Chapter 1

Mammalian Sugar Transporters

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1. Introduction

Glucose represents the major energy source of mammalian cells. Due to its hydrophilic nature, glucose requires specific transporters in order to cross cellular membranes. Such transport is, in the case of glucose and also other monosaccharides, mediated by energy-coupled as well as facilitative mechanisms represented by protein families of sodium-driven sugar cotransporters (SGLTs) and glucose transporters (GLUTs), respectively.

SGLT cotransporter family present highly diverse functions. They cotransport Na+ with glucose (SGLT1, SGLT2, SGLT4, SGLT5), or galactose (SGLT1, SGLT5) or mannose (SGLT5) or fructose (SGLT5) but also with myoinositol (SGLT6, SMIT), with iodine (NIS) or with choline (CHT). One family member is not a transporter but a glucose sensor (SGLT3). Na+ gradient for this cotransporter family is maintained by Na+/K(+)-ATPase [1].

The various members of the GLUT protein family is comprised of 14 isoforms [2]

The GLUT protein family consists of three different families that can be distinguished based on their protein sequence homologies: Class I comprises the classical transporters GLUT1-4 as well as the gene duplication of GLUT3 which is GLUT14, Class II contains the isoforms GLUT5, 7, 9, and 11, while GLUT6, 8, 10, 12 and the proton-driven myo-inositol transporter HMIT (GLUT13) belong to the Class III [3].

Current understanding of whole body glucose homeostasis under normal-and, more importantly, under disease conditions-is directly linked to the understanding of SGLT and GLUT physiology (Figure 1). The active mechanism of glucose (as well as galactose) absorption in the intestine is primarily catalysed by SGLT1 (Figure 3B), while SGLT2 represents the predominant mechanism for glucose reuptake by the kidney (Figure 3A).

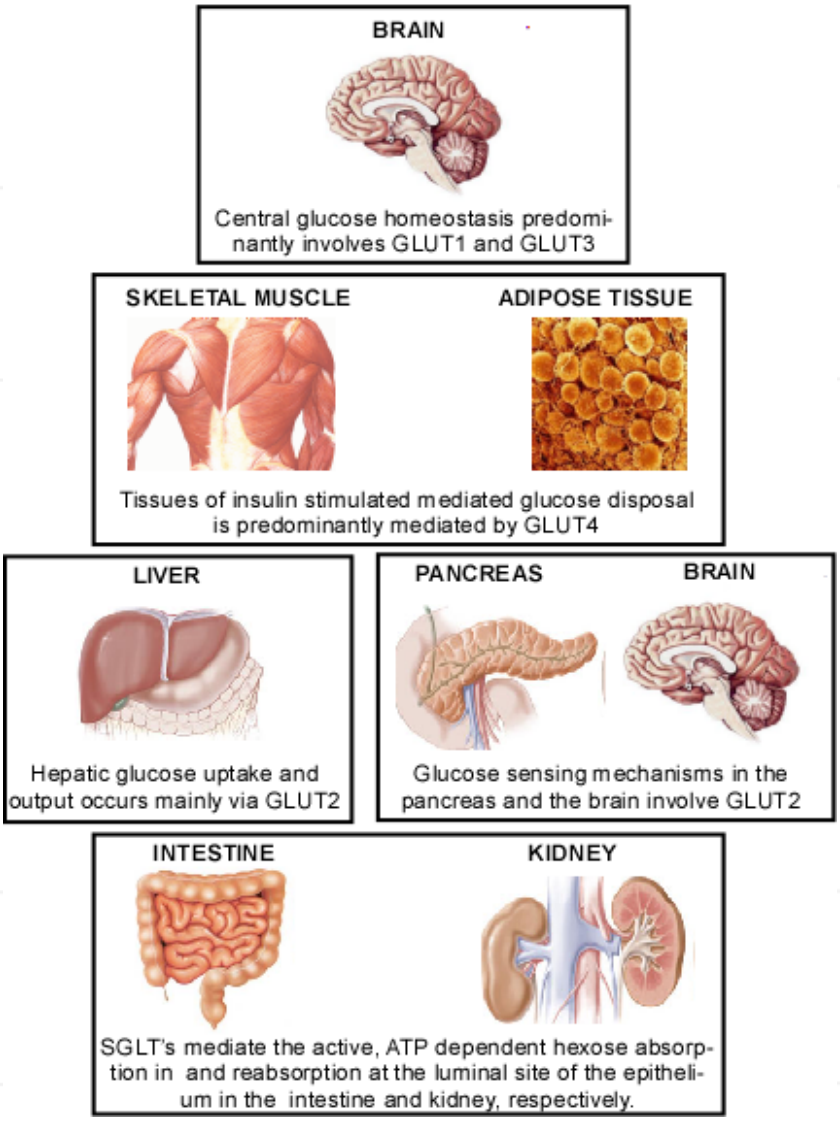

Figure 1. Overview on the principle physiological role for specific glucose transporters and their involvement in regulating glucose homeostasis in specific tissues.

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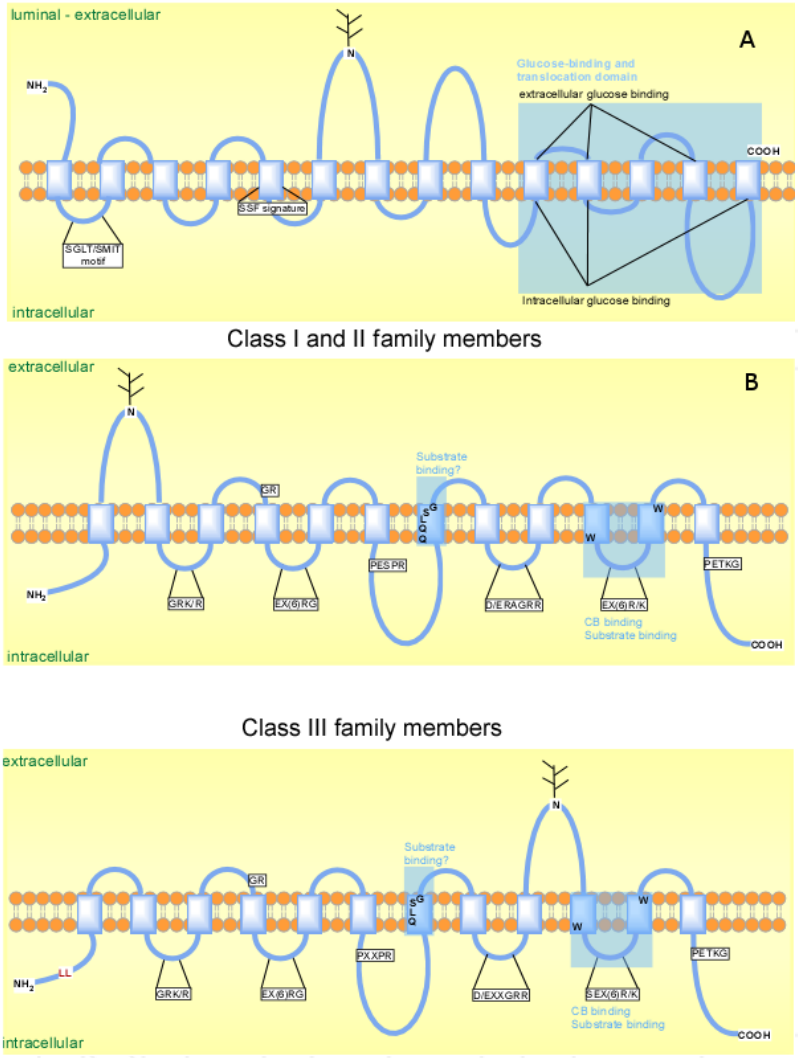

Figure 2. Putative secondary structural models for GLUT and SGLT proteins. **A-**SGLT family members contain 14 transmembrane domains [21]. Highlighted is the presence of the SSF motif present in all members of the solute symporter family (SSF) gene family. The motif that is shared between the SGLTs and sodium-myoinositol cotransporters (SMITs) is also indicated. The glucose-binding and-translocation domain is located at the COOH-terminus of the protein. The residues that are proposed to be involved in glucose binding at the extra-and intracellular sides of the membrane are highlighted. **B-**GLUT proteins contain 12 transmembrane regions. Specific structural features for class I and II (upper panel) and class III (lower panel) family members are indicated such as the proposed substrate binding site, the N-linked glycosylation sites, conserved signature sequences. The tryptophan residues implicated in cyto-chalasin B (CB) binding (positions 338 and 412 in GLUT1) and the N-terminal dileucine signal present in class III members (except for GLUT10) are also shown.

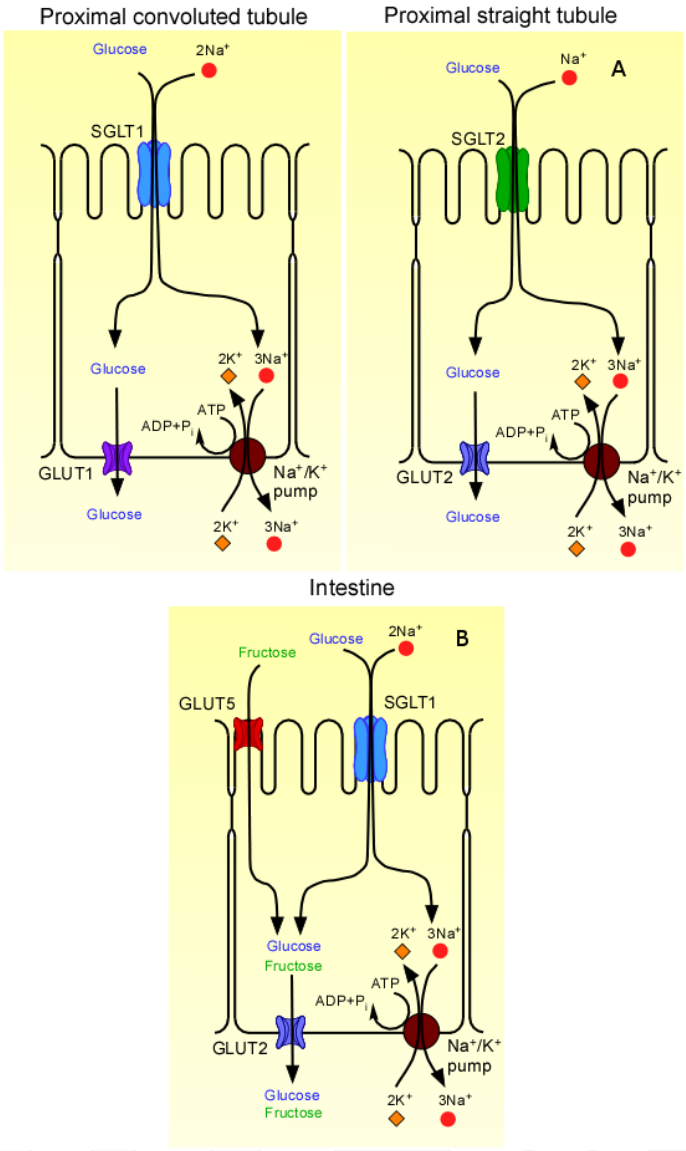

Figure 3. SGLT and GLUT family members regulate intestinal absorption and renal reabsorption of hexoses.A-Renal glucose reabsorption. In the kidney, proximal tubule transepithelial reabsorption of glucose occurs at the apical membrane by SGLT2 and GLUT2 at the basolateral membrane. In the proximal straight tubule, the remaining glucose is reabsorbed by SGLT1 at the apical site of the epithelium and GLUT1 at the basolateral membrane. **B-Intestinal glucose absorption.** In the intestine, transepithelial glucose uptake at the apical site is mediated by the Na^+-dependent glucose transporter SGLT1, while fructose is absorbed by facilitated diffusion via GLUT5. These hexoses can all exit the basolateral membrane through GLUT2.

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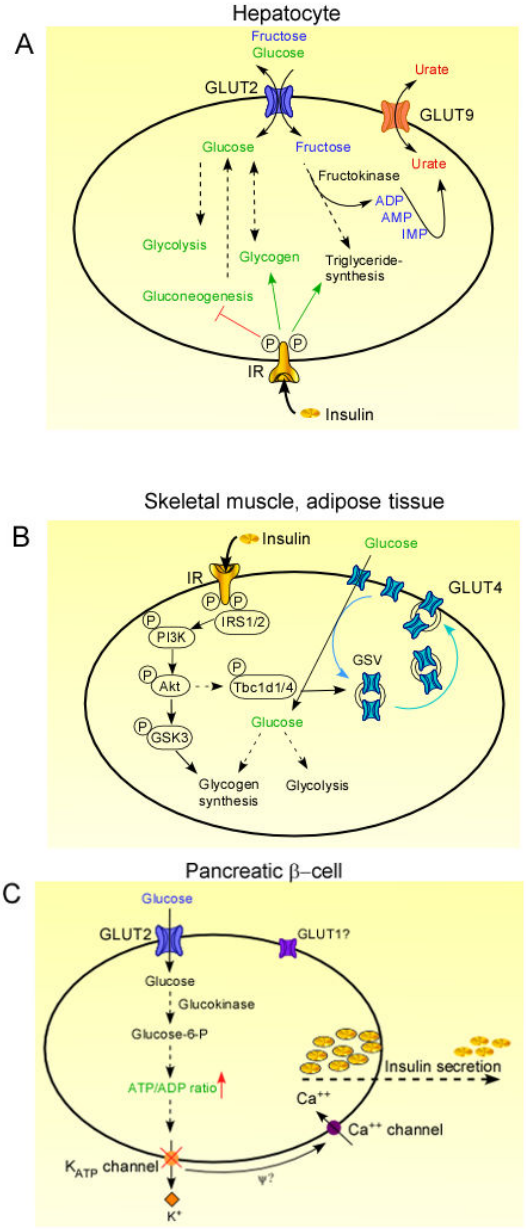

Figure 4. GLUT family members facilitate glucose transport into tissues that control glucose homeostasis such as hepatocytes, skeletal muscle, adipose tissue, and the pancreatic β-cells of the islets of Langerhans. A-Hexose transport in hepatocytes. GLUT2 mediates glucose uptake under feeding conditions into hepatocytes where glucose is metabolized by glycolysis or incorporated into glycogen. In patients with FBS fructose handling is normal, therefore

GLUT2 might not be exclusively involved in uptake of this ketohexose by the hepatocyte [38]. GLUT9 is highly expressed in liver and due to its capability to transport uric acid its proposed function in humans might be the release of uric acid from the liver. In mice GLUT9 is required for uric acid uptake into liver for further breakdown by uricase to allantoin [39]. Whether GLUT9 also contributes to hexose transport, namely fructose, in hepatocytes is currently unknown. Fructose that is taken up by the liver mainly feeds into triglyceride synthesis, and via ATP depletion stimulates AMP-deaminase and thereby purine degradation leading to an increased generation of uric acid. **B-Insulin stimulated glucose uptake into skeletal muscle and adipose tissue**. In muscle and fat, glucose uptake is stimulated by insulin. After insulin binds to its receptor, auto-phosphorylation of the receptor occurs that triggers a signalling cascade that finally leads to a translocation of GLUT4 vesicles from an intracellular pool to the plasma membrane. The acute increase of GLUT4 molecules at the cell surface leads to an increase in glucose uptake and represents the rate-limiting step in insulin-stimulated glucose uptake in adipose and muscle tissues. **C-Pancreatic β-cells secrete insulin in response to elevations in blood glucose**. GLUT2 mediates glucose uptake into β-cells. Phosphorylation of glucose by glucokinase is the rate limiting step of glycolysis which increases the ATP to ADP ratio of the cell leading to closure of the K_{ATP} channel and subsequent opening of the of Ca^{++}channels caused presumably by changes of the plasma membrane polarization. The opening of Ca^{++}channels raises intracellular Ca^{++}concentrations and induces exocytosis of the insulin granules.

As the brain's main energy source glucose needs to be transported across the blood-brain barrier. This process is facilitated by GLUT1 (Figure 1). Insulin-stimulated clearance of blood glucose through uptake into skeletal muscle, the heart and adipose tissue, the rate-limiting step is defined by translocation of GLUT4 from an intracellular compartment to the plasma membrane; and it's this signalling cascade that represents insulin sensitivity (Figure 4B). The secretion of insulin by the pancreatic β-cells of the islets of Langerhans is dependent on GLUT2 which functions as a β-cell glucose sensor (Figure 4C). However, the involvement of sugar transporters in the regulation of processes such as brain glucose sensing or glucose transport in the mammary gland are not yet well understood.

This chapter summarizes the principal characteristics of SGLT [1] and GLUT-[4] mediated sugar transport, primarily focusing on the human transporters, with emphasis on the current understanding of their physiology, based on inherited disorders and syndromes in humans and phenotypes of genetically modified mice.

2. The SGLT family

2.1. Synonyms

SGLT1-6, Gene Symbols: *SLC5*, sodium-glucose symporters

2.2. Summary

The model of active, ATP-dependent glucose transport against a concentration gradient was proposed in 1960 by Bob Crane [5]. Intestinal reabsorption of glucose by the intestinal epithelium through transporters requires sodium symport which is ATP dependent via coupling to the sodium/potassium (Na^+/K^+) pump. Mechanistically, the inward sodium gradient at the apical site of epithelial cells is maintained by the ATP-driven, active extrusion of sodium at the basolateral membrane (Figure 3, Figure 5B). The sodium dependent glucose transporters (SGLTs) are members of a larger gene family (>200 genes) of sodium:solute symporters (SSF) that contain a common SSF motif in the fifth transmembrane region [1]. The

human SGLT protein family (SLC5A) comprises 11 isoforms that structurally are characterized by 14 transmembrane domains, where the N-and C-termini face the extracellular (luminal) side of the cell. The 11 family members share an amino acid identity of 21-70%. A broad range of substrates are transported by proteins encoded by the *SLC5* genes.

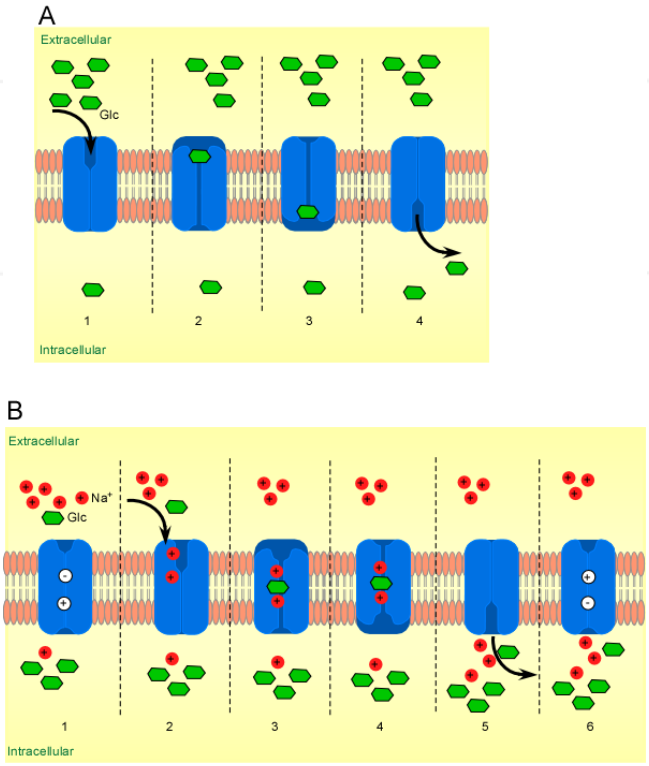

Figure 5. Proposed models for the mechanism of facilitative and active glucose transport across cellular membranes.A-A 6-state model is proposed for SGLT mediated glucose transport. The empty transporter is assumed to have a valence of-2 (1). Sugar transport is initiated upon binding of two sodium ions to the open form of the outside gate (2). In the next step, glucose binds to the transporter, which induces a conformational change from an outward to an inward occluded state (3, 4). Upon open of the inward gate, the glucose is released into the cytoplasm before the sodium (5). The transport cycle is completed by a conformational change to return the ligand-free inward facing (6) structure to the ligand-free outward-facing structure (1). **B-Model of GLUT mediated glucose transport.** Glucose binds to an outward-facing site of the transporter (1) which induces a conformational changes that allows movement of the hexose through the protein (2-3). After the release of the hexose from its inward-facing binding site into the cytosol the transporter undergoes a reverse conformational change (4-1).

The focus of the current chapter is on the sodium-dependent glucose transporters within the *SLC5* gene family, namely SGLT1-5, and the closely related, based on sequence homology and substrate specificity, sodium driven myoinositol transporter SMIT1 (SLC5A3) and SMIT2 (SGLT6/SLC5A11).

More distant relatives of the *SLC5A* gene family are the iodide transporters NIS (sodium-iodide symporter [SLC5A5]) and AIT (apical iodide transporter [SLC5A11]), the Na+/Cl-/choline transporter (CHT, [SLC5A7]) and the sodium-dependent multivitamin transporter (SMVT, [SLC5A6]). NIS and AIT are expressed in the thyroid gland. While NIS is responsible for iodide uptake which is required for production of T3 and T4, AIT is thought to catalyse the movement of iodide from the thyrocyte cytoplasm to the lumen of the gland. SMVT is widely expressed, while

CHT is mainly found in the central nervous system. Biochemically, the CHT mediates Na+/choline co-transport in a chloride dependent manner.

2.3. Structural features & substrate specificities

SGLT family members 1-6 contain between 596-681 amino acids with a 50-70% identity (67-84% similarity) where divergence in sequences can be mainly attributed to the N-and C-terminal domains of the proteins. Alternative splicing has been described for SGLT4-6, however, whether respective functional proteins with varying amino acid composition are encoded has yet to be shown. A common structural component among the large gene family of sodium:solute symporters (SSF) is the presence of a consensus pattern (Figure 2A). The consensus sequence for the six SGLTs and for SMIT1 is located near the N-terminal domain of the proteins (Figure 2A).

A secondary structural model for human SGLT1 predicts the presence of 14 transmembrane helices. The model is based on N-glycosylation and cysteine scanning mutagenesis, antibody tagging, mass spectrometry as well as computer algorithms predicting membrane-spanning regions (Figure 2A). Freeze-fracture electron microscopy provided direct evidence that both SGLT1 and vSGLT (from *Vibrio parahaemolyticus*) function as 14 transmembrane helical monomers. he recent crystal structure for the sodium/galactose symporter vSGLT demonstrated the presence of 14 transmembrane helices providing evidence for the secondary structural model of human SGLT1.

SGLT are highly glycosylated membrane proteins for example SGLT1 contains a N-linked glycosylation site at position N248. However, for SGLT1, glycosylation appears not to be required for functional expression, indicating proper folding and membrane targeting in its absence.

The transport kinetics and substrate specificities have been intensively studied for SGLT1, 2, 3, and more recently 4, mainly based on electrophysiological and biochemical studies upon heterologous expression of the transporters in *Xenopus laevis* oocytes. SGLT1-4 and SMIT1 and 2 all transport (or bind in the case of human SGLT3) D-glucose and the non-metabolizable alpha-methyl-d-glucopyranoside (α-DMG). Transport of those substrates is inhibited by the glycoside phlorizin [6].

SGLT are only able to transport sugars with a pyranose ring, cyclic polyhydroxy alcohols are not transported. The importance of the single hydroxyl groups for substrate recognition has been well characterized. The oxygen is essential for transport by human SGLT1-while sulfur substitution lowers affinity, nitrogen is not tolerated. This particular feature does not apply to

SGLT3 for which imino sugars-containing an amine group in place of a hydroxyl group-are ligands. Based on mutational analysis of SGLTs and the crystal structure of vSGLT, which is 32% identical to human SGLT1, residues that coordinate substrate recognition have been shown to be relatively conserved. An exception to that is the human SGLT3, a glucose sensor, which can be converted to a functional transporter based on a single amino acid exchange.

In addition to glucose and other monosaccharides, SGLTs also transport glycosides. Those can be either substrates such as indican and arbutin, or actual inhibitors such as the highly potent, classic competitive SGLT inhibitor phlorizin, a naturally occurring β-glucoside (see below).

The ability to recognize galactose as substrate by SLC5 family members has been attributed to the presence of a threonine corresponding to amino acid 460 in human SGLT1.

Ion selectivity and stoichiometry has been well characterized for SGLTs. The transporters are selective for the cotransport cation Na^+(K_m=4mM). While Li^+(K_m=9mM) and H^+(K_m=7μM) can replace Na^+, no other monovalent cation is accepted. The $Na^+$to glucose transport stoichiometry is established for SGLT1-3, where two Na^+ions bind to SGLT1 and 3, and only one $Na^+$is required to drive SGLT2 activity. Despite crystallographic information for vSGLT, the electron density is not sufficient to assign binding sites for small single ions such as sodium. However, using mutational analysis and superimposition of structural models from the solute symporters vSGLT, LeuT, and Mhp1, a sodium binding site for vSGLT is suggested to be close to the sugar binding residues in transmembrane domains 1 and 8. The predicted cation binding site in vSGLT appears to allow accessibility to the cytoplasmic aqueous phase.

The mechanism of sodium-driven glucose transport has been intensively investigated for SGLT1, applying various methodologies that allow the kinetics of transport to be determined using heterologous expression of the transporter in *Xenopus laevis* oocytes. From the kinetic measurements a 6-state equilibrium model is proposed, where conformational changes dependent on cation and sugar binding, transport and cytoplasmic release are integrated. The six kinetic states describe the "empty" transporter, the sodium bound form, and the sodium and glucose bound transporter at the external and internal plasma membrane surfaces (Figure 5A).

SGLT1 mediated glucose transport has been characterized regarding its kinetics, conformational changes and the significance of residues for substrate/inhibitor binding. However, many questions remain unanswered such as the precise identity of the second-sodium binding site for SGLT1, and the location of the phlorizin binding site in SGLT1 and SGLT2, which may be of relevance for SGLT2 selective inhibitors that, recently approved, represent a new treatment option for Type 2 diabetes mellitus (T2DM) (see below).

2.4. SGLT1 (*SLC5A1)*

In 1987, the laboratory of Ernest Wright cloned the first sodium-dependent glucose transporter from rabbit intestinal mRNA by an expressing cloning strategy using *Xenopus laevis* oocytes [1]. SGLT1 is primarily expressed in the brush border membrane of mature enterocytes in the small intestine and catalyses the absorption of the dietary sugars glucose and galactose from the gut lumen. SGLT1 is also expressed in the kidney on the luminal

surface of cells within the S3 segment of the proximal tubule, where it contributes to renal glucose reabsorption (Figure 3A).

SGLT1 is a high-affinity, low-capacity transporter with a K_m of 0.5 mM for the substrate αMDG in *Xenopus laevis* oocytes. Substrate transport of glucose is coupled to symport of two sodium ions. The protein is highly glycosylated which leads to an apparent molecular weight of 75 kDa.

2.4.1. SGLT1 physiology

Humans with deficiency for SGLT1 display glucose and galactose malabsorption

Mutations in the SGLT1 gene cause glucose-galactose malabsorption (GGM). GGM was first described in 1962 [7] as a severe life-threatening diarrhea in new-born children, that is fatal within weeks unless lactose, glucose, and galactose are removed from the diet. The diarrhea returns immediately upon reintroduction of the respective sugars into the diet. GGM was predicted to be caused by defective intestinal sodium-coupled glucose transport, a hypothesis that was confirmed following the cloning of human SGLT1 and the identification of homozygous carriers for the D28N mutation encoding a non-functional protein [8].

GGM is a rare autosomal recessive disease caused by missense, nonsense, frame-shift, and splice-site mutations within the SGLT1 gene. Missense mutations-even single amino acid changes-have been demonstrated to cause missorting of the protein in cells, suggesting that slight conformational changes in the protein can interfere with proper folding and/or delivery and integration of SGLT1 into the plasma membrane and thereby affecting its function. More than 80 patients with GGM have been screened for mutations in the SGLT1 gene [1].

2.5. SGLT2 (*SLC5A2)*

SGLT2 was cloned from human kidney cDNA in 1992 and was found to encode for a 672 amino acid protein with 59% similarity to SGLT1. SGLT2 is almost exclusively expressed in the kidney and localizes to the apical domain of epithelial cells that line the S1/S2 segments of the proximal convoluted renal tubule. It has been characterized as a kidney-specific transporter controlling the initial step of renal glucose reabsorption, working in concert with SGLT1, which appears responsible for clearance of residual glucose in the more distal S3 segment of the proximal tubular system (Figure 3A). In contrast to SGLT1, which transports glucose and galactose, SGLT2 represents a low-affinity, high-capacity sodium-glucose symporter with a K_m for glucose of 6 mM and a sodium-to-glucose coupling ratio of 1:1 while having no affinity for galactose.

2.5.1. SGLT2 physiology

Human Physiology-Familial Renal Glucosuria (FRG) is caused by non-functional SGLT2

Glucosuria in the absence of both generalized proximal tubular dysfunction and hyperglycemia is known as De-Toni-Debré-Fanconi syndrome. This is recognized as an inherited disorder and designated as familial renal glucosuria (FRG).

FRG is an autosomal recessive disorder and is diagnosed by persistent isolated glucosuria (urine excretion >1 g/day) with normal fasting plasma glucose levels and oral glucose tolerance.

Since the first SLC5A2 mutation in FRG was presented in 2002 [9], forty-four mutations have been identified including premature stops, frame shifts, and missense mutations. Although the pattern of inheritance for FRG is of co-dominance, a clear definition of genotype-phenotype correlation has not been established. Individuals with similar or even identical mutations display a broad range of severity in glucosuria, indicating that environmental, as well as genetic, factors affect urinary glucose reabsorption. Since, thus far, none of the FRG mutations has been tested for functional SGLT2 effects it is unknown how the various mutations relate to the severity of glucosuria.

FRG established the fundamental role of SGLT2 in renal glucose reabsorption. Since patients with FRG are not affected by severe clinical consequences, it is considered a benign condition, more a phenotype than a disease.

Mouse models of SGLT2 deficiency

The metabolic consequences of SGLT2 deficiency in mice have been investigated in a model of diet-induced obesity and associated insulin resistance and a genetic model of T2DM, the db/db mouse strain [10]. Deletion of SGLT2 leads to increased urine output and a tremendous increase in glucosuria that is associated with compensatory increases in feeding, drinking, and activity. SGLT2 knockout mice are protected from diet-induced hyperglycemia and glucose intolerance and have reduced plasma insulin concentrations. In the diabetic db/db mouse, deficiency of SGLT2 prevents fasting hyperglycemia and is associated with normalized plasma insulin levels and preserved pancreatic β-cell function. These data confirm the concept of glucotoxicity which was established by studying the anti-diabetic effects of blocking renal glucose reabsorption in diabetic rats by pharmacological means of SGLT inhibition using phlorizin [11].

2.5.2. SGLT2 inhibitors – A new concept for the treatment of Type 2 diabetes

T2DM is characterized by hyperglycemia that results both from peripheral resistance to the action of insulin and from progressive failure of the pancreatic β-cell to compensate for the increasing demand for insulin. Chronic hyperglycemia triggers glucotoxicity, a term summarizing the vicious cycle between hyperglycemia inducing β-cell dysfunction and insulin resistance that aggravates disease progression leading to micro-and macrovascular complications.

Current treatments for T2DM come with significant limitations regarding their potential to induce adverse effects. Metformin can cause gastrointestinal effects such as diarrhea and nausea, while sulfonylureas and insulin can induce hypoglycemia and are associated with weight gain. Thiazolidinediones that act as insulin sensitizers can induce weight gain, and are associated with edema and are potentially associated with an increased cardiovascular risk. GLP-1 analogues which are incretin mimicking agents can cause nausea and diarrhea. New therapeutic strategies are needed that are not only effective in terms of glucose control, but

provide excellent safety and potential add-on effects such as weight loss, lipid lowering or reductions in blood pressure.

The kidney has an important role in controlling blood glucose levels by mediating glucose reabsorption into the bloodstream. In patients with T2DM increased renal absorptive capacity has been observed, indicating that blocking the process of glucose reuptake by the kidney might be an attractive new strategy for treatment of T2DM. However, glucosuria has historically been perceived as a manifestation of the disease, which appears to make this therapeutic concept seem rather counter-intuitive.

The phenotype of subjects identified with FRG, as well as studies performed with phlorizin, indicated that correcting hyperglycemia via specific inhibition of SGLT2 might provide a new option for a safe and effective treatment of T2DM.

Phlorizin, a potent SGLT inhibitor, proved to be an important tool for investigating the mechanism and consequences of blocking renal sugar reabsorption. Its use established the concept of glucotoxicity: blocking of renal glucose reabsorption with phlorizin in diabetic rats normalized insulin levels and restored insulin sensitivity [11].

Disadvantages of phlorizin include the non-selective inhibition of SGLT2, poor bioavailability, short half-life and potential for side effects caused e.g by blocking GLUT via its major metabolite phloretin. These disadvantages that are inherited to the molecular phlorizin led into research for new compounds in order to achieve proof of concept for selective SGLT2 inhibition for the treatment of T2DM.

Although non-selective for SGLT2, T-1095 was the first orally available phlorizin derivative that was metabolically stable. When administered to diabetic animals, T-1095 corrected hyperglycemia and reduced hyperinsulinemia, and hypertriglyceridemia [12]. These findings indicated that SGLT2 inhibition might be a viable approach to treatment of T2DM.

In the following years, the selective SGLT2 inhibitors sergliflozin and remogliflozin progressed to clinical trials. While many selective SGLT2 inhibitors went into clinical testing, development of O-glycosidic SGLT2 inhibitors was halted, presumably due to their unfavorable pharmakokinetic profile. In contrast, a number of C-glycosidic compounds which differ from O-glycosides in structure and stability are in clinical development [12] or have achieved approval and represent an innovative insulin-independent treatment option for controlling blood glucose in Type 2 diabetic patients [13].

2.6. SGLT3 (*SLC5A4*)

The human SGLT3 cDNA was cloned from colon carcinoma and was found to encode a 659 amino acid protein with 70% identity to human SGLT1. SGLT3 mRNA is detected in the intestine, testes, uterus, lung, brain and thyroid, while the protein is predominantly found in intestine and skeletal muscle. Immunohistochemical analysis of the intestine identified cholinergic neurons in submucosal and myenteric plexuses as the site of SGLT3 expression. In skeletal muscle, SGLT3 co-localized with the nicotinic acetylcholine receptor indicating expression at the neuromuscular junction.

Functional characterization of human SGLT3 demonstrated a lack of sugar transport activity. Instead, human SGLT3 was found to be a glucose-sensitive ion channel where sugar binding induces plasma membrane depolarization in a saturable, sodium-dependent and phlorizin-sensitive manner [14]. Interestingly, this is in sharp contrast to pig and mouse SGLT3 which are able to transport glucose. The sodium-to-substrate stoichiometry is 2:1, which is similar to SGLT1, while substrate specificity appears closer to SGLT2 with no acceptance of galactose as a substrate. More in depth characterization of SGLT3 substrate specifities found that human SGLT3, similarly to SGLT1, interacts with various glucosides, while pig SGLT3 was found to transport imino sugars with high affinity.

The lack of transport activity by human SGLT3 has been shown to involve a specific amino acid: residue 457. This residue has been shown to be important for the function of human SGLT1, since mutations of that particular amino acid cause GGM. Structural information from vSGLT [15] revealed that the corresponding residue mediates direct interaction with the sugar. Accordingly, mutation of glutamate 457 in human SGLT3 to glutamine conferred transport activity on the transporter displaying SGLT1-like transport characteristics with respect to substrate-to-sodium stoichiometry, sugar specificities as well as affinities [15].

Physiologically, SGLT3 is hypothesized to act as a glucose sensor which, at the site of its expression in cholinergic neurons and the neuromuscular junction, might modulate action potentials of neurons/skeletal muscle cells glucose dependently. This hypothesis is supported by the observation that upon expression of human SGLT3 in sensory neurons of *C. elegans* glucose sensing *in vivo* can be monitored [16]. More recent observations indicated that SGLT3 is expressed in the proximal tubule of the human kidney and might be responsible for sodium reabsorption based on uptake studies in SGLT3 overexpressing kidney cell lines which demonstrated sodium transport that was blocked by the classical inhibitor phlorizin [17].

2.7. SGLT4 (*SLC5A9*)

SGLT4 was cloned from human small intestinal cDNA libraries. The mRNA encoding SGLT4 is almost exclusively found in the small intestine and kidney. SGLT4 exhibits Na+-dependent AMG transport with a K_m of 2.6 mM. Inhibition studies of AMG mediated transport indicated that SGLT4 appears to transport naturally occurring sugars with a rank order of mannose, glucose, fructose, and galactose. Transport studies using radiolabeled mannose indicated that SGLT4 might be physiologically relevant for intestinal absorption as well as renal reabsorption of mannose [18].

2.8. SGLT5 (*SLC5A10*)

SGLT5 was recently cloned from human kidney cDNA and characterized as a kidney specific sodium-dependent mannose transporter which is also able to transport glucose and fructose [19]. While specifically expressed in human kidney its precise localization and physiological role remains unknown. Based on amino acid sequence homology SGLT5 represents the closest homologue to SGLT4. In a manner reminiscent of the relationship between SGLT1 and SGLT2,

it can be speculated that SGLT4 and SGLT5 may act as complementary mannose transporters that regulate intestinal absorption and renal reabsorption of mannose, respectively.

Mouse models of SGLT5 deficiency

Mice deficient for SGLT5 [20] were shown not to display any renal sodium-dependent fructose uptake but increased urinary fructose indicating that SGLT5 is the major transporter responsible for fructose reabsorption in the kidney. Fructose consumption especially by corn syrup containing beverages has been epidemiologically linked to dyslipidemia, obesity, and diabetes. Despite its role in renal fructose reuptake, knockout of SGLT5 in mice paradoxically exacerbated fructose-induced hepatic steatosis.

The massive urinary fructose excretion was accompanied by reduced levels of plasma triglycerides and epididymal fat but fasting hyperinsulinemia. No difference in food consumption, water intake, or plasma fructose was described for SGLT5 deficient mice. The phenotype of SGLT5 knockout mice indicated an apparent link between renal fructose reabsorption and hepatic lipid metabolism.

2.9. SMIT1 (*SLC5A3*)

A Na(+)/myoinositol cotransporter cDNA (SLC5A3) was cloned from canine renal cells and sequenced in 1992 followed by the human SLC5A3 in 1995. The human transporter is mainly expressed in kidney, brain, placenta, pancreas, heart, skeletal muscle, and the lung.

2.9.1. SMIT1 physiology

Phenotype of mice deficient for SMIT1

Myoinositol is a precursor of the main inositol-containing phospholipids phosphatidylinositol and phosphatidylinositol-4,5-bisphosphate, a key molecule in cellular signal transduction. In addition, myoinositol has an important role in osmoregulation. The highest myoinositol levels are found in certain regions of the brain with cerebrospinal fluid levels ranging from 2-25 mM, which are higher than levels in the blood.

One hypothesis as to why lithium is effective in the treatment of bipolar disorders is based on its effect on reducing cellular concentrations of myoinositol (the inositol depletion model). Ablation of the murine *SLC5A3* gene demonstrated the significant role of this transporter in maintaining central myoinositol concentrations. SMIT1 knockout mice have significantly reduced central inositol levels with no changes in phosphatidylinositol concentrations. Besides the severe myoinositol deficiency in the brain, those animals display congenital central apnea due to abnormal respiratory rhythmogenesis leading to death shortly after birth. The neonatal lethality of SMIT1 knockout animals appears to be caused by failures in development of peripheral nerves, specifically in nerves controlling breathing. The peripheral nerve abnormalities can be corrected by prenatal myoinositol supplementation suggesting that myoinositol is required for peripheral nerve development. Phenotypic analysis of homozygous SMIT1 knockout mice indicated, that a reduction of central inositol levels is associated with lithium-

like neurobehavioral effects. Potentially, the inositol depletion hypothesis as a mode of action for lithium might be supported by the phenotypic characteristics of SMIT1 knockout mice [21].

2.10. SGLT6 /SMIT2 (*SLC5A11*)

SMIT2 was initially cloned by PCR from rabbit kidney cDNA. Sequence analysis indicated 49% and 43% protein sequence identity to SGLT1 and SMIT1, respectively. SMIT2 mRNA is detected in brain, kidney, heart, skeletal muscle, spleen, liver, placenta, lung, leukocytes, and neurons. Three transcript variants named SMIT1a, SMIT1b, and SMIT1c have been identified for the *SLC5A2* gene. It wasn't until 2002, that the cloned product was functionally characterized and identified as a sodium-coupled myoinositol transporter with a K_m of 120 μM and 13 mM for myoinositol and sodium, respectively [22]. Transport mediated by SMIT2 is phlorizin sensitive (K_i of 76 μm). The substrate specificities of SMIT1 and SMIT2 are remarkably different: SMIT2 shows stereospecific transport of D-glucose and D-xylose without affinity for fucose, while SMIT1 transports L-fucose and L-xylose (but not their D-isomers) and does not distinguish between D-and L-glucose. In contrast to SMIT1, SMIT2 transports d-*chiro*-inositol.

SMIT2 mediated myo-inositol transport has been demonstrated to occur at apical membranes of the rat intestine [23] and at the luminal side of of proximal convoluted tubules in the kidney of rabbits [24], a tissue specificity and principle role that appears similar to the sodium-dependent uptake and reabsorption of glucose mediated by SGLT-1.

3. The family of glucose transport facilitators

3.1. Synonyms

GLUT1-14, Gene Symbols: *SLC2A1-14*, solute carrier family 2A1-14

3.2. Summary

Glucose transporters are uniporters that facilitate the diffusion of their respective substrates (e.g. glucose) across cellular membranes along a concentration gradient [2, 4]. The protein family comprises 14 isoforms that share common structural features such as 12 transmembrane domains, N-and C-termini facing the cytoplasm of the cell and an N-glycosylation site within either the first or fifth extracellular loop. Based on their sequence homology (14-63% identity) three classes can be distinguished: Class I includes the 'classic' glucose transporters GLUT1-4 and GLUT14, the Class II members are GLUT5, 7, 9, 11, and the Class III transporters comprise GLUT6, 8, 10, 12 and the proton driven myo-inositol transporter HMIT (or GLUT13). Despite their structural similarities, the different isoforms are characterized by tissue specific expression and distinct characteristics such as alternative splicing and (sub) cellular localization. With respect to their substrate specificities, the protein family includes transporters of glucose (GLUT1-4, 8, 14), fructose (GLUT5, 7, 11), polyol (GLUT12), myo-inositol (GLUT13), and urea (GLUT9) transporters.

More in-depth phylogenetic analysis of the GLUT sequences implied a different clustering of GLUT6 and 8, GLUT10 and GLUT12, and HMIT as more distant family members implying the existence of 5 subclasses based on sequence identities but in addition functional properties. Based on these analysis, the evolutionary more ancient forms HMIT, GLUT10, 12, 6, and 8 are postulated to mediate substrate transport which is linked to electrochemical gradients as it has been described for HMIT and GLUT12 [25].

3.3. Structural features & substrate specificity

The protein sequences of the 14 isoforms protein sequences are between 14-63% identical and 30-79% conserved. Common to all isoforms of the GLUT protein family are the predicted 12 transmembrane helices that are based on the initial hydropathy plot for GLUT1 (Figure 2B). The 12-helix model has been supported by studies applying glycosylation scanning, the use of epitope-tags placed within the extrafacial loops as well as antibodies directed against the predicted extra-and intracellular loops and the N-and C-terminal parts of the proteins. All family members are highly glycosylated membrane proteins harbouring an N-linked glycosylation site. For Class I and II family members this is positioned in the first exofacial loop between transmembrane helices 1 and 2, while class III family members contain a shorter extracellular loop 1 and harbor the glycosylation site within the larger loop 9 [3].

Sequence comparisons between all isoforms identified conserved residues that have been termed sugar transporter signatures [3]. These include conserved glycine residues in helices 1, 2, 4, 5, 7, 8 and 10, indicating a critical role in the structure of these helices. In particular helix 7 appears to be important for substrate binding from the exofacial site.

Nonetheless, the primary sequences of the various isoforms do not allow prediction of their substrate specificity or kinetics of transport. However, on the basis of mutational analyses, several residues and motifs have been demonstrated to participate in the substrate recognition by GLUT1 as well as other isoforms. GLUT1, 3, and 4, which transport glucose but not fructose, have the QLS sequence in helix 7.

GLUT2 and 5, which both transport fructose, have a HVA or MGG in this position. Structure function analysis of GLUT2 and 3 chimeras expressed in *Xenopus laevis* oocytes demonstrated that GLUT3 can be converted to a glucose/fructose transporter with GLUT2 transport kinetics when the amino acid sequence of GLUT2 from the beginning of helix *7* to the COOH terminus is inserted into GLUT3. This demonstrates the impact of helix7 on substrate specificity and kinetics of transport. A specific feature of all Class II transporters is their ability to transport fructose, with GLUT5 representing the *bona fide* fructose transporter. Again, helix7 is important for exofacial substrate recognition, since transport of fructose by class II isoforms has been linked to the presence of a NXV/NXI motif. Mutating the isoleucine position significantly reduced fructose transport, while glucose uptake was unaltered. Interestingly, Class II isoforms were unable to transport 2-deoxy-D-glucose (2-DG) or galactose. A valine to isoleucine mutation in GLUT2 (V165I) within helix 5 abolished 2-DG transport, and introduction of the same point mutation in GLUT1 also resulted in a comparable reduction of 2-DG transport.

For GLUT1, the topology and relative orientation of the 12 transmembrane helices with the outward-facing substrate-binding sites have been been proposed by two models. More than 50% of the complete polypeptide sequence has been analyzed by cysteine scanning mutagenesis using the substituted cysteine accessibility method (SCAM) allowing a detailed prediction of the exofacial substrate-binding site and the folding of the human GLUT1. A three-dimensional model for GLUT1 has been developed based on structural information from crystallized members of the major facilitator superfamily, glycerol-3-phosphate transporter and lactose permease. Binding of glucose, forskolin, and phloretin was predicted in close proximity to the exofacial vestibule in this model. While a second binding site for forskolin and phloretin was predicted at the intracellular portion of GLUT1, cytochalasin B has been docked only at one particular endofacial position of the protein.

4. Class I family members

Isoforms of Class I GLUTs are well characterized transporters with GLUT1 being the first isoform cloned and described in 1985.

4.1. GLUT1 (*Slc2a1*)

GLUT1, also known as the HepG2 or erythrocyte sugar transporter, is highly abundant in erythrocyte membranes making up 3-5% of the total erythrocyte proteins. The high amount of GLUT1 in red blood cells allowed the generation and characterization of an antibody that was used for the molecular cloning of GLUT1 from a hepatoma cDNA expression library in 1985 [26]. Although not present in hepatocytes, however, GLUT1 represents the most ubiquitously expressed isoform. The transporter is already found throughout early mammalian embryo development from the oocyte to the blastocyst and is present at high levels in endothelial and epithelial-like barriers of the brain, the eye, peripheral nerves, the placenta and especially in certain tumor cell lines and tissues, although not in hepatocytes. Also, GLUT1 is highly expressed in most routinely used laboratory cell lines.

When assessed in *Xenopus laevis* oocytes, GLUT1 transports glucose with a K_m of ~3 mM. Under equilibrium exchange conditions GLUT1 has a K_m of 20-21 mM for 3-O-methylglucose and 5 mM for 2-DG. Other hexoses transported by GLUT1 are galactose, mannose and glucosamine. When expressed in *S. cerevisae* rat GLUT1 showed a K_m for D-glucose of 3.4 mM and transport was inhibited by cytochalasin B (IC_{50}=0.44μM), $HgCl_2$ (IC_50=3.5μM), phloretin (IC_{50}=49 μM) and phlorizin (IC_{50}=355 μM).

4.1.1. GLUT1 physiology

Human Physiology-the GLUT1 deficiency syndrome (OMIM #606777)

Mutations in the GLUT1 gene are causative for an autosomal-dominant disorder that is characterized by infantile seizures, developmental delay, acquired microcephaly, and ataxia and infantile seizures which is assumed to be caused by the decreased rate of glucose transport

from the blood into cerebrospinal fluid. Defective glucose transport across the blood-brain barrier was first described 1991 [27] and linked to GLUT1 deficiency in 1998 [28]. About a 100 cases have been identified worldwide, including a wide spectrum of heterozygous mutations, including nonsense, missense, insertion, deletion and splice-site mutations, and hemizygosity of the GLUT1 gene.

Since ketone bodies bypass the blood-brain barrier and enter the brain via a monocarboxylic acid transporter (MCT1) they provide an alternative energy source for the brain under conditions of GLUT1 deficiency. Accordingly, a ketogenic diet is effective in controlling the seizures and other symptoms of the GLUT1 deficiency syndrome. However, this treatment is less effective regarding neurobehavioral symptoms. Correlations between genotype and phenotype still remain elusive.

Mouse Models of GLUT1 deficiency

Although representing the first GLUT isoform that was discovered, and despite being well characterized, mouse models for GLUT1 deficiency were only described recently. Mice that are transgenic for a homozygous GLUT1 anti-sense transgene are lethal during gestation; heterozygosity for the GLUT1 anti-sense cDNA was associated with growth retardation and developmental malformations.

In mice, homozygous knockout of GLUT1 was associated with embryonic lethality around day E10dpc and E13-14dpc, while heterozygous animals were viable and showed no differences in body weight development and growth. Decreased brain weights were reported, however, histological abnormalities were not found. While plasma glucose levels were normal in the heterozygous animals, glucose was decreased in the cerebrospinal fluid (CSF). As shown by PET-scan analysis, glucose uptake and metabolism were reduced in brains of heterozygous GLUT1 knockout animals. These animals also showed deficits in motor activity, balance and coordination as well as spontaneous cortical seizures. Overall, heterozygosity for GLUT1 in mice resembles features of humans with the GLUT1 deficiency syndrome.

4.2. GLUT2 *(Slc2a2)*

The second transporter of the GLUT family was cloned in 1988 from human liver and kidney cDNA libraries. The initial characterization detected GLUT2 mainly in the liver, kidney and intestine but the transporter was later demonstrated to be present specifically in the insulin producing β-cells of the pancreas. GLUT2 is a low-affinity, high capacity transporter and with a K_m in the range of ~17 mM, it has the highest K_m for glucose among the known members of the GLUT family. GLUT2 also transports galactose (~92 mM), D-mannose (~125 mM), and D-fructose (~76 mM). Recently, GLUT2 was shown to transport glucosamine with high affinity ($K_{m=}$~0.8 mM). Structurally, GLUT2 lacks the QLS motif in helix 7 which is thought to confer substrate specificity of the transporter, and which may explain the high affinity for glucosamine.

GLUT2 is located in the basolateral membrane of the epithelial cells of the intestine and kidney, where it participates in the release of absorbed (via SGLT1 in the intestine) or reabsorbed (via SGLT1 and 2 in the kidney) glucose into the blood stream.

4.2.1. GLUT2 physiology

Human GLUT2 deficiency and the FANCONI-BICKEL SYNDROME (OMIM #227810)

Rare homozygous or compound heterozygous mutations within the GLUT2 gene cause a type of glycogen storage diseases (GSD), termed GSD XI. The first patient was described by Fanconi and Bickel's, therefore the GLUT2 deficiency is referred to as Fanconi-Bickel Syndrome (FBS). Thus far, 112 patients have been reported. Analysis of 63 patients revealed a total of 34 different *GLUT2* mutations with none of them being particularly frequent. The clinical symptoms of FBS are hepatomegaly secondary to glycogen accumulation, glucose and galactose intolerance, fasting hypoglycemia, tubular nephropathy, and severely stunted growth. In contrast to the metabolism of glucose and galactose, utilization of orally or intravenously administered fructose is normal in FBS patients.

Glucose homeostasis in FBS-patients is heavily disturbed and postprandial hyperglycemia is frequently observed. A few patients have been diagnosed with diabetes mellitus and have been treated with insulin. Hypoglycemia in fasting states is a feature of FBS. Hypoglycemia has very frequently been documented and plasma glucose levels as low as 18 mg/dl have been reported in FBS patients. Compared to other types of hepatic glycogen diseases hepatic adenomas or malignancies have never been observed in patients with FBS.

No specific treatment exists for patients with FBS. Symptomatic treatment is directed towards stabilization of glucose homeostasis and compensation for renal losses of various solutes. The amelioration of the consequences of renal tubulopathy includes the replacement of water and electrolytes. In order to control renal glucose loss and hepatic glycogen accumulation in FBS, patients receive a diet with adequate caloric intake with slowly absorbed carbohydrates.

The importance of GLUT2 in the regulation of glucose-stimulated insulin secretion from the β-cell of the islets of Langerhans has been well established for mice.

Investigations of GLUT2 mutations that have been linked to the Fanconi-Bickel syndrome [29] or patients with transient or permanent neonatal diabetes mellitus [30] indicated an important role for this transporter in pancreatic β-cell development and function in humans.

Mouse models of GLUT2 deficiency

The consequences of GLUT2 deficiency have been analyzed in detail for the various tissues where GLUT2 is involved and/or essential in maintaining whole body glucose homeostasis. Early on, the critical role for GLUT2 in the β-cell for glucose stimulated insulin-secretion *in vivo* became apparent in mice with transgenic overexpression of a GLUT2 antisense RNA specifically in pancreatic β-cell cells. Upon an 80% reduction of GLUT2 in β-cells those animals display hyperglycemia and develop diabetes.

Whole body GLUT2 knockout in mice results in offspring that appears completely normal at birth, but neonates develop early symptoms similar to type 2 diabetes and do not survive beyond the age of 3 weeks. Homozygous GLUT2 deficiency in mice results in hyperglycemia and elevated plasma levels of free fatty acids and β-hydroxybutyrate. *In vivo* glucose tolerance is abnormal and, *in vitro*, β-cells display a gradual loss of control of insulin gene expression by

glucose. Glucose stimulated insulin secretion in islets was impaired by loss of the first, but not the second phase of insulin secretion. GLUT2 knockout mice show marked hyperglucagonemia, and this is accompanied by alterations in the postnatal development of pancreatic islets, evidenced by a gradual inversion of the α-to β-cell ratio. A direct link between diet induced insulin resistance and β-cell dysfunction via disturbed GLUT2 plasma membrane localization has recently been demonstrated. Administration of a high-fat diet feeding in mice results in intracellular retention of the transporter due to improper glycosylation of the protein thereby leading to compromised glucose stimulated insulin secretion.

The early lethality of GLUT2 deficient mice shortly after birth hindered the analysis of GLUT2 physiology in the different tissues of its expression. Transgenic mice that overexpress GLUT1 specifically in β-cells under the control of the rat insulin promoter were generated (RIPGLUT1/GLUT2$^{-/-}$) to study the functional consequences of GLUT2 deficiency in tissues such as liver, kidney, intestine, and also in the brain.

In the liver, GLUT2 deficiency was expected to dramatically affect hepatic glucose output under fasting conditions. Interestingly, hepatic glucose output and glucagon response of the livers from mice lacking GLUT2 were normal. No counterregulation of other transporters known at that time was observed (GLUT1, 3, 4, 5 and SGLT1). Glucose output in GLUT2 deficient livers was not inhibited by cytochalasin B. An alternative membrane traffic-based pathway was proposed that releases glucose directly from the ER after glycogen breakdown or gluconeogenesis. The exact nature of this route has not been determined. In humans GLUT2 deficiency is associated with a marked hypoglycemia in the fasting state owing to a diminished hepatic glucose output and a failure of glucagon to increase plasma glucose. However, human patients with FBS do not generally develop diabetes and do not display a complete loss of their beta cell function, indicating that functionality of human pancreatic β-cells does not solely depend on GLUT2.

Deletion of GLUT2 specifically in the liver [31] or the central nervous system [32] showed, that GLUT2 mediated glucose-sensing mechanisms in both organs exist which control pancreatic β cell mass and function.

In the kidney, a role for GLUT2 in basolateral sugar reabsorption by tubular epithelial cells was postulated due the glucosuria that has been observed in RIPGLUT1/GLUT2$^{-/-}$mice. GLUT2 complements the active sugar uptake at the apical epithelium mediated by SGLT2. The functional relevance for GLUT2 in the kidney in humans is supported by the observation of impaired kidney glucose reabsorption in patients with FBS.

4.3. GLUT3 *(SLC2A3)*

GLUT3 was cloned from a human fetal muscle cDNA library. GLUT3 is considered as a neuron-specific glucose transporter due to its dominant expression in the brain in various species. However, besides the brain GLUT3 is also expressed in tissues with high demand for glucose such as testes (spermatozoa), placenta, preimplantation embryos or certain cancer cells and cancer tissues. Its tissue distribution and function correspond with its high affinity (K_m=1.4 mM for 2-DG), and transport capacity for glucose. While galactose (K_m=8.5 mM), mannose,

maltose, xylose and dehydroascorbic acid are substrates for GLUT3, fructose is not. GLUT3 is inhibited by cytochalasin B (Ki=0.4 μM), phloretin, and and phlorizin.

4.3.1. GLUT3 physiology

Single nucleotide polymorphisms (SNP's) in the GLUT3 gene have been identified and linked to dyslexia

Dyslexia is one of the most common learning disorders in school-aged children. Dyslexic children show differences in event-related potential measurements, in particular for mismatch negativity (MMN), which reflects automatic speech deviance processing. Whole-genome association analysis in 200 dyslexic children, focusing on MMN measurements, identified two SNPs that both showed a significant association with mRNA-expression levels of *SLC2A3* on chromosome 12. It was suggested that a possible trans-regulation effect on *SLC2A3* might lead to glucose deficits in dyslexic children that might cause their attenuated MMN in passive listening tasks.

Mouse Models of GLUT3 deficiency

During mouse preimplantation development GLUT3 is expressed at the apical membrane of the trophectoderm layer of the blastocyst and mediates glucose uptake by the embryo from the external (maternal) environment. Knockdown of the transporter by antisense-RNA at this time-point of development disrupts blastocyst development by diminishing uptake of glucose by the embryo. These data indicated a crucial role for GLUT3 during preimplantation embryo development and its deficiency in mice was assumed to result in embryonic lethality before implantation. Indeed, homozygous loss of GLUT3 leads to a complete loss of embryos at day 12.5. However, morulae develop normally to the blastocyst stage and implantation is not affected by loss of GLUT3.

Heterozygous GLUT3 knockout mice have been characterized especially for a potential neuron (brain) specific phenotype. These animals exhibit significantly enhanced cerebrocortical activity and are slightly more sensitive to an acoustic startle stimulus. However, behavior of these animals regarding coordination, reflexes, motor abilities, anxiety, learning, and memory is normal.

Zhao et al. described features of autism spectrum disorders in heterozygous GLUT3 knockout animals as being abnormal spatial learning, working memory, electroencephalographic seizures, and perturbed social behavior with reduced vocalization and stereotypes at low frequency.

4.4. GLUT4 *(SLC2A4)*

Besides GLUT1, GLUT4 represents one of the most intensively studied glucose transporters which is attributed to its important physiological role regulating the rate-limiting step in insulin-stimulated glucose uptake of skeletal and cardiac muscle, brown and white adipose tissue. Thereby, impaired GLUT4 translocation is causally linked to insulin resistance and consequently to the disease condition of non-insulin dependent diabetes mellitus.

GLUT4 was cloned in 1989 by various groups from human, rat, and mouse tissues. GLUT4 displays a similar affinity for glucose as GLUT1 with a K_m of ~5 mM, and is also capable of

transporting dehydroascorbic acid and glucosamine (K_m ~3.9 mM). When expressed in yeast (*S. cerevisae*) rat GLUT4 is inhibited by the classical inhibitors cytochalasin B (IC50=0.2 μM), phloretin (IC50=10 μM) and phlorizin (IC50=140 μM).

4.5. GLUT4 signalling and cell biology

GLUT4 contains unique sorting motifs at its N-terminus (FQQI) and C-terminus (dileucine) are critical for its capability to traffic between specific intracellular compartments and translocate to the plasma membrane in response to different stimuli. Insulin and exercise are able to rapidly and acutely stimulate GLUT4 translocation to the plasma membrane and thereby influence glucose uptake in muscle and adipose tissue via distinct signalling mechanisms. Activation of the insulin receptor (IR) leads to its autophosphorylation and subsequent signalling through the insulin receptor substrate proteins (IRS) and recruitment of PI 3-kinase catalyzes the formation of phosphatidylinositol (3,4,5)-3-phosphate (PIP3). PIP3 itself activates the protein kinase Akt via two intermediate protein kinases, PDK1 and Rictor/ mTOR. Akt2 rather than the Akt1 or Akt3 isoforms appears to control GLUT4 trafficking. With the identification of the Akt substrates TBC1D4 (AS160), and more recently TBC1D1, two GTPase activating proteins have been identified that appear to bridge the gap between insulin-signalling and trafficking events. For TBC1D4 the substrate is the rab GTPase rab10 which has been shown to be required for insulin-stimulated translocation of GLUT4 vesicles to the plasma membrane.

Exercise has also been shown to induce TBC1D4 phosphorylation but apparently via a distinct PI3-kinase independent mechanism that requires activation of AMPK. However, simultaneous disruption of AMPK and Akt failed to completely inhibit contraction-induced AS160 phosphorylation hinting towards alternative signalling events leading to GLUT4 translocation. A PI 3-kinase-independent pathway has been proposed that involves the adaptor molecules APS and CAP that bind to the insulin receptor and recruit c-Cbl. C-Cbl signals to the guanine nucleotide exchange factor C3G which activates the small GTP binding protein TC10. However, recent data indicated a rather minor contribution for this pathway in insulin mediated GLUT4 translocation.

4.5.1. GLUT4 physiology

Human

Although the significance of GLUT4 for insulin-stimulated glucose uptake in muscle and adipose tissue is well understood, thus far, no polymorphisms within the GLUT4 gene have been identified that would robustly be associated with impaired glucose homeostasis in humans under circumstances such as type 2 diabetes, increased fasting blood glucose levels, or obesity.

Mouse Models of GLUT4 deficiency

Conventional Knockout

GLUT4 knockout mice, surprisingly, are normoglycemic with insulin resistance and hyperinsulinemia in the fed state. The mice are growth-retarded, with markedly reduced

fat mass, cardiomegaly, and shortened lifespan, but no diabetes. In contrast, heterozygous GLUT4-null mice develop hyperglycemia and hyperinsulinemia associated with reduced muscle glucose uptake, hypertension and morphological alterations in heart and liver. Rather unexpectedly, about 50% of heterozygous GLUT4 knockout mice develop diabetes before the age of 6 months, a phenotype that can be reversed by selective overexpression of GLUT4 in skeletal muscle.

Muscle-specific GLUT4 knockout mice

In contrast to conventional GLUT4 knockout animals muscle specific GLUT4 deficient mice have normal body and fat pad weight and a normal lifespan. While skeletal muscle mass is unchanged the heart weight is increased similar to GLUT4 deficient mice and heart specific GLUT4 knockout animals. Basal and especially insulin-or contraction-induced glucose uptake into skeletal muscle is reduced, which is causative for the hyperglycemia, glucose intolerance and insulin resistance seen in those animals. A subset of animals develops diabetes. Surprisingly, insulin stimulated glucose transport in adipose tissue and insulin induced suppression of hepatic glucose production are also impaired which is assumed to be secondary to the hyperglycemia in those animals.

Adipose tissue-specific GLUT4 knockout mice

Adipose tissue selective GLUT4 inactivation, unlike the conventional GLUT4 disruption, does not affect growth, adipose mass or size. Cardiac hypertrophy is not seen in those animals. Fat specific GLUT4 null mice are insulin resistant and glucose intolerant and a subset of animals develop diabetes, which is also observed in muscle specific GLUT4 deficient mice. Muscle and liver are insulin-resistant in those animals. Surprisingly, insulin resistance in muscle was only observed in vivo but not ex vivo indicating a systemic impact of adipose tissue on insulin sensitivity.

Cardiac tissue specific GLUT4 knockout mice

GLUT4 knockout mice specifically muscle and cardiac tissue specific GLUT4 deficient mice all develop cardiac hypertrophy. While homo-and heterozygous GLUT4 null mice show cardiac dysfunction under normal conditions, cardiac specific GLUT4 knockouts have normal contractile function under basal conditions but decreased recovery after hypoxia. Metabolically, heart specific GLUT4 knockout mice are normal and have a normal lifespan.

4.6. GLUT14 *(SLC2A14)*

Searching the human genome for additional genes coding for glucose transporters, a putative gene was identified and cloned showing 95% identity to GLUT3 on the nucleotide level. The *SLC2A14* gene maps to chromosome 12p13.3 with a 10Mb distance to GLUT3 and appears to be a consequence of a gene duplication of GLUT3. GLUT14 was shown to be specifically expressed in the testes. Two alternatively spliced forms of GLUT14 were identified. Interestingly, GLUT14 has no orthologue in mice, a finding that has also been made for GLUT11.

4.7. Class II family members

With GLUT5 being identified in 1990 and established as a fructose transporter initial transport studies for GLUT7 and 9 indicated that those transporters might also transport fructose. A specific feature for class II transporters is that cytochalasin B as a classical GLUT inhibitor does not block glucose transport. Furthermore, all isoforms do not show an affinity for 2-DG and galactose. Unique for GLUT9 and GLUT11 is the alternative splicing /promoter usage, where either two or three different mRNA's are transcribed, respectively. In case of GLUT9 this leads to two proteins that only differ in their N-terminal region.

4.8. GLUT5 *(SLC2A5)*

Human GLUT5 was initially cloned from an intestinal epithelial cell line. GLUT5 is considered as the prototypic fructose transporter – when expressed in *Xenopus laevis* oocytes the human protein transports fructose with a K_m of 6 mM without any noticeable glucose transport activity. However, fructose transport is not inhibited by cytochalasin B, phloretin or phlorizin. Besides fructose, the rat GLUT5 transports glucose, an activity that can be blocked by cytochalasin B. In humans, rats and mice, GLUT5 is primarily expressed in the jejunal region of the small intestine. Lower levels of the protein are expressed in the kidney, the brain, skeletal muscle, and adipose tissue. GLUT5 mediates fructose absorption in the jejunum at the apical, and potentially, at the basolateral membrane, of the epithelial cells into the portal vein (Figure 3B).

4.8.1. GLUT5 physiology

Mouse Models of GLUT5 deficiency

GLUT5 deficiency is associated with reduced fructose absorption when animals are challenged by a high fructose diet. While wildtype mice upon high fructose feeding display an enhanced salt absorption in their jejuna and develop systemic hypertension, GLUT5 knockout mice do not show fructose stimulated salt absorption. Instead, the animals display impaired nutrient absorption that is accompanied by hypotension. Absence of GLUT5 leads to a massive dilatation of the cecum and colon, consistent with severe malabsorption. On a normal chow diet, GLUT5 deficient mice have normal blood pressure and display normal weight gain. The phenotype of GLUT5 deficient mice demonstrates that this isoform is essential for fructose absorption by the intestine and thereby fundamentally involved in fructose induced hypertension.

4.9. GLUT7 *(SLC2A7)*

The human GLUT7 was cloned from an intestinal cDNA library using a PCR-based strategy. GLUT7 is primarily expressed in the small intestine and colon, although mRNA has been detected in the testes and prostate as well. The protein has been localized to the apical membrane of the small intestine and colon. GLUT7 shows a rather high affinity for glucose and fructose (K_m for glucose=0.3mM) while galactose, 2-DG, and xylose are not transported. Sugar transport by GLUT7 is not inhibited by cytochalasin B or phloretin. Sequence alignments

between fructose and non-fructose transporting GLUT isoforms identified a motif in GLUT7 that potentially confers its ability to transport fructose. Mutational analysis of those residues in GLUT7 identified isoleucine 314 as an important determinant for fructose affinity. The finding of a specific residue within the extracellular vestibule of helix 7 that drives substrate specificity was extended to GLUT2, 5, 9 and 11 and proposed as a common NXI/V consensus motif among isoforms capable of transporting fructose.

4.10. GLUT9 *(SLC2A9)*

Human GLUT9 cDNA was isolated by PCR amplification from a human kidney cDNA library on the basis of sequence information from ESTs and from its genomic sequence. GLUT9 mRNA is detected almost exclusively in the kidney and liver and at low levels in the small intestine, placenta, lung and leucocytes. GLUT9 is localized to the insulin-secreting β-cells of human and mouse islets, where downregulation of the protein by siRNA in rat and mouse insulinoma cells leads to a reduced glucose-stimulated insulin secretion. In humans as well as in mice alternative splicing/or promoter usage results in two proteins, GLUT9a and GLUT9b, which only differ in their N-terminal region. While human and mouse GLUT9b are mainly expressed in the kidney, placenta and liver, GLUT9a shows a broader tissue distribution. The different N-termini of human GLUT9a and b determine basolateral versus apical sorting in polarized cells *in vitro*, respectively. In mouse kidney, GLUT9 is localized to apical as well as basolateral membranes of distal convoluted tubules. Initial characterization of GLUT9 determined a rather low affinity for 2-DG. However, a high affinity transport has been reported for glucose and fructose with K_m values of 0.6 mM and 0.4 mM, respectively. More recently, GLUT9 has been identified as a high-affinity uric acid transporter with a K_m of 0.9 mM and 0.6 mM for the human and mouse protein, respectively. Although transport of glucose and fructose has not been observed by some investigators, GLUT9 is thought to exchange glucose or fructose for urate. The classical inhibitor cytochalasin B does not block GLUT9 function. GLUT9 has been established to represent a major regulator of urate homeostasis in dogs, mice, and men.

4.10.1. GLUT9 physiology

In humans GLUT9 is involved in renal uric acid reabsorption-mutations in the GLUT9 gene have consequences on plasma uric acid levels

Various genome wide association studies uncovered polymorphisms in *SLC2A9* as being one of the most significantly associated genes that can be linked to gout and increased serum uric acid concentrations. Although the identified SNPs are located in intronic regions of the gene, evidence exists that increased RNA expression for GLUT9 positively correlates with increased serum uric acid concentrations. In contrast, exonic mutations in GLUT9 suggested that loss of GLUT9 function is associated with hypouricemia. Two distinct heterozygous missense mutations (R380W and R198C in GLUT9a) were described in three patients with hypouricemia. The two mutations were shown to result in loss of function when uric acid transport was studied in *Xenopus laevis* oocyes. A genome-wide homozygosity screen linked hereditary hypouricemia to two homozygous SLC2A9 mutations that lead to a missense mutation (L75R) or a 36-kb deletion. The homozygous loss-of-function mutations of GLUT9 caused a total defect

of uric acid absorption, leading to severe hypouricemia complicated by nephrolithiasis and exercise-induced acute renal failure. Therefore, GLUT9 is essential for renal reabsorption of uric acid-increased expression is associated with hyperuricemia and gout, while loss of function leads to severe hypouricemia.

Mouse Models of GLUT9 deficiency

GLUT9 deficiency in mice leads to hyperuricemia, massive hyperuricosuria, and an early-onset nephropathy, which his is in contrast to the condition in humans where dysfunctional GLUT9 is associated with hypouricemia. Hyperuricemia in mice due to GLUT9 deficiency appears to be a result of impaired urid acid uptake by the liver and therefore inability to be degraded to allantoin by uricase. The nephropathy in GLUT9 knockout animals is characterized by obstructive lithiasis, tubulointerstitial inflammation, and progressive inflammatory fibrosis of the cortex. In contrast, liver-specific GLUT9 inactivation in adult mice results in severe hyperuricemia and hyperuricosuria, in the absence of urate nephropathy or any structural abnormality of the kidney. The deficiency of GLUT9 in mice showed that it represents a functional uric acid transporter *in vivo,* allowing GLUT9 to be identified as a major player in urate homeostasis due to its dual role in uric acid handling in the kidney and in the liver. Whether GLUT9 at all plays a role as a (glucose)/fructose transporter that potentially links fructose uptake by the liver and uric acid metabolism remains to be seen (Figure 4A).

4.11. GLUT11 *(SLC2A11)*

The human GLUT11 was cloned by PCR on the basis of sequence information obtained from ESTs and a genomic sequence. Three variants of GLUT11 (GLUT11-A, GLUT11-B, and GLUT11-C) have been identified that only differ in their N-terminal sequences. Since for each of the variants the corresponding 5′ sequences upstream of exon 1 exhibit promoter activity, transcriptional regulation is assumed to occur by alternative promoter usage. The three isoforms are expressed in a tissue specific manner: GLUT11-A is present in heart, skeletal muscle and kidney; GLUT11-B is expressed in placental, adipose, and kidney tissue; while GLUT11-C is found in adipose, heart, skeletal muscle and pancreatic tissue. Glucose transport activity for GLUT11 was detected in liposomes reconstituted with GLUT11 containing membranes. The transporter shows affinity for glucose with a K_m of 0.16 mM when measured in *Xenopus laevis* oocytes. GLUT11 also transports fructose but not galactose and shows a rather low affinity for cytochalasin B. Mutational analysis of the DSV motif in GLUT11 that corresponds to the “fructose” transporter motif “NAI” also showed that also in GLUT11 this particular region of helix 7 determines substrate selectivity of the transporter. Endogenous GLUT11 protein was localized in heart and skeletal muscle tissue with an antibody raised against the C-terminus of GLUT11 that does not distinguish between the different variants. Human GLUT11 is expressed exclusively in slow-twitch muscle fibres and is unaffected by physiological and pathophysiological conditions except in primary myopathy. Surprisingly, the human SLC2A11 gene has not orthologue in the rat and mouse genome.

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4.12. Class III family members

Class III isoforms share specific features that are unique to this Class. First, structurally all Class III members carry their N-glycosylation site at the fifth extracellular loop. Common to all the isoforms is the presence of an internalization signal (dileucine or YSRI in case of GLUT10) that retains these transporters at an intracellular localization under steady state conditions. Thus far, a stimulus for translocation of Class III isoforms leading to plasma membrane localization has only been proposed for GLUT13 which has not been confirmed.

4.13. GLUT6 *(SLC2A6)*

GLUT6 (formerly designated GLUT9) was cloned from human leucoyte cDNA by (RACE)-PCR on the basis of murine ESTs and the human genomic sequence. GLUT6 mRNA is expressed predominantly in the brain, spleen and peripheral leucocytes. Hexose transport for GLUT6 was only shown when reconstituted in liposomes, where GLUT6 transport activity was found in the presence of 5 mM but not 1 mM glucose. GLUT6 exhibits a low cytochalasin B binding affinity. Thus far, no other data on kinetics of transport and potential substrates have been published for this isoform.

GLUT6 contains an N-terminal dileucine motif that is responsible for intracellular retention of the protein when overexpressed in primary rat adipocytes. GLUT6 is only detected at the plasma membrane when the dileucine residues are mutated to alanine or when clathrin dependent endocytosis is blocked by overexpression of a dominant-negative dynamin mutant. However, no cell-surface translocation of GLUT6 is observed in response to stimuli such as insulin, phorbol ester or hyperosmolarity. A gene expression profiling study aimed to identify deregulated in chronic lymphocytic leukaemia associated with Trisomy 12 identified Slc2a6 among the seven genes with the strongest correlation. Although a significant deregulation was not confirmed subsequently by real-time PCR analysis, the specific expression of GLUT6 in leucocytes and the spleen might indicate an important role for this transporter in normal physiology for this cell lineage.

The biochemistry, cell biology and physiology of GLUT6 is currently not well understood.

4.14. GLUT8 *(SLC2A8)*

GLUT8 (formerly GLUTX1) was the first isoform of the extended *SLC2A* family to be identified by database mining. The human, rat and mouse cDNA were cloned by 5′ and 3′ RACE-PCR from testis cDNA samples. The transporter is mainly expressed in the testes, and lower levels are found in the brain (cerebellum), adrenal gland, liver, spleen, brown adipose tissue and lung. Functional characterization of GLUT8 in *Xenopus laevis* oocytes immediately revealed the intracellular retention of the transporter due to the presence of a dileucine based motif in its N-terminus. When the two leucine residues are changed to alanines GLUT8 localizes to the plasma membrane in mammalian cells and *Xenopus laevis* oocytes that allowed the determination of transport kinetics for the protein. GLUT8 shows a high affinity for glucose with a K_m of ~2 mM. Glucose transport can be competitively inhibited with fructose, galactose, and cytochalasin B. The intracellular localization of

GLUT8 raised the question of whether this isoform would be insulin-responsive and thereby compensate for GLUT4 in the respective knockout mice which are lacking the insulin-responsive glucose transporter yet near normal glucose tolerance. Indeed, in blastocysts of mice, GLUT8 has been found to account for insulin-stimulated glucose uptake. However, various groups performed extensive studies in primary rat adipocytes, 3T3L1 adipocytes, insulin-responsive CHO-cells as well as neuronal cell types such as N2A, PC12, and hippocampal neurons that all failed to identify a stimulus that leads to a plasma membrane translocation of GLUT8.

The intracellular localization of GLUT8 is also observed *in vivo* under steady-state conditions. In the testis immunofluorescence microscopy shows intracellular localization of GLUT8 in a late-endosomal/lysosomal compartment. In the brain, immunogold labeling electron-microscopy localized GLUT8 in synaptic dense core vesicles of nerve terminals and secretory granules of vasopressin neurons.

The intracellular retention signal of GLUT8 has been characterized and found to contain the consensus sequence [D/E]XXXL[L/I] that represents a late-endosomal/lysosomal sorting motif. The DEXXXLL sorting signal of GLUT8 interacts with the adaptor proteins AP-1 and AP-2 and controls trafficking of the protein. Interestingly, both GLUT8 and GLUT12 contain a [D/E]XXXL[L/I] sorting signal; however, the exact composition of the XXX residues seems to fine-tune the routing and trafficking of the two proteins.

4.14.1. GLUT8 physiology

Mouse Models of GLUT8 deficiency

GLUT8 knockout mice appear healthy and exhibit normal growth, body weight development and glycemic control, indicating that GLUT8 does not play a significant role in maintenance of whole body glucose homeostasis. Offspring distribution from heterozygous matings indicated a deviation from the expected Mendelian distribution regarding birth of GLUT8 homozygous animals. This observation was attributed to a decreased sperm motility of GLUT8 deficient spermatocytes that is associated with lower ATP levels and a reduced mitochondrial membrane potential, while number and survival rate of spermatozoa is unchanged. The reduced amount of homozygous GLUT8 offspring is not related to impaired preimplantation embryo development – as might have been suggested by antisense studies in embryos-since mating of knockout mice produced viable, normally developing offspring in numbers comparable to those of a wildtype intercross. GLUT8 deficiency is associated with behavioural alterations indicating a significant physiological role for this isoform in the central nervous system. GLUT8 deficient mice have an increased proliferation of hippocampal cells and behavioral tests show increased arousal, a tendency to altered grooming, and a reduced risk assessment in those animals. However, despite the in depth characterization of the cell biology of GLUT8 and its physiology *in vivo* based on knockout mice, the function of the protein remains thus far unknown.

4.15. GLUT10 *(SLC2A10)*

GLUT10 has been cloned from human liver cDNA by 3′ and 5′ RACE PCR based on an EST sequence that was identified via a homology search with known GLUT protein sequences. GLUT10 mRNA is present in the human heart, lung, brain, liver, skeletal muscle, pancreas, placenta and kidney. Expression of GLUT10 is also detected in human and mouse white adipose tissue as well as human and mouse adipocyte cell lines SGBS and 3T3L1, respectively. One remarkable structural feature is the absence of the PESPR motif just after helix 6 that is conserved for all the GLUT isoforms. Heterologous expression of GLUT10 in *Xenopus laevis* oocytes demonstrated 2-DG transport with high affinity (K_m ~0.3 mM). Uptake of 2-DG can be competed with galactose and glucose and inhibited with phloretin.

Although no localization study has been performed for GLUT10 clearly demonstrating a plasma membrane or intracellular localization as seen for other Class III family members, immunocytochemical studies indicated an intracellular localization for GLUT10 under steady-state conditions. The presence of the potential internalization motif YSRI at the C terminus of the transporter supports these findings.

GLUT10 is located at the chromosomal region 20q12-13.1, a susceptibility locus that has been linked to type 2 diabetes in Caucasian Americans. Therefore, there has been particular interest in GLUT10 as a potential candidate or susceptibility gene involved in the disease. However, polymorphisms in GLUT10 were not associated to type 2 diabetes in Caucasian Americans, Danish, Finns, and Taiwanese populations.

4.15.1. GLUT10 physiology

GLUT10 and Arterial tortuosity syndrome (ATS, OMIM# 208050) in Humans

Deficiency for GLUT10 in humans has been found to be associated with ATS, a rare autosomal recessive connective tissue disease that is characterized by widespread arterial involvement with elongation, tortuosity, and aneurysms of the arteries. Homozygous mutations (deletion, non-sense, and missense) for GLUT10 were found in six families with ATS. It is currently unknown how loss of GLUT10 leads to this connective tissue disorder [33].

Mouse models of GLUT10 deficiency

Two groups reported the phenotypic analysis of mice with amino acid substitutions G128E or S150F in GLUT10 [34, 35]. Both substitutions are located in exon2 of GLUT10 and are conserved among the rodent and human protein. Based on predictions, the substitutions G128E and S150F were expected to interfere with the helix structure of transmembrane regions 4 and 5, respectively. The mice strains were generated after screening a mutant mouse library that was based on N-ethyl-N-nitrosurea (ENU) mutagenesis in healthy C3HeB/FeJ males. Callewaert et al. [34] did not report any of the vascular, anatomical, or immunohistological abnormalities as encountered in patients with ATS. Both mutant strains appear normal at birth, gained weight appropriately and survived to adulthood. The animals showed normal heart rhythm, heart structure, and ventricular function. No specific arterial tortuosity, stenosis, dilatation, or aneurysm in cerebral vessel pattern was noted. However, histopathology revealed thickening

and an irregular vessel wall shape of arteries with increased elastic fibers. Furthermore, the animals displayed endothelial hypertrophy and disarranged elastic fibers that resulted in disruption of internal elastic lamina in the aorta. Neither group analyzed whether the mutations caused any dysfunction or loss of the target protein, therefore the reported phenotype of those mice remains inconclusive with respect to GLUT10 function.

4.16. GLUT12 *(SLC2A12)*

GLUT12 was identified by 5′ and 3′ RACE-PCR from the human breast cancer cell line MCF-7. Strong GLUT12 expression is found in ductal cell carcinoma *in situ* when compared to benign ducts of breast cancer tissues. GLUT12 is mainly expressed in skeletal muscle, heart, small intestine, adipose tissue, and prostate. GLUT12 shows glucose transport when functionally characterized in *Xenopus laevis* oocytes that can be competed with fructose, galactose, 2-DG, and cytochalasin B. The transport activity of GLUT12 has been demonstrated to be affected by pH and in other reports by sodium, indicating its activity might be directly or indirectly coupled to an electrochemical gradient.

As a Class III isoform GLUT12 also contains a dileucine motif – both at the N-and C-terminal ends of the protein. Furthermore, the transporter does not contain GLUT12 contains a hydrophobic residue in position 314 which has been implicated in case of the isoleucine for GLUT2, 5, and 7 with the capability of fructose transport. This structural feature goes along with the substrate specificity of GLUT1. Endogenous as well as overexpressed GLUT12 protein localizes to intracellular compartments as well as to the plasma membrane in various cell lines. The N-terminal dileucine signal of GLUT12 is similar to that of GLUT8 which is the [DE]XXXL[LI] consensus sequence that represents a late endosomal/lysosomal sorting signal. However, GLUT12 does not colocalize with GLUT8, but rather resides in the Golgi network and at the plasma membrane. Plasma membrane associated GLUT12 is not endocytosed, which indicates the absence of a continuous cycling mechanism for GLUT12.

The phenotypic characterization of mice deficient in GLUT4 indicates the presence of a second transporter that facilitates insulin-stimulated glucose transport. Due to its tissue expression and its biological characteristics, GLUT12 was studied for its ability to respond to insulin-induced plasma membrane translocation of the protein. Indeed, in human skeletal muscle insulin induces an increase in plasma membrane GLUT12 which is comparable to the insulin-stimulated GLUT4 translocation. Although GLUT12 expression in skeletal muscle is unaltered under pathophysiological conditions such as of obesity and type 2 diabetes these data imply that an additional transporter is expressed in human muscle that is insulin-responsive in a PI3-kinase dependent manner. Mice with transgenic overexpression of GLUT12 display increased insulin sensitivity in insulin-sensitive tissues, while basal (non-stimulated) glucose uptake into adipose tissue and skeletal muscle was unaffected [36] indicating that GLUT12 might contribute to insulin-stimulated glucose uptake in those tissues, an effect not observed in heart muscle. Besides its potential importance for substrate transport in insulin-sensitive tissues, GLUT12 also appears relevant in prostate and breast cancer. However, the physiological role and relevance for GLUT12 remains elusive [37].

4.17. GLUT13; HMIT *(SLC2A13)*

Screening of public expressed sequence databases with the GLUT8 protein sequence identified a rat EST clone that allowed cloning of the rat and human HMIT (*SLC2A13*) cDNAs from spleen and frontal cortex cDNA libraries. Despite low level expression in adipose tissue and kidney, HMIT is predominantly expressed in the brain, with high expression found in hippocampus, hypothalamus, cerebellum and brainstem.

The HMIT amino acid sequence contains all motifs known to be important for glucose transport activity. As for other Class III GLUT family members, HMIT is restricted to an intracellular location. Functional characterization of the protein in *Xenopus laevis* oocytes and in mammalian cells has been possible through the introduction of various mutations that yielded significant plasma membrane expression. Surprisingly, no sugar transport activity has been found for HMIT. Instead, HMIT has been identified as a H^+-coupled myoinositol symporter with a K_m of about 100 μM. More recently, HMIT has been shown to transport inositol-3-phosphate (IP3). HMIT is inhibited by the common GLUT inhibitors phloretin, phlorizin and cytochalasin B, although at high concentrations. Translocation of HMIT to the plasma membrane has been demonstrated to occur in PC12 cells or primary neurons upon depolarization or protein kinase C (PKC) activation resulting in functional HMIT at the plasma membrane evidenced by increased myoinositol uptake in those cells. However, those initial findings were not reproduced by other groups leaving uncertainty about a stimulus that induces plasma membrane translocation of the transporter. In the brain, myo-inositol serves as the precursor for phosphatidylinositol, a key regulator for various signaling pathways. Dysregulation of the phosphatidylinositol signaling has been implicated in psychiatric illness such as bipolar disorder. Standard therapies (lithium, valproic acid and carbamazepine) alter neuronal growth cone morphology, a phenotype that is reversed by extracellular myo-inositol. Because of its predominant expression in the brain compared to two other myo-inositol transporters that are sodium-coupled (SMIT1 and SMIT2), interest has been raised as to whether HMIT might play a role in the regulation of myo-inositol/phosphatidylinositol physiology in neurons. Mice deficient for the transporter demonstrated that HMIT is not involved in the neuronal transport of inositol from the extracellular environment.

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