyte basolateral membranes

The presence of (Na⁺/K⁺)ATPase on the colonocyte basolateral membranes was detected by Western-blot analysis. Before Western blotting, the proteins were separated on a non-reducing 7.5% (w/v) polyacrylamide gel as described in the Methods section. Lane A: pig basolateral membranes (40 μ g of protein). Lane B: purified pig kidney (Na⁺/K⁺)ATPase (5 μ g of protein). Lane C: purified pig kidney (Na⁺/K⁺)ATPase (10 μ g of protein). The separated proteins were transferred to nitrocellulose sheets. Lanes A and B were probed with a mouse monoclonal antibody raised to the pig kidney (Na⁺/K⁺)ATPase and a peroxidase-linked secondary antibody. Lane C was a control incubated with the secondary antibody only.

Figure 2 Villin abundance in the human colonocyte basolateral membranes

The proteins were separated on a 7.5% (w/v) polyacrylamide gel containing 0.1% SDS as described in the Methods section. Lanes 1a, 1b, 1c: human colonocyte homogenate (20, 10, 5 μ g of protein respectively); lanes 2d, 2e, 2f: human colonocyte basolateral membrane vesicles (20, 10, 5 μ g of protein respectively). The separated proteins were transferred to nitrocellulose sheets, and were probed with a mouse monoclonal antibody raised to villin and a peroxidase-linked rabbit anti-mouse secondary antibody.

were electrophoresed on a 7.5%-polyacrylamide gel containing 0.1% SDS. Western-blot analysis was conducted using a monoclonal antibody against villin. The results are illustrated in Figure 2, showing that villin is clearly present in the homogenate [tracks 1(a,b,c)]. There was some villin present in the final membrane preparation [see Figure 2, tracks 2(d,e,f)]. The amount of villin was quantified by scanning each track; the amount of colour per unit amount of protein was calculated. The abundance of villin per unit of protein in the original homogenate was 3 times that in the final membrane fraction. This contrasts with

Figure 3 Time course of the uptake of D- and L-glucose by pig colonocyte basolateral membrane vesicles

Basolateral membrane vesicles were prepared from pig colon as described in the text. The transport of D- (○) and L- (●) glucose was measured in a medium containing 1 mM D-[U-¹⁴C]- or L-[1-³H(n)]-glucose, 300 mM mannitol, 0.1 mM MgSO₄ and 20 mM Hepes/Tris, pH 7.4. Each point represents the mean ± S.D. for three assays.

Table 3 Effect of hexoses on Na⁺-independent D-glucose transport in colonocyte basolateral membrane vesicles

Basolateral membrane vesicles were prepared from pig colon as described in the Methods section. The transport of D-glucose was measured at 37 °C in the presence of 1 mM D-[U-¹⁴C]glucose (10.7 GBq/mmol), 250 mM mannitol, 0.1 mM MgSO₄ and 20 mM Hepes/Tris, pH 7.4. The hexoses were each added to the incubation medium at a concentration of 50 mM. Each point represents the mean ± S.D. for three assays: *difference significant at $P < 0.005$.

Hexose	D-Glucose uptake (pmol/s per mg of protein)
D-Glucose (control)	32.9 ± 5.5
2-Deoxy-D-glucose	18.3 ± 3.0*
3-O-Methyl-D-glucopyranoside	14.1 ± 5.6*
Methyl α-D-glucopyranoside	35.7 ± 3.1
L-glucose	29.5 ± 8.9

the 13-fold enhancement of the ouabain-sensitive K⁺-activated phosphatase (see Table 1).

Transport studies

It has been shown that D-glucose is transported in a Na⁺-independent manner across the membrane of the basolateral vesicles isolated from enterocytes (Dyer et al., 1990). Thorens et al. (1990) used immunofluorescence and Western-blot techniques to demonstrate the presence of the liver glucose transport protein, the GLUT 2 isoform, on the basolateral membrane of the enterocyte.

Characteristics of D-glucose transport

We investigated the mechanism of D-glucose transport and the type of glucose transporter present within the basolateral membrane of pig and human colonocytes. The data presented in Figure 3 show that the transport of D-glucose into basolateral membrane vesicles is Na⁺-independent and stereospecific. The initial rates of transport of D- and L-glucose were 24.9 ± 9.1 and 9.4 ± 6.2 pmol/s per mg of protein (both $n = 3$) respectively. The

Figure 4 Substrate dependency of D-glucose uptake by colonocyte basolateral membrane vesicles

The uptake of D-glucose was measured in basolateral membrane vesicles prepared from pig colon. The incubating medium contained 0.1 mM MgSO₄, 20 mM Hepes/Tris, pH 7.4, and D-[U-¹⁴C]- or L-[1-³H(n)]-glucose at concentrations ranging from 0.5 to 50 mM. Osmolarity of the incubation medium was maintained at 320 m-osM by addition of mannitol. The initial rates were measured with 5 s incubations and were calculated by subtracting the uptake of L-glucose from that of D-glucose. The data are presented as a Michaelis-Menten curve and (inset) as a Hanes-Woolf transformation. Each point represents the mean ± S.D. for three determinations.

initial rate of uptake was similar in the presence or absence of Na⁺. The transport of D-glucose was temperature-dependent; rates of transport were enhanced 3-fold at 37 °C compared with 25 °C. The transport of D-glucose was inhibited by 65% by 50 μM HgCl₂.

Na⁺-independent D-glucose transport systems (GLUT isoforms) have been shown to transport analogues of D-glucose such as 3-O-methyl-D-glucose (Craig and Elliot, 1979) and 2-deoxy-D-glucose (Hopfer, 1987). They are unable to transport L-glucose and methyl-α-D-glucose (Hopfer, 1987). The ability of 50 mM concentrations of these analogues to inhibit the transport of 1 mM D-[U-¹⁴C]glucose was investigated. The data in Table 3 show that only 2-deoxy-D-glucose and 3-O-methyl-D-glucose could interact with the carrier and inhibit significantly the uptake of D-glucose. These observations indicate the presence of a GLUT-type protein within the basolateral membranes of colonocytes.

Identification of the D-glucose transport proteins

The various GLUT isoforms differ in their K_m values for D-glucose (Kasanicki and Pilch, 1990). The initial rates of L- and D-glucose uptake into basolateral membrane vesicles were investigated over a range of glucose concentrations, 0.5–50 mM. Three different preparations of basolateral membrane vesicles were used. The values for the uptake of L-glucose were subtracted from those of D-glucose to determine the carrier-mediated D-glucose uptake. The transport of D-glucose was saturable and conformed to Michaelis-Menten kinetics. A typical plot is presented in Figure 4. The inset displays a linearly regressed Hanes-Woolf transformation. The calculated value of V_{max} was 3.1 ± 0.4 nmol/s per mg of protein ($n = 3$) and K_m was 28.1 ± 0.8 mM ($n = 3$). This value for the K_m falls within the range of the reported values for a GLUT 2 isoform (Kasanicki and Pilch, 1990).

Figure 5 Detection of the GLUT 2 isoform in colonocyte basolateral membrane vesicles

The proteins were separated on a 10% (w/v) polyacrylamide gel containing 0.1% SDS as described in the Methods section. Lane A, human hepatocyte homogenate; lane B, pig colonocyte basolateral membranes; lane C, human colonocyte basolateral membranes; 100 µg of protein was applied to each lane. Lane D, human hepatocyte homogenate (200 µg of protein). The separated proteins were transferred to nitrocellulose sheets. Lanes A, B and C were probed with a rabbit antibody raised to the human liver GLUT 2 and a peroxidase-linked pig anti-rabbit secondary antibody. Lane D was probed with the secondary antibody only.

The fungal metabolite cytochalasin B inhibits the Na⁺-independent D-glucose transporters in a number of tissues (Jung and Rampal, 1977; Wardzala et al., 1978; Salter and Weber, 1979; Axelrod and Pilch, 1983). The inhibition constant for cytochalasin B can be used to distinguish the various types of GLUT isoform, the K_i for GLUT 2 isoforms being 10 times the values reported for the other isoforms (Axelrod and Pilch, 1983). We have determined the inhibition constant for cytochalasin B to be $1.70 \pm 0.15 \mu\text{M}$ ($n = 3$). This value is characteristic of a GLUT 2 isoform (Axelrod and Pilch, 1983). These kinetic and inhibition data suggest that there is a GLUT 2 isoform present on the basolateral membrane of pig colonocytes.

The GLUT isoform of the colonocyte basolateral membrane was further investigated, by Western-blot analysis, using antibodies raised against the exofacial loop (residues 47–60) of the human liver GLUT 2 isoform and the C-terminus of human liver GLUT 2 protein (Orci et al., 1990). Figure 5 shows the results obtained when the latter antibody was used to probe homogenates of human hepatocytes and the basolateral membrane vesicles isolated from pig and human colonocytes. Hepatocytes showed a cross-reaction with a species of molecular mass 54 kDa. Human and pig basolateral membrane vesicles also display a cross-reaction at a slightly lower molecular mass, 52 kDa. The slight difference in molecular mass may be due to tissue-specific glycosylation (Thorens et al., 1990). The specificity of these antibodies was tested. They did not react with membrane preparations isolated from human adipocytes and erythrocytes. These contain GLUT 4 and GLUT 1 respectively.

It is well established that most tumour cells display accelerated rates of D-glucose uptake and metabolism as compared with untransformed cells (Flier et al., 1987). The molecular mechanisms for this accelerated rate of D-glucose transport are unknown. The present study provides baseline data for our future investi-

gations addressing the colonocyte basolateral-membrane glucose transporters during the transition from normality to malignancy.

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