Mouse SGLT3a generates proton-activated currents but does not transport sugar

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Barcelona S, Menegaz D, Diez-Sampedro A. Mouse SGLT3a generates proton-activated currents but does not transport sugar. Am J Physiol Cell Physiol 302: C1073–C1082, 2012. First published February 1, 2012; doi:10.1152/ajpcell.00436.2011.—Sodium-glucose cotransporters (SGLTs) are secondary active transporters belonging to the SLC5 gene family. SGLT1, a well-characterized member of this family, electrogenerically transports glucose and galactose. Human SGLT3 (hSGLT3), despite sharing a high amino acid identity with human SGLT1 (hSGLT1), does not transport sugar, although functions as a sugar sensor. In contrast to humans, two different genes in mice and rats code for two different SGLT3 proteins, SGLT3a and SGLT3b. We previously cloned and characterized mouse SGLT3b (mSGLT3b) and showed that, while it does transport sugar like SGLT1, it likely functions as a physiological sugar sensor like hSGLT1. In this study, we cloned mouse SGLT3a (mSGLT3a) and characterized it by expressing it in Xenopus laevis oocytes and performing electrophysiology and sugar transport assays. mSGLT3a did not transport sugar, and sugars did not induce currents at pH 7.4, though acidic pH induced inward currents that increased in the presence of sugar. Moreover, mutation of residue 457 from glutamate to glutamine resulted in a Na+ presence of sugar. Moreover, mutation of residue 457 from glutamate to glutamine resulted in a Na+ presence of sugar. Additionally, sugar-induced currents are larger at acidic pH (2, 4).

In humans there is one gene that codes for SGLT3, while two genes code for two SGLT3 proteins in mouse and rat. In mouse, both genes, S1c5a4a and S1c5a4b, which code for mouse SGLT3a (mSGLT3a, gene ID: 64452) and mouse SGLT3b (mSGLT3b, gene ID: 64454), respectively, are found on chromosome 10 (26). In rat, these two genes are located on chromosome 20 (gene ID for rat type 3a: 294341 and for rat type 3b: 294342). We have previously shown that mSGLT3b transports sugar, has low apparent sodium and sugar affinities compared with SGLT1, and displays uncoupled sugar transport (1).

To date, no detailed studies of the biophysical properties of SGLT3a from any species have been reported. In this study, we have cloned mSGLT3a from mouse small intestine. By measuring and analyzing steady-state currents and sugar transport, we found that mSGLT3a was not capable of transporting sugar and that sugar did not induce currents at pH 7.4. Interestingly, mSGLT3a was sensitive to low pH, and acidic solutions induced inward currents in the presence or absence of sugar. Moreover, in the absence of Na+, protons induced comparable currents. Furthermore, a mutation of a single amino acid of mSGLT3a permitted sugar transport in a Na+-dependent manner, similar to human SGLT1, thus, revealing its SGLT characteristics.

**METHODS**

**Cloning of mouse SGLT3a.** Full-length mSGLT3a cDNA was cloned from mouse small intestine total RNA (Ambion, Life Technologies, Grand Island, NY) using oligo (dT) primers and Superscript III Reverse Transcriptase (Invitrogen, Life Technologies, Grand Island, NY). The primers used for PCR were sense-5'-CTGGCGTT-GCCTACAGCC-3' and antisense-5' TATTGCTGGACGACGTC-3' using Platinum Taq DNA Polymerase High Fidelity (Invitrogen). The cDNA was sequenced and found to be identical to the mouse genome (gi:12444905) and to a partial cDNA reported earlier (28). The full-length cDNA was then cloned into the pcR2.1-TOPO vector using the TOPO-TA cloning kit (Invitrogen). Mouse SGLT3a was then subcloned into the vector pGH19 (17, 30) for in vitro transcription.

**Real-time PCR.** Expression of mSGLT3a and mSGLT3b was determined in kidney and intestine of mouse. Total RNA was extracted from the tissues with RNaseasy (Qiagen, Valencia, CA) and transcribed to cDNA with Superscript III (Invitrogen) to be used in real-time PCR. TaqMan PCR assays were performed to measure SGLT3a or SGLT3b RNA expression using the ABI Prism 7500 Real-Time PCR System (Applied Biosystems, Life Technologies, Grand Island, NY). Relative quantification between different samples was determined as 2-ΔΔCt (27). We presented the data as relative expression of our mRNAs compared with expression of 18S.

**Cloning of rat SGLT3a.** Full-length rat SGLT3a (rSGLT3a) cDNA was cloned from rat kidney total RNA following the same protocol as above for mSGLT3a. The primers used for PCR were sense-5'-GGAGGCTGATCATTAACTTAGGA-3' and antisense-5' -TCAG-GAGGCTGATCATTAACTTAGGA-3' .
Table 1. mSGLT3a is highly expressed in the small intestine

<table>
<thead>
<tr>
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<th>Relative Abundance ([mSGLT3a][18S]) × 10^6</th>
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<tr>
<td></td>
<td>Intestine</td>
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<tr>
<td>mSGLT3a</td>
<td>148.01</td>
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<tr>
<td>mSGLT3b</td>
<td>170.03</td>
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Real-time PCR showed expression levels of mouse sodium-glucose cotransporters 3a (mSGLT3a) and 3b (mSGLT3b) relative to 18S in mouse kidney and intestine. Both mSGLT3a and mSGLT3b showed high expression in the small intestine. As a result, cloning of mSGLT3a cDNA was focused on the small intestine.

GCAAATAAGCATAGAAAA-3’. The resulting cDNA sequence was not identical to the genome entry for rSGLT3a (gi: 157823977). Silent mutations at nucleotide residues 813, 894, 1044, 1464, 1670, 1788, and 1915 were found with nucleotides C, A, T, C, T, T, and A present instead of G, G, C, T, C, C, and G, respectively.

Mutagenesis. Wild-type mSGLT3a cDNA in the plasmid pGH19 was used as a template for site-directed mutagenesis. Glutamate at residue 457 was mutated to glutamine (E457Q) using the QuikChange kit (Stratagene, Agilent Technologies, Santa Clara, CA). The primers used for mutation were 5’-CAACTGTTCCATTATACA-CAACGAATTTCAAGCTACCTT-3’ and antisense-5’-AAGGTAGCGTTGAAATTCGTTGTTATAATGGACCAGTTGG-3’. The mutated nucleotide is in bold and underlined. Sequencing of gene from start to stop codon was completed to verify the correct sequence with the mutated nucleotide.

Expression of proteins and recordings in Xenopus laevis oocytes. cDNAs for rSGLT3a, mSGLT3a, E457Q-mSGLT3a, and mSGLT3b were linearized with XhoI, and RNAs were transcribed and capped in vitro using the T7 RNA promoter (MEGAscript kit, Ambion). Mature mRNAs were injected into oocytes of X. laevis in the presence of NaCl, KCl, CaCl2, and HEPES. Sugar transport was determined using a scintillation counter. Noninjected oocytes from the same batch were used as a control.

Sugar uptake experiments. Oocytes expressing SGLT3 proteins were incubated with 50 μM α-methyl-β-glucose (αM-glc) with traces of [14C]αM-glucose in Na+ solution at pH 7.4 or 5, in Na+ free solution at pH 7.4, or in Na+ solution at pH 7.4 with Pz. After 1 h of incubation at room temperature, the oocytes were washed with cold Na+ free solution and were trypsinically solubilized with 10% sodium dodecyl sulfate (SDS). Sugar transport was determined using a scintillation counter. Noninjected oocytes from the same batch were used as control.

Transfection and patch-clamp recordings in mammalian cells. cDNAs for mSGLT3a and mSGLT3b were subcloned into a pRES2-enhanced green fluorescent protein (EGFP) vector (Clontech, Mountain View, CA). Chinese hamster ovary (CHO) cells were grown in flasks and were kept at 37°C and 5% CO2 with Opti-MEM (Invitrogen) media supplemented with 10% fetal bovine serum (Invitrogen) and penicillin-streptomycin (Invitrogen). Cells were split when confluent. Cells were plated on coverslips in 12-well plates where 500 μl of nonsupplemented Opti-MEM warmed to 37°C was added to each well. Twenty-four hours after plating, cells were transfected with lipofectamine (Invitrogen) following manufacturer’s instructions. Recording of cells took place within 24–48 h. Coverslips with transfected CHO cells were placed in a recording chamber on an inverted microscope (Axiovert 100, Zeiss, Thornwood, NY). Cells were perfused with Ringer’s solution with different concentrations of glucose and at pH 7.4 or 5. The composition of this solution was (in mM) 1 glucose, 145 NaCl, 4.5 KCl, 1 MgCl2, 1 MgCl2 and 10 HEPES at pH 7.4 or MES at pH 5. When additional glucose was included in the solution, osmolality was adjusted. The intracellular solution was composed of (in mM) 130 KGlutonate, 15 KCl, 5.8 CaCl2, 1 MgCl2, 10 EGTA, and 10 HEPES. Transfected cells were identified using an epifluorescence microscope to visualize GFP. Patch-clamp recordings were made in whole cell configuration using borosilicate glass electrodes fabricated with a microelectrode puller (model P-1000, Sutter Instruments, Novato, CA). The recordings were performed at room temperature (22°C) in voltage-clamp mode with a holding potential of −50 mV. Current recordings were amplified, low-pass filtered at 2 kHz, and digitized at 10 kHz using an Axopatch 200B Amplifier, Digidata 1440A Digitizer, and pCLAMP software (all from Axon Instruments).

Sugar uptake experiments. Oocytes expressing SGLT3 proteins were incubated with 50 μM α-methyl-β-glucose (αM-glc) with traces of [14C]αM-glucose in Na+ solution at pH 7.4 or 5, in Na+ free solution at pH 7.4, or in Na+ solution at pH 7.4 with Pz. After 1 h of incubation at room temperature, the oocytes were washed with cold Na+ free solution and were trypsinically solubilized with 10% sodium dodecyl sulfate (SDS). Sugar transport was determined using a scintillation counter. Noninjected oocytes from the same batch were used as control.
RESULTS

In this work, we cloned SGLT3a cDNAs and we studied the functional characteristics of the expressed proteins by using electrophysiology and sugar transport assays in *X. laevis* oocytes or mammalian cells that expressed the protein.

**Cloning of mouse SGLT3a.** Previous studies that have attempted to clone mSGLT3a cDNA from mouse kidney resulted only in partial cDNAs (28). To increase our odds of isolating full-length cDNA, we performed real-time PCR with cDNA obtained from total RNAs from several tissues of mouse revealing that mSGLT3a, like mSGLT3b, is highly expressed in the small intestine. Table 1 compares the expression levels of mSGLT3a and mSGLT3b mRNA relative to 18S rRNA in the small intestine and in the kidney based on our experiments. Attempts to clone mSGLT3a cDNA were then focused on mouse small intestine where mSGLT3a mRNA was found to be abundant.

We successfully cloned full-length mSGLT3a cDNA from small intestine total RNA by reverse transcription followed by PCR with gene-specific primers. We obtained a single band at 2,000 bp in an agarose gel (not shown). The cDNA was cloned in a TOPO vector (Invitrogen) and was fully sequenced. This cDNA sequence was identical to the partial cDNA sequence of mSGLT3a published in Tabatabai et al. (28) and to the complete predicted cDNA sequence of mSGLT3a published in GenBank.

**Effect of sugar in mSGLT3a at physiological pH.** To determine whether or not sugars depolarized mSGLT3a-expressing oocytes at pH 7.4, sugar-induced changes in membrane potential were recorded from oocytes injected with mSGLT3a cRNA. The sugars tested included D-glucose (glc), αM-glc, D-galactose (gal), and 1-deoxy-nojirimycin (DNJ), all of which are known to interact with other SGLT1 and SGLT3 proteins. Sugar-induced depolarizations were not observed in mSGLT3a with any of the sugars tested (Fig. 1A), while in mSGLT3b, glc and αM-glc induced large membrane depolarizations (Fig. 1B).

To verify that mSGLT3a was not sensitive to sugar at pH 7.4, glc-induced currents were recorded from voltage-clamped oocytes expressing mSGLT3a and were compared with those obtained from oocytes expressing mSGLT3b and from control oocytes. In these experiments, 100 mM glc was added to the external Na⁺ solution at pH 7.4 and currents were recorded at voltages ranging from -150 to +50 mV. Glc-induced currents were not observed from mSGLT3a at any voltage (Fig. 2A), similar to what we observed in control oocytes (Fig. 2C). In contrast, robust glc-induced currents were detected from mSGLT3b and, as expected, these currents were larger at more hyperpolarized voltages (Fig. 2B).

**pH effect on mouse SGLT3a.** It was previously found that hSGLT3 is sensitive to acidic pH, such that inward currents increase in more acidic solutions. Moreover, the sugar-induced currents were larger in acidic solutions even in the absence of Na⁺ (2, 4). To determine whether mSGLT3a responds to high proton concentrations like hSGLT3, we investigated the effects of increasing H⁺ concentration ([H⁺]) in the external solution in the presence or absence of sugar.

First, we tested how mSGLT3a responded to increasing [H⁺]. Representative currents at two pHs, pH 7.4 (Fig. 3A) or

![Fig. 2. Glucose did not induce current in mSGLT3a-expressing oocytes at pH 7.4. Glc-induced currents at voltages ranging from -150 mV to +50 mV were recorded in oocytes expressing mSGLT3a (A) or mSGLT3b (B) and in control oocytes (C). I, current. The two recordings performed in each of the representative oocytes show the currents obtained with the Na⁺ solution at pH 7.4 and in this solution with 100 mM glc added. Glc did not induce currents in mSGLT3a-expressing oocytes and control oocytes while glc induced large currents in mSGLT3b-expressing oocytes at every voltage tested.](#)

![Fig. 3. Currents in one representative mSGLT3a-expressing oocyte. A: oocyte expressing mSGLT3a was clamped at -50 mV with 100-ms test pulses to voltages ranging from -150 mV to +50 mV in a solution containing Na⁺ at pH 7.4. B: currents obtained from the same oocyte when the pH was 5. The lines at the left of the recordings in A and B indicate zero current.](#)
H⁺-induced currents at pH 7.4, 6.5, 6, 5.5, and 5 in solution with Na⁺ at voltages ranging from −150 to +50 mV were recorded. A: in mSGLT3a-expressing oocytes, lower pH induced larger currents. B: H⁺-induced currents were not observed in control oocytes. Data presented are means ± SE (n = 5 for mSGLT3a, and n = 3 for control oocytes) of the H⁺-induced currents.

Fig. 4. mSGLT3a is pH sensitive. H⁺-induced currents at pH 7.4, 6.5, 6, 5.5, and 5 in solution with Na⁺ at voltages ranging from −150 to +50 mV were recorded. A: in mSGLT3a-expressing oocytes, lower pH induced larger currents. B: H⁺-induced currents were not observed in control oocytes. Data presented are means ± SE (n = 5 for mSGLT3a, and n = 3 for control oocytes) of the H⁺-induced currents.

Lack of inhibition by phlorizin. Phlorizin (Pz) blocks glucose absorption across intestinal epithelia (7). It is a competitive inhibitor to SGLT1 (14, 24), SGLT2 (15, 23), pig SGLT3 (21), hSGLT3 (2, 4), and mSGLT3b (1). Figure 7 shows that neither H⁺-induced currents nor sugar-induced currents at pH 5 were inhibited by 200 μM Pz, indicating that, unlike other SGLT proteins, Pz may not interact with mSGLT3a.

Effect of Na⁺ absence on mSGLT3a currents. SGLT proteins are selective for Na⁺ ions. In SGLT1, H⁺ and Li⁺ can replace Na⁺ to drive sugar transport; however, the apparent affinity for sugar is affected (12). To determine the effect of Na⁺ on mSGLT3a-expressing oocytes, we recorded inward currents with and without Na⁺ in the absence (Fig. 8, A and B)
or in the presence (Fig. 8C) of glc. Figure 8 shows that the presence or absence of Na\(^+\) did not significantly affect the magnitude of the currents in each of the three conditions, pH 7.4, pH 5, and glucose at pH 5.

To further investigate the effect of H\(^+\) ions on the currents through mSGLT3a, we calculated the change in reversal potential (\(\Delta E_{\text{rev}}\)) under various conditions (Table 2). If protons were selectively transported, the reversal potential should shift to more depolarizing voltages towards the equilibrium potential for protons. For a membrane protein that is selective only for H\(^+\) ions, a positive shift of +138 mV is expected when changing the pH from 7.4 to 5. In mSGLT3a-expressing oocytes, and not in control oocytes, large shifts to more positive potentials were observed when lowering the pH of the external solution in the absence of sugar with \(\Delta E_{\text{rev}} = 46 \pm 4\) mV in Na\(^+\) solution and \(\Delta E_{\text{rev}} = 60 \pm 6\) mV in Na\(^+\)-free solution.

**Functional studies of rSGLT3a.** The function of mouse SGLT3a was different from the previous SGLT3 proteins tested. We wanted to test other SGLT3a proteins but none were available. Thus, we cloned full-length rSGLT3a cDNA. The predicted amino acid sequence of this full-length cDNA clone was identical to the predicted amino acid sequence for rSGLT3a; however, the nucleotide sequence was not identical to the genome entry for rSGLT3a (gi: 157823977). We expressed the protein in oocytes and performed similar experiments to the ones done with mSGLT3a. Like in mSGLT3a, in rSGLT3a-expressing oocytes, sugar did not induce any current in solutions at pH 7.4. In addition, inward currents were observed when increasing [H\(^+\)] and these currents were larger when sugar was added (Fig. 9A). Likewise, these currents were also observed in the absence of Na\(^+\) (Fig. 9B). Representative traces are shown in Fig. 9 of experiments repeated more than 10 times.

**Sugar transport in mSGLT3b.** Mouse SGLT3b transports sugar (1) while human SGLT3 does not, although hSGLT3 functions as a sugar sensor by depolarizing the cell in the presence of sugar (4). To determine whether ion transport observed in the presence of glucose at pH 5 corresponds to sugar transport, we performed sugar uptake experiments in the presence of Na\(^+\) at low pH using [\(^{14}\)C]M-glc in control oocytes and oocytes expressing either mSGLT3a or mSGLT3b (Fig. 10). Although sugar-induced currents were not observed in oocytes expressing mSGLT3a at pH 7.4, we performed the experiments at pH 7.4 to test that sugar was not being transported in a nonelectrogenic fashion. At pH 7.4, mSGLT3a had an uptake similar to control with 0.8 ± 0.15 pmol/h and 0.5 ± 0.04 pmol/h, respectively, while uptake with mSGLT3b was approximately 9 times higher (7.3 ± 0.6 pmol/h). At pH 5, mSGLT3a had a M-glc uptake of 0.3 ± 0.07 pmol/h similar to control (0.6 ± 0.2 pmol/h). These results show that sugar is not transported by mSGLT3a at pH 7.4 and although there is sugar-induced current when the pH is acidic, the protein does not transport sugar, suggesting that the ion transport that occurs at pH 5 is not coupled to sugar transport.

**Expression of mSGLT3a proteins in mammalian cells.** The functional data acquired for mSGLT3a in oocytes indicated that the characteristics of this protein do not resemble other well-studied SGLT proteins like SGLT1 and SGLT2, which are Na\(^+\)-sugar cotransporters. Data presented so far on SGLT3 proteins were obtained utilizing the *X. laevis* oocyte expression system. To learn whether the expression system influenced the characteristics of the protein, we tested SGLT3a’s response to glucose and H\(^+\) in a mammalian expression system. CHO cells were transfected with either mSGLT3a or mSGLT3b, and whole cell patch-clamp was performed in cells expressing these proteins. Cells clamped at −50 mV were perfused with Na\(^+\) solution with 1 or 50 mM glc at pH 7.4 or pH 5 to determine whether they displayed glc-induced or H\(^+\)-induced currents. Figure 11A shows a representative recording from a mSGLT3a-expressing cell and illustrates H\(^+\)- and glucose-induced currents at pH 5. However, glucose at pH 7.4 did not induce currents. The largest current observed with mSGLT3b (Fig. 11B) was in the presence of glc at pH 7.4, while glc

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**Fig. 7.** Phlorizin (Pz) did not inhibit H\(^+\)- or glucose-induced currents at low pH in mSGLT3a-expressing oocytes. Currents induced with 100 mM glc at pH 5 in the presence of Na\(^+\) with and without 200 µM Pz were recorded at voltages ranging from −150 mV to +50 mV in oocytes expressing mSGLT3a. The figure shows that the presence of Pz did not affect the induced currents. The recording shown is representative of six different experiments.
perfused at low pH did not induce current. Data from control recordings in cells transfected only with the vector and from additional recordings done with cells transfected with mSGLT3a or mSGLT3b are shown in Table 3. These results, which are consistent with our findings in X. laevis oocytes, clearly show that glc induced currents at pH 7.4 only in mSGLT3b-expressing cells, but not in control or in mSGLT3a-expressing cells, and that currents induced by H⁺ or glc at low pH exist only in mSGLT3a-expressing cells, but not in control or in mSGLT3b-expressing cells.

**Mutagenesis in mSGLT3a.** SGLT1 and SGLT2 in both human and mouse have a glutamine (Q) at position 457. However, human SGLT3 and mSGLT3a have a glutamate (E) at that position, while mSGLT3b has a glycine (G) (alignments in Ref. 2). The importance of residue 457 in SGLT proteins has been extensively studied. In the crystallized structure of Vibrio SGLT (vSGLT), the residue corresponding to 457 was identified as one of the residues that bind to the sugar (8). In hSGLT1, this residue has been shown to be important for sugar transport (5) and a mutation of this residue results in glucose-galactose malabsorption syndrome (38). We previously showed that mutating residue 457 in hSGLT3 from E to Q changed it from a sugar sensor to a sugar transporter with functional characteristics resembling SGLT1 (2).

Since the amino acid in mSGLT3a at position 457 is glutamate just like in hSGLT3, following the same reasoning as our earlier work, we mutated residue 457 in mSGLT3a from E to Q (E457Q-mSGLT3a). This mutation resulted in the recovery of some of the key characteristics of SGLT proteins, including the ability to perform Pz-sensitive and Na⁺-dependent sugar transport (Fig. 12). Hence, in the presence of Na⁺, transport of αM-glc by E457Q-mSGLT3a was ~24 times higher than wild-type with 12 ± 0.6 pmol/h vs. 0.5 ± 0.08 pmol/h, respectively. Addition of 100 μM Pz or removal of Na⁺ from the solution inhibited this transport.

**DISCUSSION**

Until the functional characterization of the human SGLT3 protein (4), SGLT proteins, as a class, were thought to function as Na⁺-substrate cotransporters. Human SGLT3 was the first SGLT to be described as a sensor and not a transporter after extracellular glc resulted in cell depolarization without glc transport (4). While in humans there is only one gene that codes for one SGLT3 protein, in mouse (26) and rat there are two genes coding for the proteins SGLT3a and SGLT3b. The amino acid identities between hSGLT3 and mSGLT3a, and, between hSGLT3 and mSGLT3b, are 80% and 77%, respectively, while mSGLT3a and mSGLT3b are 75% identical. We previously characterized mSGLT3b functionally and found that, like hSGLT3, mSGLT3b has low apparent sugar affinity compared with SGLT1. However, unlike hSGLT3 but similar to SGLT1, mSGLT3b is capable of transporting sugar, although the ion/sugar cotransport ratio is uncoupled compared with SGLT1 (1). In the current study, we cloned and character-

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**Table 2. Shift in reversal potential when changing extracellular solutions in oocytes expressing mSGLT3a or mSGLT3b**

<table>
<thead>
<tr>
<th>Glucose-Induced</th>
<th>Na⁺</th>
<th>H⁺-Induced</th>
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<tr>
<td></td>
<td>pH 7.4</td>
<td>pH 5.0</td>
</tr>
<tr>
<td>Control</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>mSGLT3a</td>
<td>0 ± 0</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>mSGLT3b</td>
<td>39 ± 5</td>
<td>3 ± 1</td>
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Values are means ± SE (n = 4–11) of the changes in reversal potential from individual oocytes. Two-electrode voltage-clamp experiments were performed in control oocytes and in oocytes expressing either mSGLT3a or mSGLT3b with or without Na⁺ to determine changes in reversal potential (ΔErev) under the following conditions: 1) when adding 100 mM d-glucose (glc) at pH 7.4; 2) when adding 100 mM glc at pH 5; and 3) when decreasing the pH from 7.4 to pH 5. Positive values indicate a shift to more positive potentials and negative values indicate a shift to more negative potentials. A change to more positive potentials was observed when H⁺ concentration ([H⁺]) was externally increased for both mSGLT3a and mSGLT3b with or without Na⁺ in the solution. However, when glc was added at pH 7.4, a right shift was observed for mSGLT3b only.
ized the activity of mSGLT3a to determine functional similarities and differences with other SGLT proteins.

Because of the high amino acid identity between mouse and human SGLT3 proteins, we expected mSGLT3a to function similarly to the other SGLT3 proteins and to find that sugars depolarized the cell when mSGLT3a was expressed. Surprisingly, the sugar specificity experiments showed no sugar-induced depolarization for mSGLT3a for glc, αM-glc, gal, or DNJ at pH 7.4. Although gal neither induced any response with hSGLT3 nor with mSGLT3b, we expected glc and αM-glc, both substrates of hSGLT3 and mSGLT3b, to depolarize mSGLT3a-expressing oocytes based on protein similarity (1, 4). Moreover, the inminosugar DNJ, a strong agonist of hSGLT3 with micromolar apparent affinity (33), induced no response in mSGLT3a at pH 7.4.

In addition to the lack of response to sugars at pH 7.4 by mSGLT3a, we found that several other notable characteristics of SGLT proteins were also missing. We found that in mSGLT3a there were no pre-steady-state currents when recording in the presence of Na\(^+\) (Fig. 3). For comparison, SGLT1 (3, 18–20), pig SGLT3-SAAT1 (21), and mSGLT3b (1) show large pre-steady-state currents, while in hSGLT3 these currents are almost nonexistent (2). Pre-steady-state currents have also been described in other secondary active transporters such as excitatory amino acid transporter 2 (34) and Na\(^+\)-Cl\(^-\)-GABA cotransporter (11) among others. Pre-steady-state currents are attributed to charge transfer involved in the association and dissociation of external Na\(^+\) ions and in the reorientation of the empty protein in the membrane (18, 25). In the simplified six-state kinetic model of SGLT1, the steps that generate pre-steady-state currents are the translocation of the empty transporter and the binding and unbinding of external Na\(^+\) ions (14). One explanation for the absence of pre-steady-state currents in mSGLT3a is that Na\(^+\) does not bind to the protein. Therefore, the protein would not transit through the same conformational states as SGLT1 or mSGLT3b. A lack of Na\(^+\) binding to mSGLT3a could also explain how H\(^+\) ions can induce the same magnitude of currents with and without Na\(^+\) (Fig. 8).

Interestingly, in the mutant E457Q-mSGLT3a, we observed sugar transport that was Na\(^+\) dependent, which could indicate that Na\(^+\) sensitivity is restored in this mutant, and point to position 457 as being involved in Na\(^+\) binding in SGLT proteins. SGLT1 has, and SGLT3 may also have, two Na\(^+\) binding sites. On the basis of the crystal structure of vSGLT, Faham et al. (8) suggested that its single plausible Na\(^+\) binding site is ~10 Å away from the substrate-binding site. This was supported by a mutation in the homologous Na\(^+\)-binding site in hSGLT1 that reduced the apparent Na\(^+\) and sugar affinities (8, 36).

SGLT3a and SGLT3b are expressed in primary cultures of mouse kidney cells (28, 29). In a study on the expression of SGLT3a in rat kidney, it was suggested that SGLT3 plays a role in mediating Na\(^+\) reabsorption in diabetes (16). Although SGLT1 and SGLT2 function as Na\(^+\)-glucose cotransporters in the kidney, SGLT3 behaves differently. On the basis of our results, positive currents permeate through mSGLT3a and the magnitude of the currents in Na\(^+\)-free conditions is comparable to currents in the presence of Na\(^+\). This strongly suggests that Na\(^+\) is not the ion transported. Although we suggest that the currents may be the result of the transport of H\(^+\), we cannot conclude the origin of the ions. H\(^+\) could open a pathway for the movement of other ions that results in inward current. Thus, we recommend caution in translating to SGLT3 specific biophysical characteristics based on results from other SGLT proteins.

Even though mSGLT3a does not respond to the presence of sugars at pH 7.4, we found that mSGLT3a shares some characteristics with hSGLT3. Since H\(^+\)-induced currents with and without sugar were observed with hSGLT3 (2, 4), we tested the pH sensitivity of mSGLT3a. We varied the pH in the external solution and determined that lowering the pH increased currents (Fig. 4). More importantly, several sugars

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Fig. 9. Rat SGLT3a responded to pH and glucose similarly to mSGLT3a. A: currents from an oocyte expressing rat SGLT3a were obtained with Na\(^+\) solutions at pH 7.4 or pH 5 in the presence or absence of glucose at voltages ranging from −150 mV to +50 mV. Currents at pH 5 were larger than at pH 7.4. Addition of glc to the solution at pH 5 further increased the current, but when the glc was added to the solution at pH 7.4, currents did not change. B: recordings were done in solutions in the absence of Na\(^+\), showing a pattern very similar to that observed when Na\(^+\) was present.

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Fig. 10. mSGLT3a did not transport sugar. Uptake of 50 μM αM-glc in control oocytes and in oocytes expressing mSGLT3a was measured in Na\(^+\) solution at pH 7.4 and pH 5. mSGLT3a-expressing oocytes did not transport sugar in any of the conditions tested. For comparison, the sugar uptake in mSGLT3b-expressing oocytes was also performed. Data shown are means ± SE (n = 4–5) of the sugar uptake in individual oocytes.
such as αM-gluc, gal, and DNJ that did not induce any response at pH 7.4, induced currents at acidic pH (Fig. 6). Hence, like hSGLT3, currents increased with increasing proton gradients with mSGLT3a and addition of sugars further increased those currents. Consequently, the induced inward currents were greater when gluc was present at pH 5 than at pH 7.4. Finally, neither mSGLT3a nor hSGLT3 transported sugar at physiological or acidic pH.

Contrary to mSGLT3a, mSGLT3b responds differently to changes in pH; gluc-induced currents are lower at acidic pH than at pH 7.4. Moreover, mSGLT3b at pH 5 only induced currents in response to αM-gluc and gluc but not to gal or DNJ. In addition to the decreased sensitivity to acidic conditions, mSGLT3b also transports sugar and displays pre-steady-state currents. All these suggest that the function of mSGLT3b is quite different from the function of mSGLT3a.

Table 3. Functional characteristics of mSGLT3a and mSGLT3b expressed in mammalian cells are similar to those obtained when these proteins are expressed in oocytes

<table>
<thead>
<tr>
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<th>Current, pA</th>
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<tr>
<td></td>
<td>Glucose-Induced</td>
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<tr>
<td></td>
<td>pH 7.4</td>
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<tr>
<td>Empty vector (n = 10)</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>mSGLT3a (n = 7)</td>
<td>2.2 ± 0.4</td>
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<tr>
<td>mSGLT3b (n = 20)</td>
<td>8.2 ± 1</td>
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Values are means ± SE (n = 7 to 20). Currents from patch-clamp recordings were observed in Chinese hamster ovary cells transfected with the empty vector or with a vector coding for either mSGLT3a or mSGLT3b. The cells were perfused with Na+-solution containing 1 or 50 mM gluc at pH 7.4 or pH 5 to test responsiveness to increasing concentrations of sugar or H+. Compared with control experiments with only the vector, mSGLT3a showed induced currents in response to increasing [H+] and to increasing [gluc] at low pH while mSGLT3b showed response only in response to increasing [gluc] at pH 7.4.

The interaction of mSGLT3a with sugars at pH 5 but not at pH 7.4, the absence of pre-steady-state currents, and the independence of Na+ for inward currents made mSGLT3a’s behavior very different from other SGLT proteins. Like mouse, rat also has two different genes coding for SGLT3a and SGLT3b. We cloned rat SGLT3a, studied the function of the protein, and learned that rat SGLT3a works similarly to mSGLT3a.

The outstanding differences in the function of mSGLT3a compared with other SGLT proteins drove us to look for some
characteristic that resembles a more typical SGLT protein. We took advantage of the crystal structure of vSGLT that shows that glutamine at position 428 is involved in sugar binding to explore the importance of the corresponding residue in mSGLT3a. Similar to hSGLT3 (2), a mutation from residue E to Q at position 457 in mSGLT3a permitted sugar transport at pH 7.4. This sugar transport was Na+ dependent and Pz sensitive, which are unique characteristics of the transport by SGLT proteins. This indicates that the fundamental structural elements necessary for being a Na+/substrate cotransporter are present in mSGLT3a.

From this study, it is clear that mSGLT3a is highly expressed in mouse intestine and that it may transport protons in the presence or absence of Na+. Previous studies have also investigated where SGLT3 is expressed in the gastrointestinal system. Immunofluorescence studies in human SGLT3 showed that it is expressed in cholinergic neurons in the submucosal and myenteric plexuses (4). This agrees with in situ hybridization experiments done in mouse intestine where expression was demonstrated in the cholinergic cells and not in the epithelial cells (6).

Additional studies have examined the expression of SGLT3 in enteroendocrine cells. In cultured GLUTag cells, a stable immortalized murine enteroendocrine cell line, the secretion of glucagon-like peptide in response to gluc or αM-gluc was recorded and found to be abolished by a lack of Na+ or the presence of Pz. The authors did not conclude which SGLT protein was responsible for that effect (10), but, on the basis of our results, we suggest that SGLT3a was not involved in the response since it does not recognize glucose at pH 7.4.

The pH of small intestine of rats and mice ranges from 4.7 to 6.1, depending on the segment of the intestine and the fasting state (22). On the basis of the different inward currents we obtained when working with mSGLT3a proteins at different pHs, we suggest that in vitro experiments to study mSGLT3a require adjustment of the pH to mimic the intestinal environment. If the pH of the culture media of the cells is about 7.4, as is common in in vitro experiments, this can lead to results that differ from what is likely to happen in vivo.

Two studies in rats in vivo researched the possible involvement of SGLT3 in response to glucose (9, 32). Freeman et al. (9), in addition to detecting SGLT3 by real-time PCR in intestine, suggested that SGLT3 is involved in gastric emptying and motility, as well as intestinal fluid secretion. Vincent et al. (32) suggested that native enterochromaffin cells respond to glucose via SGLT3 and activate intrinsic and extrinsic neurons. In these two studies there was no distinction made between SGLT3a and SGLT3b, likely because it was assumed that both proteins had similar functions.

In conclusion, our results on SGLT3a show that acidic conditions in the absence of sugar result in inward currents, probably carried by protons, and that currents increase in magnitude with increasing proton gradients. Importantly, these inward currents are larger in the presence of sugars. In past studies invoking SGLT3 as an explanation for physiological phenomenon, assumptions were frequently made regarding functional similarities between SGLT3a, SGLT3b, and even SGLT1, based heavily on sequence homology. Finally, we suggest that, in studies where SGLT3a or SGLT3b is involved, intestinal pH should be taken into account and a clear distinction between SGLT3a and SGLT3b should be incorporated, due to their very different functions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.B., D.M., and A.D.-S. performed the experiments; S.B. and A.D.-S. analyzed the data; S.B. and A.D.-S. interpreted the results of the experiments; S.B. and A.D.-S. prepared the figures; S.B. and A.D.-S. drafted the manuscript; S.B. and A.D.-S. edited and revised the manuscript; S.B. and A.D.-S. approved the final version of the manuscript; A.D.-S. conception and design of the research.

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