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Intestinal absorption in health and disease—sugars

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Carbohydrates are mostly digested to glucose, fructose and galactose before absorption by the small intestine. Absorption across the brush border and basolateral membranes of enterocytes is mediated by sodium-dependent and -independent membrane proteins. Glucose and galactose transport across the brush border occurs by a Na^+/glucose (galactose) co-transporter (SGLT1), whereas passive fructose transport is mediated by a uniporter (GLUT5). The passive exit of all three sugars out of the cell across the basolateral membrane occurs through two uniporters (GLUT2 and GLUT5). Mutations in *SGLT1* cause a major defect in glucose and galactose absorption (glucose–galactose Malabsorption), but mutations in *GLUT2* do not appear to disrupt glucose and galactose absorption. Studies on GLUT1 null mice and Fanconi–Bickel patients suggest that there is another exit pathway for glucose and galactose that may involve exocytosis. There are no known defects of fructose absorption.

Key words: glucose transport; glucose–galactose malabsorption; Fanconi–Bickel syndrome.

Sugars are the major source of calories at all stages of life. The dietary sources of sugars vary from the lactose in milk to complex carbohydrates. These carbohydrates are digested to monosaccharides, mostly glucose, galactose and fructose, prior to absorption in the small intestine. Digestion occurs through a complex series of reactions mediated by salivary and pancreatic amylases and by disaccharidases anchored to the brush border surface of the enterocytes lining the surface of the small intestine. Mature enterocytes at the tips of the intestinal villi are then responsible for the complete absorption of the sugars into the body. Once absorbed, galactose and fructose are mostly converted to glucose for metabolism or storage.

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Despite its importance in providing calories, glucose is not an essential nutrient. Normal growth, development and activity can be achieved on a sugar-free diet. Under such regimes the plasma glucose concentration is normal, ~4 mM, and glucose supply to vital organs such as the brain (~100 g/day), is maintained through gluconeogenesis. In complete starvation the plasma glucose concentration falls below normal level in 2–3 days, and this causes severe brain dysfunction, leading to convulsions and coma. Similar problems occur in children with mutations in the gene coding for the blood–brain barrier glucose transporter GLUT1 (Glucose Transporter Type 1 deficiency OMIM 138140).

Although not an essential nutrient, glucose plays an important role in the regulation of normal plasma glucose levels and in the control of food intake. Plasma glucose regulates insulin secretion by the pancreas which, in turn, regulates the uptake of the sugar into fat and muscle. Within 30 minutes of ingesting a carbohydrate-rich meal the plasma glucose concentration rises to 10–12 mM, and it is the insulin secretion that returns the concentration to normal within 60 minutes. On the other hand, food intake and glucose metabolism is regulated, at least in part, by glucosensors in the hypothalamus[1] and neuroendocrine cells.[2] Little is actually known about how the glucose-sensing cells other than those in the pancreas actually respond to changes in glucose concentration.

Sugar malabsorption may occur under a diverse range of circumstances. Indirectly, malabsorption may be caused by a reduction in intestinal surface area digestion, for example, short bowel syndrome or microvillus inclusion disease, an increase in intestinal motility, or defects in the digestive process, for example, pancreatic insufficiency or hypolactasia. Malabsorption may also be due to a specific defect in sugar transporter, as in patients with glucose–galactose malabsorption[3,4] and the Fanconi–Bickel syndrome.[5] Malabsorption, irrespective of the cause, results in the rather common symptoms of diarrhoea, abdominal pain and gas, and so the challenge is to divine the actual underlying cause and to give proper treatment for the disease.

The primary goal of this chapter is to present a synopsis of our current understanding of sugar absorption in healthy individuals and then to summarize what is known about the primary defects in sugar transport. Discussion of the indirect reasons for sugar malabsorption is beyond the scope of this review.

MECHANISMS OF SUGAR ABSORPTION

Carbohydrates in the diet are converted to free sugars before absorption as a result of the concerted action of salivary and pancreatic amylases and the brush border disaccharidases. The carbohydrates in the diet are then presented to the intestinal epithelium as glucose, galactose and fructose. In infants the carbohydrate is lactose in milk, 50–90 g/l, while in adults it is usually in the form of complex carbohydrates. On a 'Western diet' the amount of glucose produced by digestion in adults is about 180 g/day (~1 mol). An ever-increasing amount of fructose is added to our diets (frequently in excess of 50 g/day) through the widespread use of corn syrup as a sweetener. All of the glucose and galactose is normally absorbed, but there is a limited capacity to absorb fructose in both young children and adults. This is evident in healthy, young adults (medical students in the USA and the UK) where the ingestion of 50 g of fructose produces abdominal pain, bloating, borborygmi, flatus and a positive hydrogen breath test in 70% of the subjects (ref. 6 and Barry Hirst, personal communication). It has been noted that two 12 oz cans of some popular soft drinks contain about 50 g of fructose in the form of corn syrup.

Sugars are absorbed in the early and mid small intestine by the mature enterocytes on the upper third of the villi.[7] Glucose and galactose are 'actively' transported across the brush border membrane by Na^+/sugar co-transport (symport) and accumulate within the cell (see Figure 1). While some of this glucose fuels cellular metabolism, a sizable fraction passes out of the cell across the basolateral membrane by facilitated diffusion (uniport). In the case of glucose analogues that are transported by SGLT1, but are not metabolized, (e.g. α-methyl-D-glucopyranoside), the intracellular concentration can reach concentrations 500-fold higher than that in the gut lumen. The energy for this uphill ('active') transport comes from the Na^+ electrochemical potential gradient across the brush border membrane. This Na^+ gradient is maintained by the basolateral $3Na^+/2K^+$-ATPase, which pumps the co-transported sodium ions out across the basolateral membrane (Figure 1). The net result is that for every glucose molecule that is transported across the brush border two sodium ions (and two accompanying anions) are also transported across the epithelium. This, in turn, draws ~1100 water molecules across the epithelium to maintain iso-omolarity of the absorbate. Recall that ion and nutrient absorption across the intestine does not increase the osmolarity of the fluid remaining in the gut lumen. The coupling between glucose, salt and water absorption provides the explanation for the finding that water absorption across the upper and mid-intestine is glucose dependent, and is the rationale for the oral rehydration therapy (ORT) used so effectively to treat patients with secretory diarrhoea (ORT[8]).

Fructose is absorbed passively across the intestine by a process completely independent of glucose absorption. This is evident in patients with glucose–galactose malabsorption in which fructose absorption is normal (see below). Studies on animal models show that fructose transport across the brush border and basolateral membranes is by facilitated diffusion (uniport) (Figure 1).

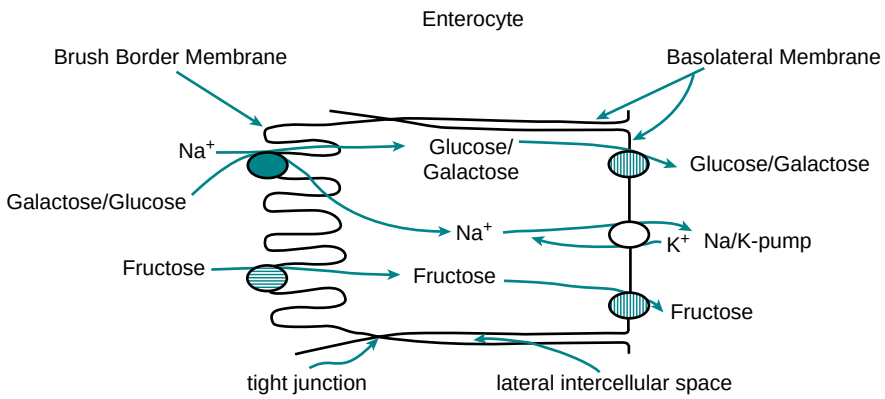

Figure 1. A model for sugar transport across an enterocyte. Sugars are transported across the cell and not through the tight junctions. At the brush border glucose and galactose are transported into the cell by a sodium glucose cotransporter, primarily SGLT1. Fructose is transported passively across the brush border by SGLT5. There are suggestions of a second low-affinity glucose transporter in the brush border: some claim GLUT2 while others claim that it may be a second SGLT (see text). Glucose, galactose and fructose are transported passively out of the enterocyte across the basolateral membrane to the blood. For non-metabolized sugar analogs such as 3OMDG the major pathway is through GLUT2. Fructose and glucose may also pass through GLUT2, but there is increasing evidence that fructose may also pass through GLUT5 and that glucose exits by an exocytosis type mechanism (see text).

Brush border glucose and galactose transport

Currently, we believe that we completely understand the molecular basis for glucose, galactose and fructose transport across the brush border and basolateral membranes. With regard to the brush border membrane we were the first to identify the Na^+/glucose (galactose) co-transporter, clone the gene, and claim that this gene product (SGLT1) accounted for most, if not all, of the transport of glucose and galactose across the intestinal brush border.[9–15] SGLT1 is a 73-kDa protein that is localized to the brush border membrane of the enterocyte. The gene encoding this protein spans some 80 kb of chromosome 22 and contains of 15 exons. The polytopic membrane protein contains 664 amino acids and 14 transmembrane alpha-helices. The N- and C-termini face the extracellular side of the membrane. The extracellular hydrophilic loop between transmembrane helices 6 and 7 bears one N-linked glycan (at Asn248) and this carbohydrate contributes another 15 000 to the apparent mass of the mature protein. The importance of SGLT1 in glucose and galactose absorption was established in the study of glucose–galactose malabsorption (GGM, see below) where mutations in the SGLT1 gene accounts for the absorption defect encountered in this disease.[3,4,13,14]

The application of biophysical and biochemical techniques, in combination with molecular engineering, has provided detailed information about how the Na^+/glucose co-transporter works. While a comprehensive discussion is beyond the scope of the present review, let it suffice to state that transport occurs by an alternating access mechanism.[16,17] Two sodium ions first bind to the outer face of the transporter and this produces a conformational change that permits subsequent sugar binding. The two Na^+ ions and the glucose molecule are then transferred to the cytoplasmic face of the membrane through another conformational change involving a co-ordinated rotation and/or tilt of transmembrane helices.[10–13] At the cytoplasmic surface glucose dissociates first and then the two Na^+ ions dissociate into the cytosol to produce a ligand-free transporter. The low affinity of the cytosolic sites for glucose and Na^+, and the low intracellular Na^+ concentration relative to the extracellular concentration (10 versus 140 m-equiv/l), promote these dissociations. The ligand-free transporter then relaxes to the outward facing conformation to complete the cycle. The complete enzymatic turnover of the transporter occurs about 1000 times a second at 37 °C.

Functional studies of SGLT1 in heterologous expression systems, for example, *Xenopus laevis* oocytes, COS cells, MDCK cells and bacteria, show that the transporter exhibits all the kinetics and selectivity of the intestinal brush border transporter in situ.[7,15,17] The transport of glucose is Na^+ dependent ($K_{0.5} \sim 6$ mM), phlorizin-sensitive (K_i 0.1 uM), and increases with increasing membrane potential. Na^+ and glucose transport are tightly coupled: two Na^+ ions per molecule of glucose. The process is reversible, i.e. the rate and direction of Na^+/glucose co-transport depends on the size and direction of the sodium electrochemical potential gradient and the glucose concentrations. Sodium can be replaced as the driver cation by H^+ ($K_{0.5} \sim 7$ uM), and to some extent by Li^+ ($K_{0.5}$ 12 mM), but the affinity for glucose is much higher in Na^+ (0.1–0.6 mM) than in either H^+ (4 mM) or Li^+ (11 mM). The apparent affinities for the natural sugars, glucose and galactose, are virtually identical, but fructose is not transported. 2-deoxy-D-glucose, a sugar analogue that is transported efficiently by the facilitated glucose transporter such as GLUT1 and GLUT2, is poorly transported by SGLT1 ($K_{0.5} > 100$ mM).

SGLT1 is a multifunctional protein in that it also behaves as an Na^+ uniporter, a water channel, a urea channel and a water co-transporter[18–20], and this raises physiological implications that are beyond the scope of this review.

There is some evidence in animals that there is a second, low-affinity glucose transporter in the brush border membrane. This stems from kinetic studies of sugar absorption in intact animals, in vitro preparations and brush border membrane vesicles.[21] Although each of these studies suffers from technical problems, the prevailing opinion is that there are two types of glucose transport across brush borders: one is a high-affinity Na^+-dependent, phlorizin-sensitive transporter, SGLT1, and the other is a low-affinity transporter which may, or may not, be Na^+-dependent and phlorizin-sensitive.[21] Some favour a facilitated glucose transporter (GLUT2), i.e. an Na^+-independent and phlorizin-sensitive carrier with a glucose K_m in excess of 25 mM.[22] Although this may account for the low-affinity transport of glucose, it should be noted that the presence of such a transporter in the brush border would short-circuit glucose transport via SGLT1, i.e. the accumulation of glucose and galactose in the enterocyte would be reduced or eliminated by the efflux of sugar out across the brush border membrane. Finally, patients with mutations in GLUT2 and animals with *GLUT2* knockouts do not appear to suffer any defect in intestinal glucose absorption.[23,24]

We favour the presence of a second SGLT in the brush border membrane. Potential SGLT candidates include SGLT2, SGLT3, SGLT4, SGLT5 and SGLT6, but in humans at least, neither SGLT2 nor SGLT3, nor SGLT5 are expressed in enterocytes.[25] While the function of SGLT4 is not known, the rabbit and Xenopus homologues of SGLT6 (SMIT2) behave as low-affinity Na^+/glucose co-transporters with K_ms in the range 6–30 mM.[26,27] Another candidate is the recently identified novel Na^+-dependent glucose transporter, rat NaGLT1, which has a glucose K_m of 4 mM and does not transport galactose.[28] At least in the rat, NaGLT1 mRNA was not detected in the small intestine. The presence of a second brush border glucose transporter in the brush border of some patients may explain why we have been unable to identify mutations in *SGLT1* in two patients, and may account partially for the less severe phenotype reported for some GGM patients with deleterious *SGLT1* mutations.

Brush border fructose transport

Animal studies indicate that fructose is transported across the brush border membrane passively by a member of the GLUT family of sugar transporters, namely GLUT5.[29] This uniporter is fairly exclusive for fructose, with a K_m of 6–14 mM.[30] GLUT5, unlike GLUT2 (see below), is insensitive to phloretin and cytochalasin B. GLUT5 is expressed in both the human brush border and basolateral membranes of human enterocytes. See Ferraris[31] for a review of intestinal fructose absorption in rodents.

Basolateral glucose, galactose and fructose transport

The accepted dogma is that all three sugars are transported out of the enterocyte across the basolateral membrane by a passive process, i.e. down their concentration gradients (Figure 1). Because GLUT2 is expressed in the basolateral membrane, and because this transporter handles glucose ($K_m > 50$ mM), galactose and fructose (K_m 66 mM)[30], it has been reasoned that GLUT2 is indeed responsible for the exit of sugar from the cell. This has been reinforced by the similarity of the sugar transport by GLUT2 and basolateral membrane vesicles[7] in terms of kinetics, sugar specificity and sensitivity to inhibitors such as phloretin and cytochalasin B. The reversibility of GLUT2 would also account for the ability of enterocytes to be fueled by blood glucose in the absence of sugar in the gut. In addition to GLUT2-mediated fructose transport across

the basolateral membrane there is some evidence that GLUT5 also mediates fructose transport from the cell.[32]

This dogma was recently challenged by studies of intestinal glucose absorption in *GLUT2* null mice[23], and in patients with GLUT2 deficiency.[24] In both cases there was no impairment of glucose absorption! A glucose-6-phosphate translocase inhibitor blocked glucose absorption in the knockout animal, but not in the control. In addition, the absorption of the non-metabolized sugar 3-*O*-methyl-D-glucose was eliminated in the knockout animal (this sugar is a substrate for both SGLT1 and GLUT2). These results clearly demonstrate that there are two separate pathways for sugar exit from enterocytes: one that involves GLUT2 and another that requires glucose phosphorylation, the transfer of glucose-6-phosphate into the endoplasmic reticulum and the release of free glucose into the blood. The release mechanism is unclear, but it has been proposed to involve vesicle trafficking. This is supported by oral tolerance tests on a patient with congenital deficiency in glucose-6-phosphate translocase 1 in whom glucose absorption was impaired but not eliminated.[24] It would be informative to know whether fructose absorption is impaired in either the GLUT2 knockout animals or the patients with GLUT2 deficiency.

GLUCOSE–GALACTOSE MALABSORPTION (OMIM 182380)

GGM is characterized by a neonatal onset of severe diarrhoea.[13,14] The diarrhoea stops immediately on removing the offending sugars (glucose, galactose and lactose) from the diet, but promptly resumes on feeding. The most comprehensive clinical evaluation of GGM was carried out on the first American patient[33], in whom it was demonstrated that there was malabsorption of glucose and galactose, while fructose and xylose absorption were normal. Laboratory studies with intestinal biopsies showed a normal mucosal histology but an impaired ability to accumulate glucose and galactose across the brush border. Autoradiographic experiments with [H^3]-phlorizin indicated that the defect was due to a reduction in Na^+/glucose co-transporters in the brush border membrane.[33,34]

We tracked down this GGM patient in 1996[35] and found that she was still intolerant of carbohydrate (one teaspoon of table sugar, 6 g, produces malabsorption symptoms). We determined that her *SGLT1* gene contained two separate mutations, one inherited from her mother (Cys355Ser) and the other from her father (Leu147Arg). The proband's two daughters each carries one of the mutations, while her brother carries neither. When the mutant proteins were expressed in *Xenopus laevis* oocytes, they were translated to the same level as the wild-type protein, but there was no sugar transport. On the basis of electrophysiological measurements and freeze-fracture electron microscopy, we concluded that the defect in sugar transport was the failure of the protein to reach the plasma membrane. This is consistent with the previous autoradiographic studies of [H^3]-phlorizin binding to the brush border membrane.[33,34] Thus the trafficking defect in the patient's enterocytes is recapitulated in the *Xenopus laevis* oocyte.

We are now aware of ~300 cases of GGM worldwide. The children that are affected are of a very diverse origin—every racial and ethnic group is represented. A high percentage of our patients are female (~70%), and about two-thirds are the product of a consanguineous relationship. Nevertheless, about 30% have unrelated parents and so have inherited different mutations from the mother and the father (complex heterozygotes).

We have examined 82 patients in 74 families and have identified the mutations that cause the defect in sugar absorption in all but three GGM patients (3, 4, 13, 14, 35 and unpublished observations). As expected for an autosomal recessive disease, virtually each GGM patient has a unique mutation(s); out of 56 mutations only one is found in three different patients, and only seven are found in two different patients. This is quite unlike cystic fibrosis in which a single mutation (delta 508) accounts for the disease in roughly 70% of the patients. Nevertheless, our studies demonstrate that in the vast majority of cases of GGM we have identified mutations in the *SGLT1* gene that result in defective transporters, and provide *circumstantial* evidence that SGLT1 is mainly responsible for the transport of glucose and galactose across the human brush border membrane.

Summary of GGM mutations

We have identified homozygous mutations in 30 GGM patients, and heterozygous mutations in 16 GGM patients (in 12 of the latter cases we have identified the mutations on both alleles). Overall, we have identified 46 mutations (Table 1). No mutations have been found in 16 patients, several of which do not have GGM (e.g. patients with primary lactase deficiency, cow mild protein allergy, chronic diarrhoea that resolved spontaneously). Only in three GGM cases have we failed to identify mutations. There are 34 missense mutations (Table 1), and of the 24 tested in oocytes, only three failed to exhibit a serious defect in glucose transport. Of the six patients with these benign mutations, five have other transport defective mutations.

Only one mutation, C355S, was found in three different kindreds, and another missense mutation, Q457R, was found in three patients in one kindred. Seven other mutations are found in two GGM patients from different families, for example, C292Y is found in two Indian families, C255W in two Arabic families, R379X in Japanese and German families, and S159P in two American families.

All of the nonsense, frame-shift, and splice-site mutations are predicted to produce truncated proteins, and we have demonstrated that the shortest and longest proteins (premature stops at codons 191 and 379) do not transport glucose.[4]

Our DNA analysis of GGM patients and their near relatives has confirmed the autosomal recessive nature of the disease. For example, the parents of children with the D28N mutation only carry the mutation on one allele symptoms. Likewise, in a study of a family in which the proband was a complex heterozygote, C355S and L147R, the mother, father and daughter did not show symptoms even though each was a carrier of one of these mutations.

The autosomal recessive nature of GGM is consistent with other evidence demonstrating that SGLT1 functions as a monomer.[36–38]

Only one other laboratory so far has conducted a molecular genetic analysis of a GGM patient.[39] This Japanese patient is a compound heterozygote with a missense mutation, R135W, on the allele inherited from the father and a novel splice site mutation inherited from the mother. In oocyte expression experiments the R135W mutant was unable to transport sugar. We also have identified this mutation in a European–American patient, and found that it was inactive.[4] There is one difference between the two studies, i.e. on the basis of a light microscopic immunocytochemical analysis Kasahara concluded that R135W is in the oocyte plasma membrane, whereas we cannot detect the protein in the membrane.

Known GGM mutations are distributed throughout the protein and the residues are conserved in all of six mammalian SGLT1s that have been cloned, and also in many of

Table 1. Mutations in *SGLT1*.

Missense mutations		*AA*	*% Uptake*[a]	*Kindred*	*Genotype*
1		Ala → Val	nd	40	Het
2		Asp → Asn	0	1	Hom
3		Asp → Gly	0	12	Hom
4		Gly → Gln	nd	71	Het
5		Asn → Ser	70	45/9	Hom/Het
6		Gly → Val	nd	61	Hom
7		Ala → Pro	nd	62	Hom
8		Arg → Trp	0	16	Het
9		Leu → Arg	0	28	Het
10		Ser → Pro	0	6/24	Het
11		Ala → Thr	9	11	Hom
12		Cys → Trp	nd	60/67	Hom
13		Asp → Gly	nd	44	Het
14		Trp → Leu	4	30	Hom
15		Cys → Tyr	0	8/48	Hom
16		Gln → Arg	0	5	Hom
17		Arg → Ser	0	23	Het
18		Ala → Val	2	41	Hom
19		Isl → Leu	Nd	71	Het
20		Gly → Arg	0	56	Het
21		Cys → Ser	0	10/28/69	Het
22		Tyr → Cys	Nd	70	Hom
23		Leu → Ser	0	46	Het
24		Arg → Gln	0	24	Het
25		Ala → Val	0	15	Het
26		Phe → Ser	0	15	Het
27		Ala → Thr	66	30/66/73	Hom
28		Gly → Arg	0	6	Het
29		Gln → Arg	0	40/49/50	Het
30		Ala → Val	4	56	Het
31		Val → Asn	10	10	Het
32		Arg → His	30	38	Het
33		Arg → Cys	Nd	73	Hom
34		His → Gln	67	45	Hom
Nonsense mutation		*Exon*	*AA*	*Kindred*	*Genotype*
35		2	Arg63 Stop	64	Hom
36		6	Tyr191 Stop	39	Hom
37		8	Lys254 Stop	2	Hom
38		8	Trp276 Stop	54	Hom
39		11	Arg379 Stop	38/72	Het
40		12	Trp477 Stop	69	Het
Frame shift					
41	248delG	3	FS → Stop	47	Hom
42	273delC	3	FS → Stop	55	Het
43	799delC	8	FS → Stop	3	Hom
44	1098ins15	10	ins LVGCT	44	Het
45	1265del4	11	FS → Stop	7/9	Hom

(*continued on next page*)

Table 1 (continued)

46	1286insT	11	FS → Stop	45	Hom
47	1404insT	12	FS → Stop	35	Hom
Splice site		*Intron*			
48	145 + 1	1		26	Hom
49	382 + 2(del/ins)	4		23	Het
50	382 + 1(Dupli)	4		55	Het
51	497 + 2	5		73	Het
52	593 + 1	6		43	Hom
53	1458 + 5	12		18	Hom
54	1458 + 2	12		16	Het

Data from refs. 3,4,35,48 and unpublished observations.

[a] Sugar uptakes for the mutants were determined in oocytes and are given as a percentage of that obtained for the wild-type transporter.

the homologs of the SGLT1 gene.[40] Even though the level of protein in the cell is comparable to that of the wild-type protein, only Q457R is directed properly to the plasma membrane.[41]) In 22 out of 23 missense mutant proteins the defect in sugar transport is a defect in targeting of the protein to the plasma membrane (Figure 2). In three of these mutants, the phenotype in the oocyte expression system has been confirmed for the patient by using intestinal biopsies.[13]

It was quite unexpected to find that conservative mutations in SGLT1 cause miss-trafficking of the protein to the plasma membrane.[4,13,14] For example, there are three Ala to Val mutations (A304V, A388V and A468V) and one Ala to Thr mutant (A166T) that are not trafficked to the plasma membrane. At least with A166T there was sufficient residual trafficking (9% of wild type) to determine function using electrophysiological assays: the K_m for transport was 3.8 mM for alpha-methyl-D-glucopyranoside (aMDG) and 24 mM for Na^+; and the Ki for phlorizin was 0.4 uM (all two to four times higher than for the wild-type). However, introducing a cysteine (A166C) resulted in a normal level of expression.[42] Although the level of A166C present in the plasma membrane was comparable to that of the wild type, the maximum rate of Na^+/sugar co-transport was only about 20% of normal (reduced turnover number), and the affinities for glucose and galactose were lower than for the wild-type protein (1.5–5.5 mM versus 0.4 mM).

While the transport activity of A468V was only 4% of that for the wild-type protein, introducing a cysteine (i.e. A468C) produced a remarkable restoration of expression. Whereas no sugar-stimulated currents were observed for A468V, currents of up to 200 nA were observed for A468C with an aMDG K_m of 0.8 mM and a Na^+ K_m of 2.5 mM, i.e. the maximum rate of transport was about 25% of that for wild-type and the sugar and sodium affinities were comparable. The major difference in structures between Ala and Val are their volume (87 versus 140 A^3) and the presence of two, rather than one, carbon radical attached to the beta carbon. The volume of Cys is intermediate (109 A^3). This suggests that increasing the bulk and branching of the side-chain at position 468 in transmembrane Helix 11 cause the defect in SGLT1 targeting.

Given these results and the diverse range of mutations throughout the secondary structure of SGLT1, it is reasonable to postulate that it is the subtle parameters of tertiary structure of SGLT1 that play a dominant role in determining the trafficking of the co-transporter to the plasma membrane.

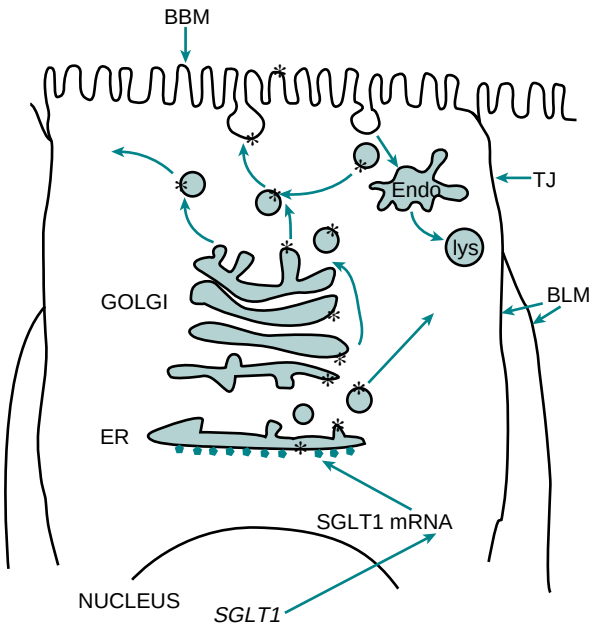

Figure 2. The biosynthetic pathway for SGLT1 in enterocytes. The *SGLT1* gene in the nucleus is transcribed, the mRNA is translated and the protein inserted into the ER membrane where the core sugars are added before passing through to the *trans*-Golgi apparatus were complex glycosylation occurs. The fully mature protein is trafficked to the brush border membrane in 100 nm diameter transport vesicles. After some residency time in the plasma membrane, SGLT1 is internalized by endocytosis where it either enters a transport pool for recycling to the brush border or an endosomal compartment where it is destined for degradation. In GGM we have not observed any defect in transcription or translation of the protein, or any abnormal degradation of the protein. Most GGM mutants either do not pass through the biosynthetic machinery from the ER, or they are trafficked poorly from the GOLGI to the brush border membrane. Only one of 24 GGM mutant proteins reaches the brush border at a normal rate, but in this case the transport is defective. BBM, brush border membrane; BLM, basolateral membrane; TJ, tight junctions.

Regulation of mutant expression

On the basis of our previous report that SGLT1 expression is regulated by protein kinases[43], we set out to see whether we could enhance expression of GGM mutants. We chose two GGM mutants, A166T and W470N, which supported sugar transport in oocytes to a modest degree—10% of wild-type. With these it was possible to use electrophysiological techniques to measure the kinetics of sugar transport, including the maximal rate of transport (V_{max}) and the number of transporters in the plasma membrane. Activation of protein kinase A (PKA) increased the maximal rate of transport by A166T by 40% within 15 minutes, but there was no effect on the expression of V470N. Unlike the wild-type transporter, activation of protein kinase C lowered A166T transport owing to a decrease in the number of mutant transporters in the plasma membrane. Activation of protein kinase C (PKC) resulted in a 40% stimulation of V470N transport. We concluded that the effects of PKA and PKC on sugar transport by GGM mutants were modest and critically dependent on the specific missense mutation.

Clinical implications

The majority of patients with GGM present with a severe diarrhoea in the first days of life and if they are not diagnosed and treated in a timely manner the diarrhoea can be fatal. It can be a challenge to conduct a differential diagnosis of a neonate with diarrhoea, but three key tests can be used to establish GGM as the cause.[13] (1) Eliminate glucose, galactose, sucrose and lactose from the diet—this will resolve the diarrhoea within an hour or two, but the diarrhoea promptly resumes on feeding any of these sugars. (2) An oral glucose (or galactose) tolerance test and/or a breath hydrogen test. A flat plasma glucose (galactose) response and/or a abnormal breath hydrogen ($\gg$ 20 ppm). This, combined with an oral fructose tolerance test, is very informative. (3) An intestinal biopsy showing normal histology. It should be clear from the discussion above about the molecular genetics of GGM that current genetic testing is not particularly useful, owing to a plethora of possible mutations. The only time that it may be useful is in prenatal diagnosis on a family at risk for GGM.[44]

The management of these patients is, in theory, straightforward as the diarrhoea resolves on a diet free of glucose, galactose and lactose. Commercial formulae are available in the USA and Europe and these are generally necessary only in early childhood. One cautionary note is that these commercial formulae are frequently supplemented with fructose to provide calories and this may not be beneficial because diarrhoea may result owing to the low capacity of young children to absorb fructose. It is useful to recall that sugars are not essential nutrients and that the children grow and thrive normally on a sugar-free diet. It is beneficial for the parents to consult a dietitian experienced in guiding GGM patients through infancy.

CONGENITAL GLUT2 AND GLUT5 DEFICIENCIES

A congenital defect in glucose transport by GLUT2 has been identified (Fanconi–Bickel syndrome OMIN 227810) and characterized largely by Santer and his colleagues.[5,24,45] Because GLUT2 is normally expressed in the liver, pancreas and kidney as well as in the intestine, defects in this transporter are expected to have a widespread effect on glucose homeostasis. Indeed, the Fanconi–Bickel patients exhibit tubular nephropathy, fasting hypoglycaemia, rickets, stunted growth, and hepatomegaly secondary to glycogen accumulation.

The link to GLUT2 was promoted by the gene knockout studies on mice, which showed a diabetic-like phenotype that resulted in their early death due to a defect in insulin secretion.[46] Interestingly, these mice did not show an abnormal oral glucose tolerance test even though that had renal glycosuria. When the pancreatic defect was rescued the mice survived, but again they showed a normal oral glucose tolerance test even though GLUT2 was not expressed in the intestine. However, these mice did not absorb a non-metabolized sugar analogue, 3-O-methyl-D-glucoside (3-O-MDG). Further, a glucose-6-phosphate translocase inhibitor, which had little effect in the wild-type mice, inhibited glucose absorption. As mentioned above, it was concluded that although GLUT2 played a role in sugar absorption, there was a second mechanism for glucose to passively exit the enterocyte.[23] Studies by Santer's group on patients with GLUT2 deficiency and G6PT1 deficiency[24] extended these observations to humans. What still remains to be fully explained is why the children that present with the Fanconi–Bickel syndrome have symptoms of glucose and galactose intolerance.[24]

Irrespective of the intestinal symptoms, it should be noted that Santer's group have carried out an exhaustive analysis of the mutations in the GLUT2 gene in some 50 patients diagnosed with the Fanconi–Bickel syndrome.[45] As with other autosomal recessive diseases such as GGM, multiple mutations were found (nine missense, seven nonsense, 10 frame shift and seven splice site), and in a high percentage of the patients the mutation is homozygous. Functional studies of these mutant GLUT2s have yet to be conducted.

Inherited disorders of fructose transport (GLUT5) have not yet been reported.

SUMMARY

Much progress has been made in our understanding of intestinal sugar absorption in heath and disease in both animals and humans. For over 40 years glucose transport across the intestinal epithelium has been the paradigm for the transport of molecules across other epithelia ranging form the renal proximal tubule to the choroid plexus. In the intestine the major players have been identified and cloned, and mutations in the genes that cause congential disease have been discovered. In addition, the physiology of intestinal sugar absorption has been exploited to treat major diseases (secretory diarrhoea), and the molecular genetics of inherited diseases has been used to test physiological principles. While the physiology and pathophysiology of intestinal sugar transport has benefited immensely from molecular genetics, new challenges have arisen with the completion of the first phase of the human genome project. Challenges include unravelling the physiological implications of multiple genes in the human SGLT and GLUT gene families (six SGLTs and more than 11 GLUTs), the undiscovered sugar transporters that are not in these gene families, and the unexpected ancillary functions of sugar transporters. Examples include the recent isolation of a gene encoding a novel Na^+/glucose co-transporter in the rat kidney[28] and another is the anticipated role of human SGLT3 as a glucose sensor.[47]

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