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SGLT2 Inhibitors: Physiology and Pharmacology

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Abstract: SGLTs are sodium glucose transporters found on the luminal membrane of the proximal tubule, where they reabsorb some 180 grams (one mole) of glucose from the glomerular filtrate each day. The natural glucoside phlorizin completely blocks glucose reabsorption. Oral SGLT2 inhibitors are rapidly absorbed into the blood stream where they remain in the circulation for hours. On glomerular filtration, they bind specifically to SGLT2 in the luminal membrane of the early proximal tubule to reduce glucose reabsorption by 50-60%. Because of glucose excretion, these drugs lower plasma glucose and glycosylated hemoglobin levels in patients with type 2 diabetes mellitus. The drugs also protect against heart and renal failure. The aim of this review is to summarize what is currently known about the physiology of renal SGLTs and the pharmacology of SGLT drugs.

Disclosures: The author has no current disclosures to report, but acknowledges that in the past, 2006-2015, has been a consultant on SGLTs for various pharmaceutical companies, including Boehringer Engelheim.

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SGLT2 Inhibitors: Physiology and Pharmacology

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ABSTRACT

SGLTs are sodium glucose transporters found on the luminal membrane of the proximal tubule, where they reabsorb some 180 grams (one mole) of glucose from the glomerular filtrate each day. The natural glucoside phlorizin completely blocks glucose reabsorption. Oral SGLT2 inhibitors are rapidly absorbed into the bloodstream where they remain in the circulation for hours. On glomerular filtration, they bind specifically to SGLT2 in the luminal membrane of the early proximal tubule to reduce glucose reabsorption by 50-60%. Because of glucose excretion, these drugs lower plasma glucose and glycosylated hemoglobin levels in patients with type 2 diabetes mellitus. The drugs also protect against heart and renal failure. The aim of this review is to summarize what is currently known about the physiology of renal SGLTs and the pharmacology of SGLT drugs.

Introduction

Rather unexpectedly to endocrinologists, SGLT2 transporters in the kidney have become a major target for the treatment of type 2 diabetes mellitus (T2DM). As a background, the kidneys normally filter 120-180 grams (0.67 – 1 mole) of
glucose from plasma every 24 hours, and less than half a gram (3 m-moles) is excreted in the urine. Homer Smith and his colleagues reported in 1933 that the reabsorption of the filtered load of glucose was completely inhibited by intravenous phlorizin (phloretin-2’-β-D-glucopyranoside), a naturally occurring plant glucoside [1]. Fast forwarding half a century, Ralph DeFronzo and his colleagues further found that “phlorizinising” diabetic rats diminished their hyperglycemia and restored their insulin sensitivity, suggesting that phlorizin could be used as a therapy for T2DM [2]. Oral phlorizin was not considered to be a viable therapy owing to its hydrolysis by intestinal lactase, and the diarrhea caused by inhibition of intestinal SGLT1.

The pharmaceutical industry set its sights on developing an oral SGLT2 inhibitor to treat T2DM after the cloning and characterizing of the intestinal and renal SGLTs in 1987 - 1992 [3-10]. This was encouraged by reports that familial renal glucosuria (FRG OMIM 233100) is a benign disorder [11]. The initial effort was pioneered by Kenji Tsujihara at Tanabe Seryaku Co Ltd in Japan [12], who tested oral phlorizin derivatives on renal glucose excretion in rodents that culminated in the prodrug T-1095 [13]. Other drug houses in Japan, the USA and Europe rapidly followed (see, for example, [14-16], resulting in three high-affinity SGLT2 drugs, dapagliflozin, canagliflozin, and empagliflozin, approved in 2013-2014. This class of drugs are sometimes referred to as Gliflozins.
Common features of these drugs are that they are aryl-C-glucosides (Figure 1) that have low Nano molar affinities for SGLT2, and a high selectivity over SGLT1 (Table 1). Another key to their success is that SGLT2 is found on the luminal membrane of the early segments of the renal proximal tubule, and virtually nowhere else in the body. For these reasons, it was reasonable to expect that these drugs would not encounter adverse side-effects.

SGLT Biology

SGLT1 and SGLT2 are members of the SLC5 gene family, a subdivision of an ancient superfamily of sodium cotransporters [8-10, 17]. SGLT1 (SLC5A1) is predominantly expressed the small intestine, and to a lesser extent the kidney cortex, while SGLT2 (SLC5A2) is virtually restricted to the renal cortex. The genes code for near identical proteins with ~670 amino acids organized in 14 transmembrane helices. A core of 10 TM helices coding for the glucose, and sodium binding sites are shared with other sodium transporters in bacteria and animals. SGLT2 is located solely in the luminal membrane of the S1 and S2 segments of the proximal tubule, and SGLT1 in the luminal membrane of the S3 segment [18]. In the intestine SGLT1 is restricted to the brush border membrane of mature enterocytes [8, 18].

The functional properties of both transporters have been documented in studies of SGLT1 and SGLT2 expressed in heterologous expression systems.
Table 1 summarizes the results obtained in cultured cells at 37°C. Both sodium glucose cotransporters show a similar affinity for glucose, $K_m$ 2 and 5mM, and similar turnover numbers of 30-60 s$^{-1}$ (the number of complete kinetic cycles per second). The major differences are in the number of sodium ion needed to drive glucose transport, the Na$^+$/glucose coupling ratio is 2 for SGLT1 and 1 for SGLT2, and the sugar and inhibitor selectivity.

Figure 1 shows the chemical structure of phlorizin and three of the first inhibitors approved for the treatment of T2DM. All are glucosides, but empagliflozin ((Jardiance), dapagliflozin (Forxiga) and canagliflozin (Invokana) are aryl-C-glucosides that are not cleaved by lactase. These inhibitors are more potent against SGLT2 than SGLT1 with inhibitors constants in the range of 1 to 12 nano-molar, and are more selective for SGLT2, 2,700-fold for Jardiance (Table 1). This difference in potency between SGLT2 and SGLT1 must be due to differences in structure of the aglycones, but this has not been rigorously addressed. However, subtle differences in the structure of aglycones determine whether they are SGLT1 substrates or inhibitors [19]. Although the SGLT2 drug inhibitor constants are quite similar, the oral clinical dose is 100-300 mg for Invokana but only 10 -25 mg for Jardiance and Forxiga.

The distribution of SGLTs in human subjects has been limited to gene expression and immunohistochemistry on post-mortem and/or surgically resected
tissues. However, these methods do not provide information about their functional activity in tissues. To remedy this we have turned to Positron Emission Tomography (PET) with a specific, non-metabolized SGLT tracer, Me4FDG ($\alpha$-methy-4-[F-18]-fluoro-4-deoxy-D-glucopyranoside), in both animal and human subjects[20-22]. PET is a non-invasive imaging method with high spatial (2 mm) and temporal resolution (seconds) to follow F-18 distribution in living subjects [23]. Me4FDG is transported by SGLT1 and SGLT2, but not by facilitated glucose transporters, GLUTs [21]. Figure 2 shows the steady-state distribution of Me4FDG injected intravenously into two subjects, a control (A) and (B) a patient with Familial Renal Glucosuria (FRG, OMIM 2333100, [8, 11]. This male FRG patient has a homozygous SGLT2 mutation, Ala305Val, associated with a defect in renal glucose reabsorption resulting in the urinary excretion of 66 grams (370 m-moles)/1.73 m$^2$/24 hours [24].

In the control subject, accumulation of Me4FDG is observed in the renal cortex, regions of the liver, with modest accumulations in skeletal muscle and testis. None is found in the brain or the urinary bladder, the former is due to the lack of SGLTs in the blood brain barrier, and the latter is due to the reabsorption of Me4FDG from the glomerular filtrate by SGLTs in the proximal tubule. A similar distribution is seen in the FRG patient with the exception that in the kidney Me4FDG is shifted from the cortex towards the renal pelvis, and is excreted into
the urinary bladder. The time course of Me4FDG excretion into the bladder of the FRG patient is shown in Figure 3 relative to that for a control subjects with or without treatment with Jardiance (empagliflozin). Me4FDG excretion for the FRG patient reached 3.5% of the injected dose in 75 minutes compared with none for the control, and 7% after treatment with Jardiance. The SGLT2 inhibitors also inhibited Me4FDG reabsorption in mouse microPET studies [25].

To distinguish between SGLT1 and SGLT2 in PET studies we developed a [18-F]-labelled SGLT2 inhibitor, 4-[18-F]fluoro-dapagliflozin, F-DAPA, where 18-F replaces the -OH group on C#4 in the pyranose ring [25]. In rodent, i.v. injection results in rapid F-DAPA binding to the kidney that is displaced after i.v. dapagliflozin or phlorizin. The specific binding of F-DAPA to the whole kidney was 6X-higher than non-specific binding, and no specific binding was observed for other parts of the body, brain, heart, skeletal muscle, liver or salivary glands. In human subjects, Jardiance significantly reduced the modest Me4FDG uptake into liver and skeletal muscle, but not the heart (unpublished).

The time course of F-DAPA distribution in the rat is shown in Movie 1 [25]. After i.v. injection there was a rapid accumulation of F-DAPA in the renal cortex that approached a steady state in 20 minutes. Following i.v. injection of dapagliflozin (or phlorizin, not shown) there was a rapid displacement of F-DAPA from the kidney cortex, reabsorption of the tracer into blood and excretion into the
intestine. Of particular note, there was no excretion of F-DAPA into the urinary bladder, before or after displacement with dapagliflozin or phlorizin. The mechanisms of F-DAPA reabsorption from the glomerular filtrate and secretion by the liver are unknown. Similar F-DAPA PET experiments were conducted on human subjects, but these were not successful owing to a very rapid metabolism of the tracer.

The location of specific F-DAPA binding in mouse kidney was refined by autoradiography (Figure 4). In this experiment, F-DAPA was injected intravenously, the kidney was harvested after 60 minutes, and the distribution of F-DAPA was examined in thin frozen sections [25]. As shown for the whole kidney (Figure 4A), F-DAPA binding was restricted to the outer cortex, and at higher magnification to tubules close to the glomeruli (Figure 4B & C). This distribution of F-DAPA binding mimics SGLT2 gene expression and SGLT2 immunocytochemistry in the mouse, rat and human kidneys, i.e. in S1 and S2 segments of the proximal tubule [6, 18].

The results shown in the movie, Figures 2, 3 and 4, are explained by the activity of SGLTs in the reabsorption of glucose in the proximal tubule, specifically SGLT2. Me4FDG micro-PET experiments on wild-type, SGLT1-null and SGLT2-null mice confirmed the importance of SGLT2 and SGLT1 in renal glucose reabsorption, and SGLT1 in intestinal absorption. The lack of SGLT
activity in mouse heart, liver, and skeletal muscle may be due to a species
difference from human [21, 26].

**Glucose reabsorption**

The current understanding of glucose handling by the human kidney is that
after glomerular filtration glucose virtually all the filtered load is reabsorbed in
healthy subjects ([Figure 5A](#)). Reabsorption occurs in the proximal tubule as no
glucose is found at the end of straight S3 segment [27]. The SGLT2 inhibitors are
believed to be freely filtered, as for phlorizin [28], where they then bind to SGLTs
in the luminal membrane of the epithelial cells in the proximal tubule. In the dog
kidney, the clearance of [3-H]-phlorizin was identical to that for creatinine, and
none was excreted in the urine.

The SGLT2 drugs, even at saturating plasma concentrations only inhibit
glucose reabsorption by 40-50% ([29]. The remaining amount in the glomerular
filtrate is largely salvaged by SGLT1 activity in S3. This has been confirmed in
mice with knockouts of the SGLT1 and SGLT2 genes [21, 30], and it is also
evident in patients with SGLT2 “knock-out” mutations, where glucose excretion is
less than the filtered load (see [8]).

SGLTs are expressed in the luminal membrane of the proximal tubule,
SGLT2 in the S1/S2 segments ([Figure 5B](#)) and SGLT1 in the S3 segment. Glucose
uptake into the epithelia across the luminal membrane is driven by the Na⁺
electrochemical potential gradient across the membrane, and then glucose diffuses out into blood across the basolateral membrane through the facilitated glucose transporter GLUT2 [21]. Na\(^+\) that enter the cells through SGLTs is transported out across the basolateral membrane by Na\(^+\)/K\(^+\)-pumps. In the S1 and S2 the net result is that one Na\(^+\) is absorbed for every glucose molecule (and one anion to maintain electroneutrality), whereas in S3 two Na\(^+\) ions are absorbed for every glucose molecule. Given a glucose reabsorption of 120-180 grams (0.67 – 1 moles) of glucose per day, the stoichiometry of the SGLTs, glucose coupled sodium absorption only accounts for less 10% of the total sodium reabsorption in the proximal tubule. At clinical doses, the SGLT2 inhibitors only reduce total glucose absorption by some 50%, and so there is only a modest effect on urinary sodium excretion given the ability of the distal tubule to reabsorb the increased salt load.

**Mechanism of Na\(^+\) glucose transport**

Much more is known about the mechanism of glucose transport by SGLT1 than for SGLT2, simply due to the much higher activity of SGLT1 in heterologous expression systems such as Xenopus laevis oocytes [6, 8, 9]. This has been partially rectified by the discovery that co-expression of MAP17 increased SGLT2 expression in oocytes [31]. MAP17 (PDZK1IP1) is small protein expressed in the plasma membrane, endoplasmic reticulum, and Golgi apparatus of many cells types, and is involved in cargo transport between the Golgi and plasma membrane.
In the mouse proximal tubule, MAP17 is expressed in the brush border membrane of the S1 and S2 segments where it is involved in the endocytosis of the sodium phosphate cotransporter NaPi-IIa from the brush border into the trans-Golgi network [32]. MAP17 interacts indirectly with the cytoplasmic C-terminal domain of NaPi-IIa through a PDZ protein. However, there is no comparable C-terminal domain of SGLT2, but there is evidence that MAP17 may be a β-subunit of SGLT2 in the plasma membrane of the S1 and S2 segments [31, 33].

SGLT2 is robustly expressed in cultured mammalian cells at 37°C [34-37], there is no MAP17 in these cells [32, 33]. The kinetic properties of SGLT2 in cultured cells and oocytes are similar [35, 38], and those for HEK293T cells are summarized in Table 1.

The kinetics of SGLT2 and SGLT1 are very similar in that they are sodium cotransporters with an obligatory coupling of glucose and sodium transport that is specifically blocked by phlorizin. The glucose affinities are 5 and 2 mM for glucose, with maximum enzymatic turnover numbers of 30-60 s⁻¹. Such data refutes earlier claims that these are low affinity high capacity, and high affinity, low capacity, transporters. The major kinetic differences between SGLT2 and SGLT1 are: 1). Stoichiometry of Na⁺ and glucose transport (1 for SGLT2, and 2 for SGLT1); 2). Sugar specificity (galactose is transported by SGLT1 but rather poorly for SGLT2); 3). The requirement of SGLT2 for MAP17; 4). The high
selectivity of SGLT2 for inhibitors; and 5). High SGLT1 capacitive currents are evident for SGLT1 but not SGLT2 (these have been instrumental in developing kinetic models, see Figure 6).

A kinetic model for Na\(^+\) and glucose transport by SGLT2 is presented in Figure 6. This is largely based on the wealth of experimental biochemical, molecular, and biophysical data collected for SGLT1 (see [8, 17]. The transporter is represented as a double gated membrane protein that exists in a minimum of interconvertible five states (C1, C2, C3, C4, C6). The distribution of these depends upon the Na\(^+\) and glucose concentrations on each side of the membrane, and the voltage across the membrane. Under physiological conditions, with the extracellular Na\(^+\) and glucose concentrations of 145 m-equiv/l and 5 mM, and a membrane potential of -60mV, and the direction of Na\(^+\)/glucose is into the cell. In the kinetic scheme extracellular Na\(^+\) binds first to open the extracellular gate (C1 - C2) and following extracellular glucose binding external gate closes (C2 - C3). The intracellular gate then opens to permit Na\(^+\) and glucose to exit into the cytoplasm (C4 - C6). The intracellular release of ligands is presumed to be stochastic, unlike our original symmetrical model with a C5 state [8, 17, 39]. The final step is the transition of the inward to outward facing conformations (C6 –C1). The apo-transporter is charged, valance -1, and the major effect of voltage in on
the distribution between C6 and C1, and this is the origin the fast, transient SGLT1 capacitive currents in the absence of ligands [8, 40] [41].

The model accounts for the strict coupling between Na\(^+\) and glucose transport that is completely reversible - the direction and rate of transporter simply depends on the ligand concentrations on each side of the membrane and the membrane potential. In the case of SGLT1, the capacitive currents provide an entree into estimating the rate constants for the each step in the kinetic cycle, and determining the total number of transporters in the plasma membrane. This topic is beyond the scope of the present review, but interested readers are directed elsewhere (see[8, 17]. Suffice to say that a single set of rate constants account for SGLT1 kinetics.

**SGLT Inhibitors**

Phlorizin and other SGLT blockers are high affinity, competitive inhibitors. that are only active from the extracellular surface in the presence of Na\(^+\) [17, 37, 39, 42, 43]. Inhibition is reversible, but the OFF-time constants are slower for the specific SGLT2 inhibitors than phlorizin [15, 36]. Phloretin, the phlorizin aglycone, is a low affinity non-specific inhibitor of SGLTs [8]. This, together with the high affinity of phlorizin relative to glucose, has led to the view that the glucose moiety of phlorizin binds to the glucose binding site of SGLTs, and the phloretin binds at an adjacent site within 15 Å [44].
A molecular interpretation of inhibitor interactions with SGLTs is illustrated in Figure 7. Phlorizin and the SGLT2 drugs bind to the external surface of SGLTs in the outside open Na\(^+\) bound conformation. As shown in Figure 7A phlorizin enters with the glucose moiety approaching the glucose binding site, and the aglycone approaching an external vestibule. Once bound, the inhibitors lock the transporter in an outward facing conformation. Although binding is reversible, the OFF rate is slow due to the interactions the aglycone and the vestibule, and this accounts for the high affinity of phlorizin relative to glucose.

Structural models based on the atomic structure of the bacterial isoform, vSGLT [45, 46] provide additional insight into inhibitor binding [47]. The transporters have a core structure composed of 10 trans-membrane helices with a central sugar binding site (N75, H80, E99, K321, Y290, & W291), external (L84, F98, F453), and internal (Y290) gates, and a Na\(^+\) sodium binding site centered around S393 (Figure 7B). All these residues are conserved between SGLT2, SGLT1 and vSGLT (See [8, 17], and their functional importance in SGLT1 has been confirmed by studies of cysteine mutants[48] [40, 49, 50]. Similar studies with SGLT2 have not yet been carried out.

The precise location of the inhibitor-binding site shown in Figure 7B-D. This has been determined by molecular docking studies on SGLT1 and SGLT2 structural models, and verified for SGLT1 by mutations of coordinating residues
The glucose moiety of phlorizin binds in the glucose binding site, and the aglycone is found in an external vestibule bounded by the external ends of TM1, TM2, TM3 and TM10. Phloretin overlaps with the aglycones. Mutation of SGLT1 side-chains predicted to interact with inhibitors, e.g. F101, reduces phlorizin binding by 200-fold without influencing glucose transport. In SGLT2 the bound inhibitors are capped by H80, F98, and H268 on the long extracellular loop connecting TM5 and TM6. This cap forms an aromatic cage around the central aromatic ring of the aglycone tail in SGLT2 but not SGLT1 and so may contribute to the differences in selectivity of SGLT inhibitors.

Dapagliflozin binding also involves the glucose moiety binding to the glucose site, and the aglycone tail overlaps with the phlorizin tail in the external facing vestibule, but with the minor difference that dapagliflozin lies deeper the in the pocket. Simulations of inhibitor binding also indicate that the external gate partially closes after binding due to a rigid rotation of TM9, an inward bend of the outer half of TM10, and the inward movement of the extracellular loop between TM5 and TM6. These helical rearrangements have been observed in real time by fluorescence measurements on fluorophores covalently bound within the sugar binding site [17, 50].

Unfortunately, the structural models do not account for the selectivity of inhibitors for SGLT2 over SGLT1 (Table 1). Nevertheless, they do account for the
sidedness of inhibitor binding as cytoplasmic phlorizin and SGLT2 drugs fail to gain access to the sugar binding site due to steric clashes [47].

To date, there are few reports of SGLT1 specific inhibitors, but there has been some interest in the pharmaceutical companies in dual SGLT1/SGLT2 inhibitors, e.g. sotaglifozin (Table 1). The objectives were to control blood glucose, especially in Type 1 diabetes, by reducing intestinal absorption, and inhibiting renal SGLT2 and SGLT1 (see [51].

**SGLT2 inhibitors in Heart and Kidney Failure**

An unanticipated outcome of the Jardiance cardiac safely trial was that diabetic patients treated with Jardiance showed a remarkable reduction in hospitalization and mortality for heart failure [52]. This cardio-protection is a SGLT2 inhibitor class effect, and it persists in non-diabetic patients [53-55]. SGLT2 inhibitors are now approved for the treatment of heart failure. While there is no direct evidence for a relationship between the kidney and heart in the effects of SGLT2 inhibitors, there is rodent data suggesting and off-target effect on cardiac sodium proton exchangers (NHE1). The most compelling experiments come from Coert Zuurbier’s group, who tested the effect of SGLT2 inhibitors on the intracellular Na\(^+\) and Ca\(^{++}\) concentrations in isolated cardiac myocytes, and the onset of contraction in an ischemic mouse heart model [56-58]. At pharmacologically relevant doses, 0.25 to 1 uM, the inhibitors produce fast
lowering of the intracellular Na and Ca concentrations that are consistent with inhibition of NHE1. Indirect support was achieved by molecular docking studies of the drugs with a homology model of NHE1. The glucose moiety of the drugs reportedly binds to the Na\(^+\) binding site and the aglycone to the adjacent extracellular vestibule (our preliminary studies also find vestibule leading canonical glucose binding site, Bisignano, P., Grabe, M. & Wright, E.M. unpublished). The effect of SGLT2 inhibitors on cardiac NHE1 are consistent with the current thinking that elevated myocyte Na and Ca intracellular concentrations are drivers of heart failure and cardiac death. Whether or not these rodents studies will translate into patients in unknown, but I do note that clinical trials on other potent NHE1 inhibitors have not been successful, e.g. cariporide GUARDIAN and EXPEDITION TRIALS (see [59].

Following the cardiac safely trials, it emerged that the SGLT2 inhibitors produced a comparable reduction in the progression of chronic kidney disease in both diabetic and non-diabetic patients (see [60, 61]. The underlying mechanisms are not understood, as it is unclear at what points the inhibitors act in the natural progression of kidney disease. In particular, the potential importance of renal sodium protein exchangers, principally NHE3 in the proximal tubule, has yet to be clarified.
Summary

Oral SGLT inhibitors are rapidly absorbed from the GI tract into blood, where they bind to plasma proteins and circulate throughout the vascular tree with a half life of 10-14 hours. The free drugs are filtered from plasma at the glomerulus where they then bind to the luminal membrane of the proximal tubule. The SGLT2 inhibitors bind to SGLT2 in the luminal membrane of the early (S1 and S2) segments of the nephron, where they may block up to 60% of glucose reabsorption. Glucose that escapes reabsorption in S1 and S2 segments is normally salvaged by SGLT1 in the late proximal tubule (S3). The non-specific inhibitor phlorizin binds to SGLT2 in S1 and S2, and to SGLT1 in S3 causing complete inhibition of glucose reabsorption. In mice, this has been confirmed using SGLT2 and SGLT1 null-mice, and may be inferred in humans with “knock-out” mutations of SGLT2 (Familial Renal Glucosuria). There have been efforts to develop dual SGLT2/SGLT1 inhibitors, e.g. sotagliflozin (Table 1), but these have not yet gained FDA approval for the treatment of diabetes. Considerable progress has been made in understanding the mechanism of action of SGLT inhibitors at a molecular level, but additional work remains to account for their selectivity for SGLT2 over SGLT1.
There is a remarkable ability of SGLT2 drugs to lower the progression of heart and renal disease, and, at least for heart disease, this may be due to an off-target effect on the cardiac sodium proton exchanger NHE1.

Disclosures
The author has no current disclosures to report, but acknowledges that in the past, 2006-2015, has been a consultant on SGLTs for various pharmaceutical companies, including Boehringer Engelheim.

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Author Contributions
Ernest Wright: Conceptualization; Investigation; Writing - original draft
References


TABLE 1

Kinetics of human SGLT2 and SGLT1

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<tr>
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<td>IC₅₀ (nM)***</td>
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All data collected on human SGLT2 and SGLT1 clones expressed in cultured cells at -60 mV and 37°C [35, 36], except for the turnover of SGLT2 that was in *Xenopus laevis* oocytes at 22°C (* [38]). IC₅₀ values are for inhibition of aMDG uptakes (** [15]; and *** [62].
FIGURE LEGENDS

Figure 1. The chemical structure of phlorizin, empagliflozin (Jardiance), dapagliflozin (Forxiga) and canagliflozin (Invokana). Note that phlorizin is an O-glucoside that can be hydrolyzed by the intestinal brush border lactase, whereas the aryl-glucosides are not. The selectivity of these SGLT inhibitors is given in Table 1. Modified from Wright, 2013[9].

Figure 2. Distribution of SGLT activity as visualized by Me4FDG PET in a control subject (A) and a patient with familial renal glucosuria, FRG (B). Me4FDG is a specific substrate for SGLTs [21](Sala-Rabanal et al 2016). Each subject was injected intravenously with 10 mCi of Me4FDG and whole-body Positron Emission Scans (PET) were collected 60 minutes later. The scans were normalized to the highest region of Me4FDG uptake (red). In both, there was no uptake into brain (or lungs) owing to the absence of SGLTs in the blood brain barrier, but there was modest activity in kidney, skeletal muscle, and testis. In the control, there was no Me4FDG in the urinary bladder, while Me4FDG was excreted into the bladder in the FRG patient. Taken, in part, from Wright et al 2011 [8]; and Wright, Halabi, Hirayama, Kepe, Huang & Barrio (In preparation).
Figure 3. The time course of Me4FDG excretion into the urinary bladder in control subject, a control treated with oral Jardiance (Empagliflozin), and the FRG patient sown in Figure 2. In each, 10 mCi of Me4FDG (370 MBq) was injected intravenously and a dynamic scan over the abdomen was taken for up to 75 minutes. The total activity in the urinary bladder in MBq is plotted against time. The plot + Jardiance is for a control subject given a 25 mg oral dose of Jardiance 2 hours prior to the PET scan. Wright, Ghezzi, Kepe and Barrio, unpublished.

Figure 4. The distribution of SGLT2 in the mouse kidney as determined by 4-[\(^{18}\text{F}\)]fluoro-dapagliflozin (F-Dapa) autoradiography. A mouse was injected intravenously with 4mCi of F-Dapa and the kidney was harvested after 60 minutes and prepared for autoradiography. The figure shows the grain density and H and E staining for a section of the whole kidney (A), a higher magnification view of the outer cortex (B), and a higher magnification view of tubules surrounding a single glomerulus. The scale bars are 1 mm in A, and 100 um in B and C. These result shows that SGLT2 is confined to the outer cortex in the mouse kidney, specifically to some tubules surrounding the glomeruli. Taken from Ghezzi et al 2017[25].

Figure 5 A drawing showing the organization of a cortical nephron (A), and a model for glucose reabsorption from the glomerular filtrate by the epithelium
lining the S1 and S2 segments of the proximal tubule (B). Glucose is freely filtered from plasma by the glomerulus, and then completely reabsorbed in the proximal tubule (highlighted in green). No glucose is detected in tubular fluid by the end of the proximal tubule. A major fraction of the filtered glucose is reabsorbed in S1 and S2 by SGLT2, and then any remaining glucose in S3 is mopped up by SGLT1. B. Glucose is absorbed from the tubules by SGLTs in the luminal membrane, driven by the sodium electrochemical potential gradient. Glucose accumulated in the epithelium then diffuses into blood through GLUT2 in the basolateral membrane. The sodium gradient across the luminal membrane is maintained by the Na\(^+\)/K\(^+\)-pump in the basolateral membrane. The net result is the reabsorption of glucose and salt from the glomerular filtrate. Major differences between SGLT2 in S1/S2 and SGLT1 in S3 is that SGLT2 drugs specifically block glucose transport in S1 and S2, whereas phlorizin blocks transport in S1, S2, and S3.

Figure 6. A model for glucose transport by SGLT2. The transporter in the plasma membrane is shown as a double-gated protein where in the absence of ligands both gates are closed. The binding of external sodium (green circle) to the Na2 site opens the external gate to allow external glucose (yellow hexagon) to bind. After glucose is bound, the external gate closes after sugar binding to trap the sugar in
the middle of the protein before the inner gate opens to permit Na\(^{+}\) and glucose to exit into the cytoplasm in one-step. Finally, the transporter returns to the starting position. The net result is strict coupling of one sodium and one glucose transport across the membrane during one transport cycle.

Figure 7. Inhibitor binding to SGLT2. A. External phlorizin approaching the glucose binding site binding in the sodium bound outward open conformation. The glucose moiety of phlorizin binds to the glucose binding pocket and the aglycone moiety is stuck in the outer vestibule. B. A structural model of SGLT2 with glucose in the occluded binding site, and the location of the Na2 binding site. From Ghezzi et al 2018 [27]. The protein has a core structure of 10 transmembrane helices (TM 10 not shown for clarity) with glucose. We propose that the external gate formed by residues L84, F98, F453, and the extracellular helical loop EL8b. Glucose is bound through H-bonds to N75, H80, E99, K321, and W291, and hydrophobic binding to Y290. These residues are conserved in SGLT1. Y290, at least in part contributes to the internal gate. C. The SGLT phlorizin binding site in the center of the membrane protein. The glucose moiety binds to the glucose binding site, and the aglycone occupies an outer vestibule bounded by the external ends of TM1, TM2, TM3 and TM10 [17]. Y290, the internal gate is highlighted in
yellow. D. An atomic model of phorizin and dapagliflozin bound to SGLT2 [47]. This shows the superimposition of the glucose moiety of dapagliflozin and phlorizin with glucose in the sugar site, and the superimposition of the aglycones in the external vestibule. H80, F98 and H268 form a cage above the inhibitor (H268 is replaced by D268 in SGLT1).

**Movie 1** (See supplemental file) The time course of the distribution of F-DAPA in abdomen of the rat. Following i.v. delivery there was rapid distribution throughout the vascular tree, peaking at 2 minutes (3% I.D. per gram of plasma) before falling to a quasi-steady value of 0.35 % I.D. per gram). In contrast, there was rapid binding to the kidney cortex, at 5.5 % I.D. per gram, which is displaced by i.v dapagliflozin (and phlorizin, not shown). No F-DAPA appeared in the urine throughout the experiment, but it was transiently found in the renal vein, and then the liver and small intestine. Taken from Ghezzi et al 2017[25].
Figure 1

A) Phlorizin

B) Empagliflozin

C) Dapagliflozin

D) Canagliflozin
Figure 3

Me4FDG in bladder (MBq) vs. t (min)

- Control
- FRG
- +Jardiance

Graph showing the increase in Me4FDG in the bladder over time for different conditions.