Biophysical Characteristics of the Pig Kidney Na⁺/Glucose Cotransporter SGLT2 Reveal a Common Mechanism for SGLT1 and SGLT2*

Bryan Mackenzie‡, Donald D. F. Loos§, Mariana Panayotova-Heiermann, and Ernest M. Wright

From the UCLA School of Medicine, Department of Physiology, Los Angeles, California 90095-1751

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The Na⁺-dependent, low affinity glucose transporter SGLT2 cloned from pig kidney is 78% identical (at the amino acid level) to its high affinity homologue SGLT1. Using two-microelectrode voltage clamp, we have characterized the presteady-state sugar-evoked currents as a function of external Na⁺ and α-methyl-D-glucopyranoside (αMG) concentrations, which were consistent with an ordered, simultaneous transport model in which Na⁺ binds first. Na⁺ binding was voltage-dependent and saturated with hyperpolarizing voltages. Phlorizin was a potent inhibitor of the sugar-evoked currents (Kd, Na⁺ ~ 10 μM), and blocked an inward Na⁺ current in the absence of sugar. SGLT2 exhibited Na⁺-dependent presteady-state currents with time constants 3–7 ms. Charge movements were described by Boltzmann relations with apparent valence ~ 1 and maximal charge transfer ~ 11 nC, and were reduced by the addition of sugars or phlorizin. The differences between SGLT1 and SGLT2 were that (i) the apparent affinity constant (Kd,α) for αMG (~3 mM) was an order of magnitude higher for SGLT2; (ii) SGLT2 excluded galactose, suggesting discrete sugar binding; (iii) Kd,α for Na⁺ was lower in SGLT2; and (iv) the Hill coefficient for Na⁺ was 1 for SGLT2 but 2 for SGLT1. Simulations of the six-state kinetic model previously proposed for SGLT1 indicated that many of the kinetic properties observed in SGLT2 are expected by simply reducing the Na⁺/glucose coupling from 2 to 1.

In the proximal tubule of the kidney, reabsorption of filtered glucose at the apical membrane is mediated by one or more Na⁺/glucose cotransporters. The low affinity glucose transporter (SGLT2) (1), cloned from a pig kidney cell line (2), bears 78% identity at the amino acid level to its high affinity homologue SGLT1 (3), yet exhibits functional characteristics (1) that clearly distinguish it from SGLT1.

In the present study we examined the mechanisms of Na⁺/glucose cotransport via the pig SGLT2 transporter expressed in Xenopus oocytes. We determined the dependence of the presteady-state kinetics on Na⁺ and αMG concentrations, and examined presteady-state charge movements associated with SGLT2. We have also extended our analysis of the substrate selectivity of SGLT2, its cation activation, and the effects of phlorizin upon both the uncoupled and the sugar-coupled Na⁺ pathways through the transporter.

EXPERIMENTAL PROCEDURES

Stage V–VI oocytes from Xenopus laevis were injected with pSGLT2 cRNA as described previously (1). Electrophysiological experiments were performed using a two-microelectrode voltage clamp (1, 4, 5). Oocytes were superfused at 20–22 °C with experimental medium in which the Na⁺ concentration varied between 0 and 100 mM by equimolar replacement with choline or Li⁺. Test solutions (additionally containing sugars and/or phlorizin) were always washed out by superfusing the oocyte with substrate-free, choline chloride medium (pH 7.5). Sugar-evoked currents (I) were fitted to Equation 1, for which Imax is the current maximum, S is the substrate (sugar or cation) concentration, and Kd,α is the substrate concentration for half-maximal current. The Hill coefficient (nH), an empirical description of cooperativity, for S refers to the least number of molecules of S transported in each cycle and is, in general, the same as the coupling coefficient (n).

\[
I = \frac{I_{\text{max}} \cdot S^{nH}}{(K_{d,\alpha})^{nH} + S^{nH}} \quad \text{(Eq. 1)}
\]

Charge movements were obtained as the integral of the presteady-state currents and fitted to the Boltzmann relation (Equation 2) for which Qmax = Qdep - Qhyp (Qdep and Qhyp represent the charge Q at depolarizing and hyperpolarizing limits), z is the apparent valence, and V0.5 is the potential for 50% Qmax, F, R, and T have their usual thermodynamic meaning.

\[
Q - Q_{\text{hyp}} = \frac{1}{1 + \exp[(V0.5 - V)/(F \cdot R \cdot T)]} \quad \text{(Eq. 2)}
\]

Model simulations for SGLT2 (see Fig. 7) were performed as described for SGLT1 (6). The steady-state concentrations of each transporter state (Cj) were estimated using the King-Altman procedure (in Parent et al. (6), see Fig. A1 and Equations A14–A19). The steady-state currents and the kinetic parameters Kd,α and Imax were derived as described (Equations A20–A43) in Parent et al. (6), but with the empty transporter valence (zj) changed to -1, Na⁺-coupling (n) = 1, and internal concentrations [Na⁺]i = 10 mM and [αMG]i = 0. The steady-state current is given by Equation 3, for which the rate kdep describes the reaction step Cj -> Ci (see Fig. 7).

\[
I = -zC \cdot F[k_{\text{dep}} \cdot [C] - k_{\text{hyp}} \cdot [C]] \quad \text{(Eq. 3)}
\]

The presteady-state current (I) is given by Equation 4.

\[
I_t = -F[z_{\text{dep}} \cdot \alpha(k_{\text{dep}}[C] - k_{\text{hyp}}[\text{C}]_\alpha) + zC \cdot \delta(k_{\text{dep}}[C] - k_{\text{hyp}}[C])] \quad \text{(Eq. 4)}
\]

and was obtained by numerical integration of Equations A44–A49 in Parent et al. (6). The phenomenological constant α describes the fraction of the membrane electric field between bulk solution and the Na⁺-binding site at the external face, α is its internal equivalent, and δ is the fraction of the membrane field sensed by the empty binding site on the carrier during translocation; microscopic reversibility requires that α + α + δ = 1 (7). I was integrated over time to yield the...
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Simulated Q/Vm data. The slow (τ1) and fast (τ2) time constants of transient current decay are given by

\[ \tau_1 = \frac{0.5}{R_4 - W} \]  
(Eq. 5)

and

\[ \tau_2 = \frac{0.5}{R_4 + W} \]  
(Eq. 6)

where

\[ R_4 = k_{16} + k_{41} + k_{12} + k_{21} \]  
(Eq. 7)

and

\[ W = \sqrt{(R_4^2 - 4[k_{16}k_{41} + k_{23}k_{41} + k_{12}k_{21}])} \]  
(Eq. 8)

RESULTS

Currents Associated with pSGLT2—Following step-changes in membrane potential (V_m) from the holding potential (V_h) of −50 mV, we observed pSGLT2 transporter-mediated currents in oocytes (Fig. 1A) in the presence of Na⁺. After the onset of the pulse (left panel), the transient currents comprised two components, (i) a capacitive current (also observed in control-injected oocytes), which decayed with time constant \( \sim 0.7 \) ms, and (ii) a more slowly decaying current (time constant, 3–6 ms). At the end of the 100-ms voltage pulse, the “off” currents again included similar transients with opposite polarity to the “on” currents, before returning to the steady-state current at −50 mV. Following the addition of sugar (right panel) the transporter-mediated transients were attenuated (especially obvious from the off response) and there was a large increase in inward current. The glucose-dependent steady-state currents (at 20 ms) were roughly linear as a function of voltage between +50 and −150 mV (Fig. 1B). Phlorizin abolished the transporter-mediated presettlement-state and steady-state currents (not shown).

Steady-state aMG Kinetics—The sugar-evoked currents were measured as a function of extracellular sugar at fixed [Na⁺]_o (between 1 and 100 mM) in a single oocyte. I_max and K_0.5 were determined for αMG and Na⁺ as functions of [Na⁺]_o and [αMG], (Equation 1). K_0.5 increased with progressive Na⁺ replacement (Fig. 2A), and a similar relationship was observed for K_0.5 as a function of [αMG]_o (Fig. 2B). Whereas I_max was independent of [Na⁺]_o between 100 and 5 mM Na⁺ (Figs. 2C and 3C), I_max showed a marked dependence upon [αMG]_o (Figs. 2D and 3C), and fell by 90% as [αMG]_o was reduced from 20 to 5 mM.

Selected data were plotted as a function of V_m. At 100 mM Na⁺, K_0.5 was 3 mM and independent of voltage (Fig. 3A), whereas at 5 mM Na⁺, K_0.5 increased with depolarization. At −150 mV, K_0.5 (0.8 mM) was essentially independent of [αMG]_o (Fig. 3B); K_0.5 was relatively unchanged with depolarization in the presence of high αMG, but increased with depolarization at low αMG. The I_max/V_m relationship was independent of [Na⁺]_o (Fig. 3C). The relationship of I_max to V_m was similar at each [αMG]_o (Fig. 3D) however the magnitude of I_max fell markedly

FIG. 1. Currents associated with pSGLT2 expressed in a single oocyte, in the presence and absence of d-glucose, at 100 mM Na⁺. A. The oocyte was held at V_h = −50 mV (the holding current is indicated by the dotted horizontal line) and V_m was stepped to between +50 mV and −150 mV (in 20-mV increments) for 100 ms (for clarity, alternate voltage steps are omitted), first in 100 mM NaCl and 100 mM NaCl, and then in the presence of 20 mM glucose (+Glc, left panel) then in the presence of 20 mM glucose (+Glc, left panel) and (ii) a more slowly decaying current (time constant, 3–6 ms). At the end of the 100-ms voltage pulse, the “off” currents evoked currents were absent in choline chloride (not shown).

FIG. 2. Steady-state kinetics of pSGLT2-mediated Na⁺/αMG cotransport at −70 mV. The kinetic parameters K_0.5 and I_max were each derived for αMG and Na⁺ as a function of [Na⁺]_o and [αMG]_o, respectively, by fitting data to Equation 1 (error bars represent the error in the estimates). All data were derived from a single oocyte superfused with test solutions containing 0.2, 0.5, 1, 2, 5, 10, 20, or 50 mM αMG at each of 1, 5, 10, 20, and 100 mM Na⁺ (in random order). The results presented were all at a test potential (V_m) of −70 mV; however, the kinetics were qualitatively no different at any V_m between −150 and −30 mV. The same oocyte was used for measurement of presteady-state charge movements (Fig. 4B).

FIG. 3. Voltage dependence of the steady-state parameters for pSGLT2-mediated Na⁺/αMG cotransport. Selected kinetic data, all derived from the same experiment as in Fig. 2, were plotted as a function of membrane potential (V_m). A and C, αMG kinetics at 5 mM (●) and 100 mM (○) NaCl; B and D, Na⁺ kinetics at 1 mM (●), 5 mM (△), and 50 mM (■) αMG. Error bars represent the error in the estimates of kinetic parameters calculated using Equation 1. Dotted lines represent the corresponding predictions from the model simulation, using the rate constants given in Fig. 7.
**FIG. 4.** Presteady-state currents associated with pSGLT2. Presteady-state currents were recorded in pSGLT2 cRNA-injected oocytes in Na\(^+\) medium in the absence of sugar following step changes in \(V_m\) from the holding potential (\(V_h\)) of \(-50\) mV (as shown in the top panel). A, compensated membrane current records (with capacitive transient and the steady-state current subtracted; filtered at 100 Hz for display) from 3 ms after the voltage step, in 10 mM Na\(^+\). B, charge/voltage relationship at 10 mM Na\(^+\). The presteady-state currents were integrated with time and averaged for off and on currents; data are the mean ± S.E. from 6 determinations in a single oocyte (the same oocyte as in Figs. 2 and 3). The data were fitted to the Boltzmann relation (solid line, Equation 2), from which we determined \(V_{1/2}\) (the \(V_m\) for 50% charge transfer) to be \(-34\) mV. The fitted Boltzmann relation was then compared with the model prediction (dotted line, \(V_m\) was \(-28\) mV) using the rate constants determined for pig SGLT2 (Fig. 7). The predicted values of \(Q\) were normalized by setting \(Q_i\), i.e. \(Q\) at extreme depolarization \(V_m\) equal to the observed \(Q_o\). The \(Q/V_m\) relationship shifted to hyperpolarized \(V_m\) as \([Na^+]_o\) was decreased, as indicated by the dependence of \(V_{1/2}\) upon \([Na^+]_o\) (inset). To obtain estimates of \(V_{1/2}\) at 1 and 20 mM Na\(^+\), \(Q_{max}\) was constrained at 11.3 nC (the \(Q_{max}\) determined for 5 and 10 mM) since \(Q_{max}\) did not appear to change with \([Na^+]_o\). \(\Delta V_{1/2}\), (the change in \(V_{1/2}\) per 10-fold increase in \([Na^+]_o\)) was \(\approx -96\) mV (\(r = 0.99\)), as \([\text{aMG}]_o\) was reduced. The Na\(^+\):aMG coupling stoichiometry of 1:1 was inferred from the Hill coefficients (\(n_H\)) for Na\(^+\) and for aMG. At \(-70\) mV, \(n_H\) for Na\(^+\) activation of the 20 mM aMG-evoked currents was 1.3 ± 0.2, and that for aMG activation (100 mM Na\(^+\)) was 1.2 ± 0.2; \(n_H\) for Na\(^+\) did not vary with either \(V_m\) or \([\text{aMG}]_o\) (not shown).

**DISCUSSION**

A molecular basis for the kinetic heterogeneity of Na\(^+\)/glucose cotransport activity in renal brush-border membrane vesicles (8, 9) was established with the cloning of two distinct glucose cotransporters from kidney, SGLT1 (high affinity for glucose) and SGLT2 (low affinity). The amino acid sequence identity among the SGLT1 isoforms from rat, rabbit, and man exceeds 87% (10), and pig SGLT2 is 76% identical to pig SGLT1 (1). The residues which differ for SGLT2 but are conserved throughout the SGLT1 subfamily must account for the different characteristics of SGLT2. Several of the SGLT1 isoforms

**FIG. 5. Substrate selectivity for pSGLT2.** The substrate selectivity of pSGLT2 was investigated by measuring the currents evoked by a range of glucose isomers, analogues, and phenylglucosides, each at 20 mM. Selected data are presented, all at \(V_m = -150\) mV. Data (mean ± S.E. from between 5 and 14 individual oocytes) were normalized to the current evoked by 20 mM aMG (312 ± 63 nA, \(n = 21\)) in the same oocyte. The pattern of substrate selectivity showed no appreciable differences at any other \(V_m\) tested.

As \([Na^+]_o\), had no effect on \(Q_{max}\) or \(z\).

**Substrate and Ion Selectivity**—The current evoked by 20 mM glucose (Fig. 5) was 25% less than that for aMG (\(p < 0.001\)) and the \(K_{0.5}\) for glucose (~6 mM at \(-150\) mV, data not shown) was twice that for aMG. The 20 mM galactose-evoked currents were only 3% of those with aMG, d-Mannose, d-allose, l-glucose, 2-deoxy-d-glucose, 3-O-methyl-d-glucopyranoside, and d-xylene failed to evoke a significant current (not shown). Furthermore, no current was obtained at 100 mM 3-O-methyl-d-glucopyranoside. Of the phenyl-\(\beta\)-glucosides tested, arbutin (4-[hydroxy]phenyl-\(\beta\)-d-glucopyranoside) evoked a current which was not significantly different from aMG, but the 2-substituted phenyl-\(\beta\)-glucosides helicin, salicin and 2-[nitrophenyl]-\(\beta\)-d-glucopyranoside were not transported. 4-[Nitrophenyl]-\(\alpha\)-d-glucopyranoside evoked a significant current (~33% of aMG), but maltose, sucrose, and amygdalin did not.

Li\(^+\) and H\(^+\) were effective substitutes for Na\(^+\) in driving aMG transport via pSGLT2 (not shown). At \(-150\) mV, the current evoked by 50 mM aMG in Li\(^+\) was identical to that in Na\(^+\), and that with H\(^+\) (i.e. choline chloride at pH 5.5) was 36% of that in Na\(^+\). The \(I/V_m\) relationships in Li\(^+\) and H\(^+\) were shifted in the hyperpolarizing direction compared with Na\(^+\).

**Inhibition by Phlorizin**—Phlorizin blocked the uncoupled Na\(^+\) current observed in the absence of sugar (Fig. 6A); this current was up to 8% of the maximal aMG-evoked current, with a reversal potential at \(\approx +10\) mV. Phlorizin inhibited the aMG-evoked currents; the inhibition constant (\(K_{p}^\text{m}\)) was determined by Dixon analysis of the currents evoked by 3 and 20 mM aMG in the presence of 0.3–100 \(\mu\)M phlorizin (Pz). At \(-50\) mV (Fig. 6B) \(K_{p}^\text{m}\) was 9 \(\mu\)M, and between \(-150\) mV and \(-10\) mV \(K_{p}^\text{m}\) was 8–14 \(\mu\)M. The \(I/V_m\) value (~260 nA) inferred from the Dixon plot (Fig. 6B) was consistent with the \(I/V_m\) measured (~230 nA) in the same oocyte. An identical \(K_{p}^\text{m}\) (~7 ± 3 \(\mu\)M) was obtained from plots (not shown) of the phlorizin concentrations that inhibited the 1, 2, 5, and 20 mM aMG-evoked currents by 50% (by extrapolating to zero aMG).
evoked (3-axial –OH at C-3 (as indicated by the exclusion of allose and was the case for SGLT1 (11). However, SGLT2 also required there were a number of striking differences, including sugar
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tation of 100 mM (not shown).
2 Hazama, A., Loo, D. D. F., and Wright, E. M. (1997) J. Membr. Biol., in press.

have been extensively characterized in terms of presteady-state and steady-state kinetics and cation/substrate selectivity, and a kinetic model has been proposed (4–6, 10–15).2 In this study of the functional mechanism for SGLT2, we found that, although SGLT1 and SGLT2 share many similar properties, there were a number of striking differences, including sugar specificity and Na+/glucose coupling.

**Substrate Selectivity**—The reactivity of SGLT2 with αMG (K_{iso,αMG} ~ 3 mM), glucose (K_{iso,glc} ~ 6 mM) and galactose (K_{iso,Gal} >> 20 mM), and its exclusion of 3-O-methyl-D-glucopyranoside, l-glucose and mannose, is entirely consistent with the substrate selectivity of the low affinity glucose transport system previously described by Turner and Moran (8, 9) for brush-border membrane vesicles from rabbit outer cortex. SGLT2 reacted with its favored substrates (αMG, glucose) with much lower apparent affinity than SGLT1: K_{0.5,αMG} was 6–15-fold higher for SGLT2 than for the SGLT1 isoforms. That SGLT2 failed to react with either mannose or 2-deoxy-D-glucose indicated a requirement for the axial –OH at carbon-2 (C-2) in glucose, as was the case for SGLT1 (11). However, SGLT2 also required axial –OH at C-3 (as indicated by the exclusion of allose and 3-O-methyl-D-glucopyranoside) and at C-4 (as indicated by the weak interaction with galactose). Substrate recognition in SGLT2 may also involve hydrophilic interactions around C-6 since xylose and the 1-enantiomer of glucose were both excluded.

Phlorizin, the classical inhibitor of Na+–coupled glucose transport, is a phenyl-β-glucoside, which prompted us to investigate the interactions of a range of phenylglucosides with pig SGLT2. Our group previously determined for rabbit SGLT1 (14) that conjugation of the phenyl ring to glucose in the β configuration decreased the apparent affinity but that this was partially restored by the addition of –OH at the C-4 in the phenyl ring (i.e. arbutin). Arbutin evoked a greater current in SGLT2 than did glucose (Fig. 5), suggesting that the phenyl ring can nest in a vestibule possessing both a hydrophobic and a polar region near C-4 of the phenyl ring. SGLT2, in contrast to SGLT1 (14), also tolerated one of the phenyl-α-glucosides, suggesting differences in the molecular architecture of the hydrophobic vestibule.

Phlorizin potently inhibited the αMG-evoked currents mediated by SGLT2. The phlorizin inhibition constant (K_{i,αMG}) for SGLT2 was ~10 μM, higher than that for each of the SGLT1 isoforms (0.01–1.4 μM) (10, 15), consistent with the 10-fold lower affinity of SGLT2 for sugar.

These studies indicate that the substrate binding site in SGLT2 is distinct from that of SGLT1 with respect to (i) the residues interacting with the sugar at C-3 and C-4, and (ii) the dimensions of the binding site vestibule. The characteristics of a pig SGLT2-pig SGLT1 chimera indicate that sugar binding is mediated by the carbonyl-terminal half of the protein (16). In particular, the hydrophilic loops between putative membrane domains M10/11, M12/13, and M13/14 possess substituted residues in pSGLT2 which are otherwise conserved throughout the SGLT family and may underlie the observed differences in sugar binding.

**Mechanisms of Na+ /Glucose Cotransporter**—Despite differences between SGLT1 and SGLT2 in Na+ coupling and voltage dependence, their steady-state properties were very similar. As for SGLT1 (13), Li+ and H+ could each substitute for Na+ in driving sugar transport mediated by SGLT2, most effectively at hyperpolarized V_m. Na+ binding appears to be V_m-dependent and saturable with hyperpolarization. K_{0.5} was more steeply voltage-dependent in SGLT1 than in SGLT2: K_{0.5} for SGLT1 was ~3-fold higher than SGLT2 at ~150 mM, but 5–15-fold higher at ~50 mV. However, in general the changes in K_{0.5} and I_{max} for SGLT2 arising from varying V_m or cosubstrate concentration were very similar to SGLT1.

The data for SGLT2 are consistent with an ordered, simultaneous transport mechanism (Fig. 7) in which Na+ binds first. The K_{0.5} increased sharply with diminishing cosubstrate concentration, indicating that both substrates were translocated simultaneously, rather than consecutively in which case the K_{0.5} values are expected to decrease (17). The order of binding, Na+ first, sugar second, was revealed by the I_{max} data: I_{max} was dependent upon [αMG], but I_{max} was not affected by varying [Na+]o, i.e. saturating αMG could always drive the transporter to its maximal rate regardless of [Na+]o, whereas [αMG], imposed a limitation upon the maximal rate of the transporter even at saturating Na+. The magnitude of the sugar-uncoupled Na+ influx being <8% of the maximal sugar-evoked current suggests that the preferred configuration for translocation across the membrane is the fully loaded “carrier-Na+”-sugar complex (Fig. 7, states 3 – 4).

Thus SGLT2 and SGLT1 share a common mechanism in which the cation (Na+, Li+, and H+) binds before glucose, followed by a simultaneous translocation step, but with distinct Na+: glucose coupling stoichiometry, 1:1 for SGLT2 and 2:1 for SGLT1. Subtle differences in the shapes of the I/V_m curves for SGLT2 and SGLT1 imply that the rate-limiting steps may differ between the two transporters.

**Transient Charge Movements**—The characteristics of the presteady-state charge movements for pig SGLT2 (Fig. 4) were similar to those obtained for SGLT1 and the other Na+ - and H+–driven transporters (see Table I in Ref. 18). As for SGLT1,2 we attribute the transient currents to movement of SGLT2 charged particles within the transmembrane electric field since these transients are (i) absent in control-injected oocytes and (ii) abolished by the addition of sugar or the specific inhibitor phlorizin. Transient currents decayed with time constants of

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**Fig. 6. Specific inhibition of pSGLT2 by Pz. A, the phlorizin-sensitive, sugar-uncoupled Na+ influx (I) was determined by the addition of 100 μM phlorizin (at 100 mM Na+) in the absence of sugar to a single oocyte expressing pSGLT2. The current evoked by 20 mM αMG in the same oocyte was ~472 nA (at ~150 mM). (Similar data were obtained from another 5 oocytes.) B, the phlorizin inhibition constant (K_{i,Pz}) was determined over a range of V_m using Dixon analysis. Representative data are shown for ~50 mV at 3 mM αMG (○) and 20 mM αMG (○) in the presence of phlorizin (at 0.3, 1, 3, 10, 20, 30, and 100 μM) in 100 mM Na+. The intersection of the regression lines (3 mM, r = 0.99; 20 mM, r = 0.98) revealed K_{i,Pz} ~ 9 μM and I_{max,αMG} ~ 260 nA, cf. the current evoked (~230 nA) by 20 mM (saturating) αMG in the same oocyte at ~50 mV (not shown).

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FIG. 7. Six-state kinetic model describing the operation of the Na+/glucose cotransporter pSGLT2. Six-state kinetic model in which the empty carrier is negatively charged (the apparent valence of the movable charge is \(-1\)) and the Na\(^+\)-coupling coefficient is \(1\). The reaction scheme is described by 14 rates (where the rate \(k_{xy}\) represents the reaction step \(x \rightarrow y\). Each rate is defined by its potential-independent rate constant \(k_{xy}^\text{m}\), \(V_m\), and ligand (Na\(^+\)) concentration, as well as the coefficients \(a\), \(a'\), \(\delta\), and \(\delta'\), which describe the fraction of the electric field sensed by the Na\(^+\) binding to its external site \((a')\) or internal site \((a)\) and by the empty ion binding site on the carrier during membrane translocation \(\delta\), where \(\alpha' + \alpha + \delta = 1\). \(\mu = FV_m/RT\). Model simulations (shown as dotted lines in Figs. 3 and 4) used \(a' = 0.3\), \(a = 0\), \(\delta = 0.7\), \(k_{10} = 100\) s\(^{-1}\), \(k_{21} = 55\) s\(^{-1}\), \(k_{32} = 2 \times 10^4\) M\(^{-1}\) s\(^{-1}\), \(k_{43} = 400\) s\(^{-1}\), \(k_{54} = 50\) m\(^{-1}\) s\(^{-1}\), \(k_{65} = 48\) s\(^{-1}\), \(k_{76} = 0.01\) s\(^{-1}\), \(k_{87} = 0.17\) s\(^{-1}\), \(k_{98} = 10^2\) m\(^{-1}\) s\(^{-1}\), \(k_{10} = 20\) s\(^{-1}\), \(k_{11} = 6.7 \times 10^3\) m\(^{-3}\) s\(^{-1}\), \(k_{12} = 800\) s\(^{-1}\), \(k_{13} = 50\) s\(^{-1}\), \(k_{14} = 50\) s\(^{-1}\); intracellular Na\(^+\) concentration ([Na\(^+\)]) was set at 10 mm, [lAMG] = 0, \(C_t = 8.4 \times 10^9\) oocyte, and at 20 °C.

3–7 ms, within the same range determined for the other cotransporters; in considering species differences, the SGLT2 time constants may not differ from SGLT1 (cf. 4–25 ms for the rat, rabbit, and human isoforms). Whereas \(V_{0.5}\) at saturating activator concentration for most other cotransporters ranged from \(-50\) mV to 0 mV (including the SGLT1 isoforms), we estimated that \(V_{0.5}\) for SGLT2 at saturating Na\(^+\) (100 mm) was \(\approx 60\) mV. This indicated that at resting membrane potential a greater proportion of the SGLT2 ligand binding sites are oriented extracellularly compared with the other transporters. For SGLT2, the shift in \(V_{0.5}\) brought about by a 10-fold increase in activator (Na\(^+\)) concentration was \(\approx 100\) mV, similar to that for other cotransporters. For both SGLT2 and SGLT1, the apparent valence \((z)\) was \(\approx 1\) and did not change with [Na\(^+\)].

Using the relation \(Q_{\text{max}} = C_p z e\) (for which \(e\) is the elementary charge) the density \((C_p)\) of functional SGLT2 carriers in the oocyte membrane was in the order of 10\(^3\)/oocyte. The validity of estimating transporter density from charge movement data for transporters and ion channels expressed in oocytes was confirmed using freeze-fracture electron microscopy (19). The turnover rate of the fully loaded transporter, given by \(I_{\text{max}}/Q_{\text{max}}\), was \(\approx 60\) s\(^{-1}\) and was similar to that determined for the other cotransporters. Thus we conclude that the presteady-state kinetic properties of SGLT2 are very similar to SGLT1.

Model Predictions Resulting from Changing Na\(^+\)/Glucose Coupling from 2:1 to 1:1—Our conclusion that the kinetic behavior of SGLT2 is very similar to SGLT1 suggests a common mechanism, but with different stoichiometry. What changes in kinetic parameters should we expect as a direct result of reducing the Na\(^+\)/glucose coupling stoichiometry from 2:1 (as for SGLT1) to 1:1 (as for SGLT2)? We simulated the six-state kinetic model of rabbit SGLT1 (6, 12) and determined the specific consequences of changing the Na\(^+\)-coupling coefficient \((n)\) from 2 to 1 (Fig. 8) without altering any other parameters. Allowing for some quantitative discrepancies that may arise from comparing SGLT homologues from different species, the \(n = 1\) model predicts a number of changes which are consistent with experimental observations for pig SGLT2: (i) the observed reduction in \(K_{\text{Na}}^\text{m}\) and the reduced voltage dependence on \(K_{\text{Na}}^\text{m}\) (Fig. 8A). The rabbit model predicted \(K_{\text{Na}}^\text{m}\) to decrease by an order of magnitude upon decreasing \(n\) from 2 to 1, whereas the observed change for pig SGLT2 was \(-5\)-fold; however, this difference was reconciled by a reduction in \(k_{43}\) (the rate constant for external sugar binding) by an order of magnitude (see below for justification, and Fig. 3B); (ii) no change in the apparent affinity for oAMG at negative \(V_m\), and a less marked

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voltage dependence (Fig. 8B). This predicted voltage dependence was observed for rabbit SGLT1 when $[\text{Na}^+]_o$ was low; when each were compared at 10 mM Na⁺, $K_{o.5}^{\text{MG}}$ for pig SGLT2 displayed significantly less voltage-dependence than that for rabbit SGLT1 (Fig. 6A of Ref. 6). At all $V_m$, $K_{o.5}^{\text{MG}}$ was higher for SGLT2 than that predicted by the $n = 1$ model, but since the molecular architecture of the SGLT2 sugar binding site is discrete from that of SGLT1 (see above, and Fig. 5), we should not expect such a change to arise simply from the reduction in $n$; (iii) shifts in the $I/V_m$ and $Q/V_m$ relationships toward more positive $V_m$ (Fig. 8, C and D). These predictions were consistent with presteady-state data, although the steady-state evoked currents in SGLT2 varied over the $V_m$ range as for SGLT1 rather than shifting to depolarized $V_m$; (iv) a 50% reduction in $Q_{\text{max}}$ (Fig. 8D), which may in part account for the lower sugar-dependent currents typically obtained for SGLT2 compared with SGLT1 assuming similar levels of expression; and (v) a reduction in $z$ from 1.4 to 0.9 (Fig. 8D), close to the measured value for SGLT2. Thus, collectively, the kinetic properties of SGLT2 are largely accounted for by the change in Na⁺-coupling from 2 to 1.

Model Simulation of Presteady-state Kinetics and Steady-state Na⁺/aMG Cotransport Kinetics for SGLT2—Presteady-state and steady-state currents for pig SGLT2 were simulated according to the six-state model (Fig. 7) assuming 1 Na⁺: 1 aMG coupling stoichiometry as suggested by Hill analysis; the model is otherwise identical to that proposed for SGLT1 (6, 12). We found that the model could account qualitatively for the steady-state (Fig. 3) and presteady-state (Fig. 4) phenomena without changing the rate constants for rabbit SGLT1 (12), except for two conservative modifications: (i) $k_{6,3}^{\text{aMG}}$ was reduced by an order of magnitude to reflect the 10-fold increase in $K_{o.5}^{\text{aMG}}$ for SGLT2; (ii) $k_{5,6}^{\text{aMG}}$, a principal rate-controlling step in rabbit SGLT1 (6, 12, 20) at negative $V_m$, was increased from 16 s⁻¹ to 48 s⁻¹ to account for the > 3-fold increase in turnover rate for SGLT2 compared to rabbit SGLT1.

The model closely predicted the $Q/V_m$ relationship (Fig. 4C) with $V_{m,0.5} = -28$ mV at 10 mM Na⁺ (cf. −34 mV determined experimentally). The kinetic data in the $V_m$ range −150 to −50 mV indicated that the Na⁺ binding and charge transfer steps in the cycle were at saturation, since $K_{o.5}^{\text{aMG}}$ was $V_m$-independent within this range (at saturating aMG) and $V_{m,0.5}$ was extremely positive. However, that the measured (but not predicted) $I/V_m$ curves still displayed $V_m$ dependence within this $V_m$ range implies that the model requires an additional voltage-dependent step in the transport cycle, e.g. Na⁺ dissociation at the internal face (states 5 → 6).

Conclusion—We have presented kinetic data demonstrating that Na⁺/glucose cotransport mediated by SGLT2 can be described by an ordered, simultaneous transport model in which Na⁺ binds first. In the absence of sugar, SGLT2 exhibited Na⁺-dependent transient charge movements which we attribute to reorientation of the charged carrier within the membrane. Taken together, these observations suggest that SGLT1 and SGLT2 share a common mechanism despite their functional differences. Among these apparent differences are the change in apparent Na⁺: aMG coupling from 2:1 for SGLT1 to 1:1 for SGLT2, and the reduction in $K_{o.5}$ observed for SGLT2. However, our model predicts that the reduced $K_{o.5}$ is a direct consequence of the change in Na⁺ coupling altering the rate $k_{12}$ (according to Equation A1). Since this change need not involve a change in the rate constant $k_{12}$ (which is a reflection of ligand binding affinity per se), it is likely that the Na⁺ binding site in SGLT2 is identical to one of the binding sites in SGLT1. The general reduction in apparent affinities for sugar and the effective exclusion of galactose suggests that the molecular architecture of the sugar binding site in SGLT2 is quite distinct from that of SGLT1. In ongoing structure-function studies of the Na⁺/glucose cotransporters, the challenge is now to identify the molecular basis for these functional differences between SGLT1 and SGLT2.

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