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Relationships Between Na^+/Glucose Cotransporter (SGLT1) Currents and Fluxes

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Abstract. The relationships between currents generated by the rabbit Na^+/glucose cotransporter (SGLT1) and the fluxes of Na^+ and sugar were investigated using *Xenopus laevis* oocytes expressing SGLT1. In individual voltage-clamped oocytes we measured: (i) the current evoked by 10 mM αMG and the $^{22}Na^+$ uptake at 10 mM Na^+; (ii) the currents evoked by 50 to 500 μM [^{14}C]αMG and the [^{14}C]αMG uptakes at 100 mM Na^+; and (iii) phlorizin-sensitive leak currents in the absence of sugar and $^{22}Na^+$ uptakes at 10 mM Na^+. We demonstrate that the SGLT1 leak currents are Na^+ currents, and that the sugar-evoked currents are directly proportional to both αMG and Na^+ uptakes. The Na^+/αMG coupling coefficients were estimated to be 1.6 at –70 mV and 1.9 at –110 mV. This suggests that the rabbit SGLT1 Na^+/αMG stoichiometry for sugar uptake is 2 under fully saturating, zero-*trans* conditions. Coupling coefficients of less than 2 are expected under nonsaturating conditions due to uncoupled Na^+ fluxes (slippage). The similarity between the Na^+ Hill coefficients and the coupling coefficients suggests strong cooperativity between the two Na^+ binding sites.

Key words: Transport stoichiometry — Secondary active transport — Na^+/glucose cotransport — Leak currents — *Xenopus* oocyte — Phlorizin

Introduction

We have examined the relationships between cotransporter currents and the fluxes of Na^+ and sugar for rabbit SGLT1 over-expressed in oocytes. The rationale being that: (i) cotransporter currents are commonly used to study the kinetics of cotransport (Umbach, Coady & Wright, 1990; Birnir, Loo & Wright, 1991; Parent et al., 1992*a;* Hirsch, Loo & Wright, 1996; Loo et al., 1996; Mackenzie et al., 1996; Hirayama, Loo & Wright, 1996; Eskandari et al., 1997); (ii) currents generated by several neurotransmitter cotransporters exceed those expected for the transport cycle (Mager et al., 1994; Wadiche, Amara & Kavanaugh, 1995; Wright et al., 1996); and (iii) there are internal leak currents through SGLT1 that may result in slippage in coupling (Parent et al., 1992*b;* Brown, 1995). The results demonstrate that SGLT1 currents are proportional to Na^+ uptakes in the presence and absence of sugar, and the sugar-evoked currents are proportional to sugar uptakes. The coupling coefficient for Na^+ to sugar transport was close to 2 under saturating conditions, and less than 2 under nonsaturating conditions. This suggests that there is significant internal slippage in the coupling of sugar to Na^+ through SGLT1.

Materials and Methods

Our experimental strategy was to overexpress the rabbit SGLT1 cotransporter in oocytes and then measure the initial rates of $^{22}Na^+$ or ^{14}C-αMG uptake into voltage-clamped cells. This permitted us to directly compare unidirectional ligand uptakes to the cotransporter currents over the same time course in a single cell.

Stage VI *Xenopus laevis* oocytes (Nasco, Fort Atkinson, WI) were defolliculated, injected with rabbit intestine SGLT1 cRNA and maintained at 18°C in modified Barth's medium (Parent et al., 1992*a*) with 10 mg · l^{-1} gentamicin sulfate.

Experimental media contained 0, 10 or 100 mM NaCl — complemented by 100, 90 or 0 mM choline chloride — with 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM HEPES (pH 7.5 with Tris), plus α-methyl-D-glucopyranoside or phlorizin as indicated.

Charge/Na^+ Stoichiometry

A two-microelectrode voltage-clamp technique was used to measure sugar-evoked currents in combination with simultaneous, unidirec-

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tional radiotracer accumulation in individual oocytes expressing SGLT1. Each oocyte was placed in a chamber of functional volume <100 μl and voltage-clamped at a single holding potential (V_h) throughout the experiment. The oocyte was superfused with 10 mM Na^+ medium (at 20–22°C) at a flow rate of ≈200 μl · min^{-1}. Baseline current was recorded in Na^+ medium, after which the oocyte was superfused with 10 mM ^{22}Na (final specific activity 0.3–0.5 MBq · μmol^{-1}, DuPont NEN, Wilmington, DE) and 10 mM alpha-methyl-D-glucopyranoside (αMG) for 10 min. The oocyte was then superfused with Na^+ medium until the current returned to baseline (≈3 min), recovered from the chamber, rinsed in ice-cold choline medium, and solubilized with 5% SDS for liquid scintillation counting (Ikeda et al., 1989).

Current was filtered at 20 Hz and sampled every 0.1 sec. The αMG-evoked current was obtained as the difference in current between baseline and after addition of sugar, and was integrated (trapezoidal rule) to obtain the total sugar-dependent charge ($Q^{\alpha MG}$). $Q^{\alpha MG}$ was converted to a molar equivalent using the Faraday (assuming monovalency). To correct for endogenous Na^+ uptake (i.e., not specific to SGLT1), we also measured ^{22}Na accumulation in control (H_2O-injected) oocytes.

Phlorizin-sensitive (Leak) Current

The SGLT1 leak current in the absence of sugar was measured by the addition of 500 μM (saturating) phlorizin (Pz) at 10 mM Na^+ (V_h = −70 mV) and compared to the current evoked by 10 mM αMG. ^{22}Na-uptake via the SGLT1 Na^+ leak pathway in the absence of sugar was determined as described above, and corrected for the endogenous Na^+ uptake in control, noninjected oocytes.

Charge/Sugar Stoichiometry

Charge/sugar stoichiometry was determined as described for the charge/Na^+ stoichiometry with the following changes. Test solutions containing 50, 200 or 500 μM α-methyl-D-[U-^{14}C]glucopyranoside (Amersham, Arlington Heights, IL) in 100 mM Na^+ were superfused for 10 min (final specific activity 0.7–1.1 MBq · μmol^{-1}) or 1 min (final specific activity ≈2.0 MBq · μmol^{-1}), at V_h of −70 or −110 mV. [^{14}C]αMG accumulation was determined in control H_2O-injected oocytes (from the same batch) under equivalent conditions, and used to correct for endogenous total αMG uptake. No endogenous Na^+-dependent αMG uptake was detected in any batch of oocytes tested.

Results

We measured sugar-evoked SGLT1 currents and either ^{22}Na or [^{14}C]-αMG uptakes in the same oocyte over the same time course. To increase the reliability of the data, we: (i) only used oocytes with high expression levels (>5 × 10^{10} cotransporters per oocyte as determined by Q_{max} measurements, Loo et al., 1993; Zampighi et al., 1996); (ii) minimized the nonspecific ^{22}Na uptakes and ^{22}Na counting errors by reducing the external Na^+ concentration to 10 mM, and increasing the external sugar to 10 mM to maximize the rate of sugar transport (*see* Parent et al., 1992*a*); (iii) measured the ^{22}Na leak through SGLT1 in the absence of sugar, and the ^{22}Na uptake into control (H_2O-injected) oocytes from the same batch of oocytes;

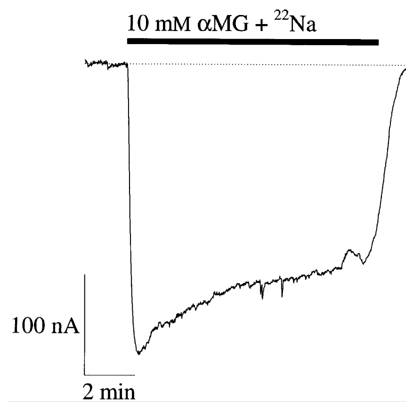

Fig. 1. The sugar-dependent current in an oocyte expressing rabbit SGLT1. Current was continuously monitored in a single rSGLT1-cRNA-injected oocyte clamped at holding potential (V_h) −70 mV. A stable baseline current was obtained in 10 mM Na^+ before superfusing 10 mM α-methyl-D-glucopyranoside (αMG) together with 10 mM ^{22}Na for 10 min (shown by the *solid bar*) and washing out with 10 mM Na^+ (without tracer). The sugar-dependent charge ($Q^{\alpha MG}$), i.e., the integral of the sugar-dependent current over 10 min, was −1,400 × 10^{-4} Coulombs, equivalent to 1,425 pmol of monovalent charge. The ^{22}Na accumulation in this oocyte was 1,398 pmol (having subtracted mean basal ^{22}Na accumulation over 10 min in control oocytes, 33 pmol), yielding a charge/^{22}Na stoichiometry of 1:1.

(iv) maximized the accuracy of the [^{14}C]-αMG uptakes by using sugar concentrations close to the sugar $K_{0.5}$ (50–500 μM) at the highest Na^+ concentration possible, 100 mM; and (v) measured the [^{14}C]-αMG uptakes in control (H_2O-injected) oocytes from the same batch of oocytes. This enabled us to take into account both the external and internal SGLT1 Na^+ and sugar uptakes. In this series of experiments we did not detect any significant Na^+-dependent αMG uptakes into noninjected oocytes (<<0.01 pmoles/minute), and the $^{22}Na^+$ uptakes in control oocytes were insignificant relative to the uptakes in SGLT1 cRNA injected oocytes (<10 pmoles/min). It should be noted that all isotope uptakes were measured under initial rate conditions, i.e., uptakes were linear for at least ten times longer than the 1–10 min uptakes reported here.

The addition of 10 mM αMG to a 10 mM Na^+ medium, resulted in large, reversible inward currents in oocytes expressing rabbit SGLT1 (Fig. 1). The reason for the slow decline in the sugar-induced currents with time (Fig. 1) is unclear. This decay is invariably observed with other cotransporters expressed in oocytes, especially at substrate concentrations above the apparent Km. However, this decay has little impact on the present study since currents and radioactive uptakes are integrated over the same time interval in the same oocytes, and the ratio of charge to uptake is the same at 1 and 10 minutes (*see* below). Current was integrated with time to determine the sugar-dependent net charge influx ($Q^{\alpha MG}$), converted to a molar equivalent assuming monovalency,

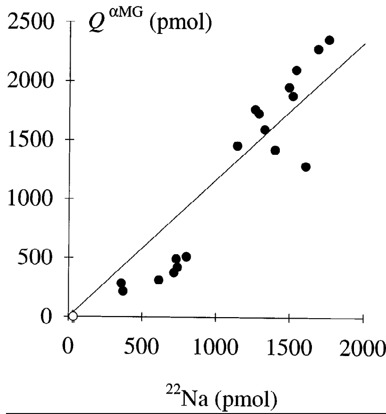

Fig. 2. Charge/^{22}Na stoichiometry for rSGLT1. The sugar-dependent charge ($Q^{\alpha MG}$) and ^{22}Na accumulation over 10 min at 10 mM Na^+ were simultaneously determined at $V_h = -70$ mV in 18 oocytes expressing SGLT1 (●); basal ^{22}Na accumulation (over 10 min) in control-injected oocytes (○) (mean ± SEM from 6 oocytes) has been subtracted. The charge/^{22}Na stoichiometry was 1.0 ± 0.1 (mean ± SEM).

and compared with the unidirectional accumulation of ^{22}Na (in the same oocyte over the same period) to obtain the net charge/Na^+ stoichiometry. In this oocyte the sugar dependent inward charge movement was 1.4×10^{-4} Coulombs (1,425 pmoles of a monovalent cation) and the Na^+ uptake was 1,400 pmoles. For the 18 oocytes expressing SGLT1 shown in Fig. 2, the $Q^{\alpha MG}/Na^+$ stoichiometry was 1.0 ± 0.1 (SEM).

Since phlorizin blocks the SGLT1 leak pathway in the absence of sugar (Umbach, Coady & Wright, 1990; Parent et al., 1992*a;* Lostao et al., 1994), we investigated the relationship between the phlorizin-sensitive, sugar-independent current and Na^+ uptake. There was a close correlation (Fig. 3) between the magnitude of the phlorizin-sensitive, uncoupled Na^+ current (I^{Pz}) and the sugar-evoked current ($I^{\alpha MG}$). At 10 mM Na^+, I^{Pz} was ≈5% of $I^{\alpha MG}$ and this is less than the standard error of the mean $Q^{\alpha MG}/Na^+$ stoichiometry (Fig. 2).

The charge associated with the uncoupled Na^+ pathway (Q^{Pz}) was estimated from I^{Pz} (since I^{Pz} did not change over time, *not shown*). According to this manipulation and assuming a valence of +1, the molar equivalent of Q^{Pz} was identical to the SGLT1-specific ^{22}Na accumulation in the absence of sugar (Fig. 3, *inset*).

We then determined the charge/sugar stoichiometry by measuring the currents evoked by [^{14}C]αMG at 100 mM $[Na^+]_o$. In 13 oocytes expressing SGLT1, measuring the current evoked by 200 μM [^{14}C]αMG over 10 min (in 100 mM Na^+ and at $V_h = -70$ mV), the $Q^{\alpha MG}$/αMG ratio was 1.4 ± 0.1 (SEM). Since we invariably observed a slight decay in the αMG-evoked current over a 10-min time course at sugar concentrations higher than the $K_{0.5}$ (*see* Fig. 1), we checked if this had any effect upon the determination of coupling. The $Q^{\alpha MG}$/αMG stoichiometry determined over 1 min was 1.5 ± 0.1 (9). We also

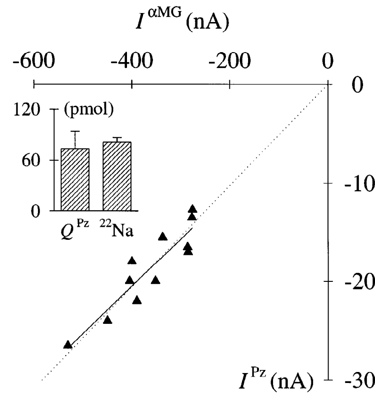

Fig. 3. The phlorizin-sensitive Na^+ current in rSGLT1. The current (I^{Pz}) sensitive to 500 μM phlorizin at $V_h = -70$ mV was correlated with the peak current evoked by 10 mM αMG ($I^{\alpha MG}$) each at 10 mM Na^+ in 11 oocytes: data were fitted by a linear regression (*solid line,* $r^2 = 0.84$, $P < 0.001$). The magnitude of the phlorizin-sensitive current was 5.2% ± 0.2% (*dotted line,* mean ± SEM) of the sugar-evoked current. (*Inset*) The estimated charge over 10 min corresponding to the phlorizin-sensitive current (*see text*) was compared with the rSGLT1-specific ^{22}Na accumulation over 10 min in the absence of sugar: the paired data (mean ± SEM) were identical. The rSGLT1 specific Na^+ uptakes were obtained from the difference between the uptakes in cRNA and water-injected oocytes from the same batch of oocytes.

failed to detect any clear effects on the stoichiometry when $[\alpha MG]_o$ was varied between 50 and 500 μM, and so all data at $V_h = -70$ mV were pooled (Fig. 4), and linear correlation between the sugar-dependent charge ($Q^{\alpha MG}$) and sugar accumulation gave a $Q^{\alpha MG}$/αMG stoichiometry of 1.6 ± 0.3 (1 SD).

The $Q^{\alpha MG}$/αMG stoichiometry at hyperpolarized potentials was much closer to 2 (Fig. 5). At $V_h = -110$ mV (in 100 mM Na^+), we superfused 200 μM [^{14}C]αMG for 10 min and obtained a $Q^{\alpha MG}$/αMG stoichiometry of 1.9 ± 0.1 (mean ± SEM, 6 oocytes).

Discussion

We have directly determined the relationship between the currents and the sugar fluxes mediated by the Na^+/glucose cotransporter by simultaneously comparing the sugar-dependent Na^+ current with the Na^+-dependent sugar flux in individual, voltage-clamped oocytes. Overexpression of the SGLT1 transporter in oocytes virtually eliminated experimental error arising from nonspecific substrate fluxes, i.e., those due to passive diffusion or mediated by other transporters (''external leaks''): the nonspecific uptakes of [^{14}C]αMG and ^{22}Na in control oocytes were typically only 0.7% and 3% of the SGLT1-specific cosubstrate-dependent uptakes. No endogenous Na^+-dependent [^{14}C]αMG uptake was observed in any batch of oocytes tested. In addition, our procedure (i) involved measuring charge and tracer accumulation over

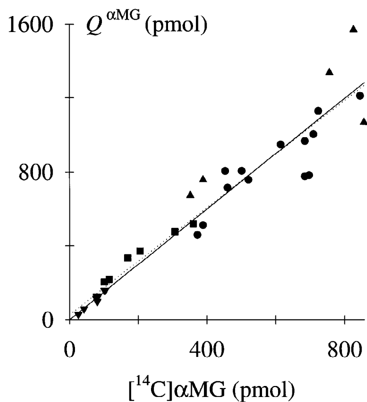

Fig. 4. Na^+/αMG coupling stoichiometry of rSGLT1. The sugar-dependent charge ($Q^{\alpha MG}$) was compared with the accumulation of 200 μM [14C]αMG over 10 min, at V_h = −70 mV in 100 mM Na^+ (●). Since no difference in the coupling coefficient was found with identical conditions over 1 min (▼) and no consistent effects of varying the sugar concentration could be discerned with $[\alpha MG]_o$ of 50 μM (■) or 500 μM (▲) all data were pooled together. Pooled data were fitted to a linear regression (r^2 = 0.92, $P < 0.001$); equating charge to Na^+ flux, the mean Na^+/αMG coupling coefficient was 1.6 ± 0.3 (1 SD).

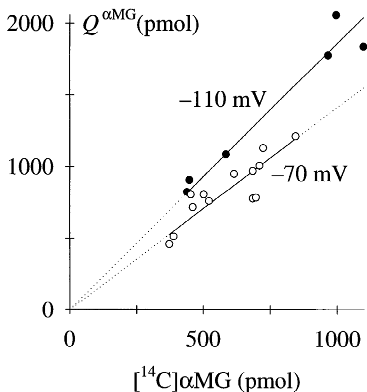

Fig. 5. Effect of membrane potential on Na^+/αMG coupling stoichiometry of rSGLT1. The Na^+/αMG coupling coefficient was determined at V_h = −110 mV (●) and at V_h = −70 mV (○), at 100 mM Na^+ and 200 μM [14C]αMG. At −110 mV, the Na^+/αMG coupling coefficient was 1.9 ± 0.1 (mean ± SEM, 6 oocytes), and at −70 mV, the coupling coefficient was 1.4 ± 0.1 (mean, SEM, 13 oocytes).

the same time course in the same oocyte, therefore taking into account changes over time (*see* Fig. 1); and (ii) eliminated errors arising from cell-to-cell variability in transporter expression.

Since all radioactive isotope uptakes were obtained during the initial linear phase, they represent unidirectional influxes of both Na^+ and αMG. Furthermore, since we have failed to detect outward sugar-dependent current in SGLT1 oocytes (e.g., Parent et al., 1992a, 1993) unless oocytes are preloaded with sugar for 24 hr (Umbach et al., 1990), the sugar-dependent inward currents through SGLT1 represent unidirectional inward currents in these experiments. This view is supported by: (i) the low concentration of intracellular glucose in oocytes, <50 μM (Umbach et al., 1990), and (ii) the high glucose $K_{0.5}$ for outward sugar-dependent currents in rabbit SGLT1 oocytes, >>20 mM (S. Eskandari, D.D.F. Loo and E.M. Wright *unpublished results;* and *see also* Chen et al., 1995). Thus under the experimental conditions reported here, both the uptakes and currents give fair estimates of the SGLT1 unidirectional influxes.

Comparison of the sugar-dependent net charge ($Q^{\alpha MG}$) and the simultaneous accumulation of ^{22}Na in individual oocytes expressing SGLT1 indicated that the sugar-evoked current was identical to the sugar-dependent Na^+ influx ($Q^{\alpha MG}/Na^+$ influx = 1.0 ± 0.1, Fig. 2). This validated the use of the sugar-evoked current as a quantitative index of Na^+ influx in the determination of Na^+/glucose kinetics. In the absence of sugar, we observed a phlorizin-sensitive current (I^{Pz}) the magnitude of which at 10 mM Na^+ was 5% of that evoked by saturating sugar. The identity between the charge corresponding to I^{Pz} and the SGLT1-specific ^{22}Na uptake in the absence of sugar also confirmed that the internal leak current through SGLT1 is a Na^+ current. We also demonstrated that the sugar-dependent currents were proportional to sugar uptake (Fig. 4), again validating the use of the sugar-evoked current as a direct measure of sugar transport.

The ion-to-substrate coupling stoichiometry of cotransporters is of physiological significance since it: (i) determines the energetic cost of transport, and (ii) sets the thermodynamic limit to the concentrative capacity (Kimmich & Randles, 1984). The extent to which a system reaches this limit depends on the influence of uncoupled pathways through the transporter (''internal leaks'') and of other nonspecific pathways (''external leaks'') within the cell (Turner, 1985). With the cloning and overexpression of cotransporters in oocytes (Hediger et al., 1987; Hediger, Turk & Wright, 1989) it is now possible to reexamine coupling for specific transporters with minimum interference from external leaks. In the case of the high-affinity Na^+/glucose cotransporters (SGLT1), both indirect and direct methods suggest a Na^+/glucose coupling of 2:1 (Ikeda et al., 1989; Parent, 1992*a;* Lee et al., 1994; Chen et al., 1995). Indirect estimates have relied on Hill coefficient determinations (rabbit SGLT1, Ikeda et al., 1989; Parent, 1992*a*), whereas the more direct methods have included the comparison of [14C]-αMG uptakes with αMG-evoked currents in different oocytes (rat SGLT1, Lee et al., 1994), and measurement of reversal potentials (human SGLT1, Chen et al., 1995). Given that the inward sugar-evoked currents are proportional to the unidirectional uptake of Na^+, it is then possible to estimate the stoichiometry of Na^+ and sugar transport. The Na^+/αMG coupling coef-

ficient was ≈1.6 at −70 mV (Fig. 4) and ≈1.9 at −110 mV (Fig. 5).

Our six-state kinetic model (Parent et al., 1992*b;* Brown, 1995) predicts that under saturating conditions, i.e., at saturating Na^+ and sugar concentrations and hyperpolarizing membrane potentials (−150 mV) where the leak pathway for Na^+ is insignificant, the Na^+/αMG coupling coefficient should be 2. The results at −110 mV (Fig. 5), and those obtained in a study of SGLT reversal potentials (Chen et al., 1995), agree with this prediction. Furthermore, the close agreement between these estimates of the Na^+/αMG coupling coefficient and our previous estimates of Na^+ Hill coefficients for rabbit SGLT1 (1.9 ± 0.2, Parent et al., 1992*a*) leads to the conclusion that there is strong interaction between the two Na^+ binding sites on the protein.

There is ample data in support of our contention that SGLT1 operates in two modes, as a Na^+-uniporter and as a Na^+/sugar cotransporter (Umbach et al., 1990; Parent et al., 1992*a,b;* Loo et al., 1993; Hazama, Loo & Wright, 1997). In the absence of sugar the rate of Na^+ transport through SGLT1 is 5–18% of the maximum in the presence of saturating sugar (5% in 10 mM Na^+ and at −70 mV, Fig. 3; and 19% in 100 mM Na^+ at −150 mV, M. Panayotova-Heiermann et al., submitted). How does this internal Na^+ leak impact our estimates of Na^+/αMG coupling? According to the model (Parent et al., 1992*b*), the addition of external sugar stimulates the coupled influx of Na^+ and reduces the Na^+ influx via the uncoupled mode. Therefore, the sugar stimulated inward Na^+ current underestimates the coupled Na^+ influx, and underestimates the coupling coefficient by up 5–18% depending on the voltage and Na^+ concentration. This would account for less than a 20% underestimate of the coupling. A further factor that may underestimate the Na^+ influx is the fact that the dissociation of Na^+ from the cytoplasmic face of the transporter is rate limiting under these experimental conditions (Parent et al., 1992*b;* Brown, 1995), and this may result in a recycling of the Na^+ back to the external surface of the membrane via the leak pathway (C_5Na_2–C_2Na_2 in the terminology of our model, Parent et al., 1992*b*). This would result in underestimates of the Na^+ influx, Na^+ currents, and Na^+/sugar coupling, but this underestimate is predicted to get larger, not smaller, as the membrane potential is hyperpolarized from −70 to −110 mV (*see* Fig. 2, Brown, 1995). The physiological importance of the Na^+ leak (''slippage'') is unclear, other than it places a penalty on the concentrative ability of the transporter. Another model for ion-coupled transporters, the multisubstrate single-file model (Su et al., 1996), does predict a voltage-dependence of the Na^+ to glucose flux ratio: simulations of SGLT1 at 10 mM Na^+ and 6 mM sugar indicate that the flux ratio increases linearly from 1.2 at −60 mV to 1.9 at −100 mV. However, this model does not account for the observed Na^+ and voltage-dependent conformational changes of SGLT1 or the temperature dependence of the presteady-state currents (Hazama et al., 1997).

In summary, this study confirms that both the sugar-dependent and independent steady-state currents exhibited by SGLT1 are inward Na^+ currents, and that the magnitude of the sugar-dependent current is directly proportional to the rate of Na^+-dependent sugar transport. We estimate that in the coupled transport mode 2 Na^+ ions are transported along with 1 sugar molecule. However, coupling coefficients of less than 2 are observed and this is due to internal Na^+ slippage through SGLT1. Substrate independent Na^+ fluxes and currents have also been reported for other cloned cotransporters, including serotonin and glutamate (Mager et al., 1994; Fairman et al., 1995). Unlike the neurotransmitter cotransporters (e.g., Mager et al., 1994; Wadiche et al., 1995; Sonders et al., 1997), the inward charge movement by SGLT1 in the presence of substrate does not exceed that expected for coupled Na^+ transport. Therefore, SGLT1 does not appear to behave as a substrate-gated ion channel.

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References

Birnir, B., Loo, D.D.F, Wright, E.M. 1991. Voltage clamp studies of the Na^+/glucose cotransporter cloned from rabbit small intestine. *Pfluegers Arch.* **418:**79–85

Brown, G.C. 1995. Rate control within the Na^+/glucose cotransporter. *Biophys. Chem.* **54:**181–189

Chen, X-Z., Coady, M.J., Jackson, F., Bertelott, A., Lapointe, J-Y. 1995. Thermodynamic determination of the Na^+:glucose coupling ratio for the human SGLT1 cotransporter. *Biophys. J.* **69:**2405–2414

Eskandari, S., Loo, D.D.F., Dai, G., Levy, R., Wright, E.M., Carrasco, N. 1997. Thyroid Na^+/I^- symporter: mechanisms, stoichiometry, and specificity. *J. Biol. Chem.* **272:**27230–27238

Fairman, W.A., Vandenberg, R.J., Arriza, J.L., Kavanaugh, M.P., Amara, S.G. 1995. An excitatory amino-acid transporter with properties of ligand-gated chloride channel. *Nature* **375:**599–603

Hazama, A., Loo, D.D.F., Wright, E.M. 1997. Presteady-state currents of the Na^+/glucose cotransporter (SGLT1). *J. Membrane Biol.* **155:**175–186

Hediger, M.A., Coady, M.J., Ikeda, T.S., Wright, E.M. 1987. Expression cloning and cDNA sequencing of the Na^+/glucose cotransporter. *Nature* **330:**379–381

Hediger, M.A., Turk, E., Wright, E.M. 1989. Cloning and expression of the human intestinal Na^+/glucose cotransporter. *J. Cell Biol.* **107:**364a

Hirayama, B.A., Loo, D.D.F., Wright, E.M. 1997. Cation effects on protein conformation and transport in the Na^+/glucose cotransporter. *J. Biol. Chem.* **272:**2110–2115

Hirsch, J.R., Loo, D.D.F., Wright, E.M. 1996. Regulation of Na^+/

glucose cotransporter expression by protein kinases in *Xenopus laevis* oocytes. *J. Biol. Chem.* **271:**14740–14746

Ikeda, T.S., Hwang, E-S., Coady, M.J., Hirayama, B.A., Hediger, M.A., Wright, E.M. 1989. Characterization of a Na^+/glucose cotransporter cloned from rabbit small intestine. *J. Membrane Biol.* **110:**87–95

Kimmich, G.A., Randles, J. 1984. Sodium sugar coupling stoichiometry in chick intestinal cells. *Am. J. Physiol.* **247:**C74–C82

Lee, W.S., Kanai, Y., Wells, R.G., Hediger, M.A. 1994. The high affinity Na^+/glucose cotransporter. *J. Biol. Chem.* **269:**12032–12039

Loo, D.D.F., Hazama, A., Supplisson, S., Turk, E., Wright, E.M. 1993. Relaxation kinetics of the Na^+/glucose cotransporter. *Proc. Nat. Acad. Sci. USA* **90:**5767–5771

Loo, D.D.F, Zeuthen, T., Chandy, G., Wright, E.M. 1996. Cotransport of water by the Na^+/glucose cotransporter. *Proc. Natl. Acad. Sci. USA* **93:**13367–13370

Lostao, M.P., Hirayama, B.A., Loo, D.D.F., Wright, E.M. 1994. Phenylglucosides and the Na^+/glucose cotransporter (SGLT1) Analysis of interactions. *J. Membrane Biol.* **142:**161–170

Mackenzie, B., Loo, D.D.F., Panayotova-Heiermann, M., Wright, E.M. 1996. Biophysical characteristics of the pig kidney Na^+/glucose cotransporter SGLT2 reveal a common mechanism for SGLT1 and SGLT2. *J. Biol. Chem.* **271:**32678–32683

Mager, S., Min, C., Henry, D.J., Chavkin, C., Hoffman, B.J., Davidson, N., Lester, H.A. Conducting states of a mammalian serotonin transporter. *Neuron* **12:**845–859

Parent, L., Supplisson, S., Loo, D.F., Wright, E.M. 1992*a.* Electrogenic properties of the cloned Na^+/glucose cotransporter. Part I. Voltage-clamp studies. *J. Membrane Biol.* **125:**49–62

Parent, L., Supplisson, S., Loo, D.F., Wright, E.M. 1992*b.* Electrogenic properties of the cloned Na^+/glucose cotransporter: Part II. A transport model under non rapid equilibrium conditions. *J. Membrane Biol.* **125:**63–79

Parent, L., Wright, E.M. 1993. Electrophysiology of the Na^+/glucose cotransporter. *In:* Molecular Biology and Function of Carrier Proteins. Annual Meeting of SGP. L. Reuss, J.M. Russel, M. Jennings, editors. pp. 263–281. Rockefeller Univ. Press

Sonders, M.S., Zhu, S.J., Zahniser, N.R., Kavanaugh, M.P., Amara, S.G. 1997. Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. *J. Neurosci.* **17:**960–974

Su, A., Mager, S., Mayo, S.L., Lester, H.A. 1996. A multi-substrate single-file model for ion-coupled transporters. *Biophys. J.* **70:**762–777

Turner, R.J. 1985. Stoichiometry of cotransport systems. *Ann. N.Y. Acad. Sci.* **456:**10–25

Umbach, J.A., Coady, M.J., Wright, E.M. 1990. The intestinal Na^+/glucose cotransporter expressed in *Xenopus* oocytes is electrogenic. *Biophys. J.* **57:**1217–1224

Wadiche, J.I., Amara, S.G., Kavanaugh, M.P. 1995. Ion fluxes associated with excitatory amino acid transport. *Neuron* **15:**721–728

Wright, E.M., Loo, D.D.F., Turk, E., Hirayama, B.A. 1996. Sodium cotransporters. *Current Opinion in Cell Biology* **8:**469–473

Zampighi, G.A., Kreman, M., Boorer, K.J., Loo, D.D.F., Bezanilla, F., Chandy, G., Hall, J.E., Wright, E.M. 1995. A method for determining the unitary functional capacity of cloned channels and transporters expressed in *Xenopus laevis* oocytes. *J. Membrane Biol.* **148:**65–78