SAAT1 Is a Low Affinity Na+/Glucose Cotransporter and Not an Amino Acid Transporter

A REINTERPRETATION*

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Bryan Mackenzie,
Mariana Panayotova-Heiermann,
Donald D. F. Loo, Julia E. Leverš, and
Ernest M. Wright

From the Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024 and the
Department of Biochemistry and Molecular Biology,
University of Texas Medical School,
Houston, Texas 77035

Recently a member of the Na⁺/glucose (SGLT1) gene family of cotransporters was isolated from a pig renal cell line and was thought to be the neutral amino acid transporter (SAAT1). This cDNA (Kong, C., Trost, S. E., and Lever, J. E. (1993) J. Biol. Chem. 268, 1509–1512) encodes a 660-amino acid protein with 76% identity to SGLT1. To confirm and extend the kinetic characterization of SAAT1, we have expressed this clone in Xenopus oocytes and measured transport using both radiotracer and electrophysiological techniques. SAAT1 did not stimulate either 50 µM 2-(methylamino)isobutyrate uptake or 2-(methylamino)isobutyrate-evoked inward Na⁺ currents, but instead stimulated 50 µM ß-methyl-α-D-glucopyranoside uptake 27-fold from 2 ± 1 pmol·h⁻¹/oocyte (n = 9) to 55 ± 6 pmol·h⁻¹/oocyte (n = 9) and ß-methyl-α-D-glucopyranoside-evoked inward Na⁺ currents (I) by up to 1000 nA/oocyte. The apparent affinity constant for ß-methyl-α-D-glucopyranoside (Kₚ) was 2 mM and was independent of membrane potential from -30 to -150 mV but was voltage-sensitive between -30 and +30 mV. The relative sugar specificity for the transporter was ß-methyl-α-D-glucopyranoside >> ß-galactose >> 3-O-methyl-α-D-glucopyranose, 1-glucose. The sugar-evoked currents were Na⁺-dependent (Kₛ = 10 ms at -50 mV) and the Hill coefficient was 1. Kₛ decreased with hyperpolarization of the membrane from -50 to -150 mV. Phlorizin inhibited the ß-methyl-α-D-glucopyranoside-evoked current with apparent Kₛ of 18 µM at -50 mV. We conclude that the SAAT1 cDNA encodes a renal low affinity Na⁺/glucose cotransporter and propose that pig SAAT1 be renamed pSGLT2.

A full-length porcine cDNA sequence designated SAAT1 (1), cloned by screening an LLC-PK₁ cell cDNA library using the rabbit intestinal SGLT1 clone (2), was previously thought to encode the Na⁺-dependent neutral amino acid transporter System A. This conclusion was based upon expression studies in dexamethasone-induced COS-7 cells transiently transfected with the SAAT1 cDNA: uptake of 2-(methylamino)isobutyrate (MeAIB), a paradigm substrate for System A, was doubled 24 h after inducing the transfected cells, whereas uptake of the glucose analogue ß-methyl-α-D-glucopyranoside (ß-MG) was not affected (1). In order to confirm and extend these preliminary findings, we have expressed SAAT1 in Xenopus oocytes and attempted to provide a detailed analysis of its substrate scope and kinetics. Because of the amino acid sequence homology (76% identity) with the high affinity Na⁺/glucose transporter (SGLT1), we have tested both sugars and amino acids as potential substrates, measuring radiotracer uptakes and evoked currents.

EXPERIMENTAL PROCEDURES

Preparation of Oocytes—Oocytes, isolated from Xenopus laevis (Nasco, Fort Atkinson, WI) under anesthesia induced by immersion in ice/water, were defolliculated using a standard procedure with collagenase B, followed by collagenase-D regiment (3). Defolliculated stage VI oocytes were selected and maintained at 18 °C in modified Barth's medium (3) with 10 mg·l⁻¹ gentamicin sulphate.

Subcloning of the SAAT1 Sequence—In preliminary experiments in oocytes injected with cRNA synthesized from the pCTK plasmid (1), we did not identify any transporter expression, presumably because SAAT1 cDNA synthesized from pCTK lacks a poly(A) tail. To provide the 3' end of the cDNA with a poly(A) tract, the 2036-base pair SAAT1 sequence (1) was cut from pCTK using BamHI and EcoRI, blunt-ended, and subcloned into the polylinker region in Multiscribe plasmid (Pharmacia Biotech Inc.). The SAAT1-poly(A) insert was then cut out using the RanHi and EcoRI sites of the pASK2 plasmid and ligated into the Multiscr ipt kit (Ambion Inc.). The resulting construct we have named pBM1. Correct insertion of the sequences into the Multisc ript kit and addition of the poly(A) sequence were verified by restriction mapping and sequencing of around 150–200 bases through each of the 3' and 5' ends of the insert, using the Sequenec version 2.0 kit (U. S. Biochemical Corp.) according to the manufacturer's protocol.

Expression of SAAT1 in Oocytes—pBM1 was linearized with EcoRI and cRNA synthesized and capped using the Ambion Multisc ript kit with T7 RNA polymerase (Ambion, Austin, TX). Oocytes were injected 1 day after isolation with 50 ng of cRNA (at 1 µg·µl⁻¹ in sterile water), or water only, and incubated at 18 °C in modified Barth's medium plus gentamicin for up to 6 days before assay of radiotracer uptake or electrophysiological investigation.

Solutions—Experimental medium (100 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM Hepes, pH 7.5, with Tris base) was used for radiotracer and electrophysiological studies; NaCl was replaced by choline chloride for Na⁺-free or low Na⁺ solutions.

Measurement of Radiotracer Uptake and Amino Acid Uptake—After injection, oocytes were incubated 1 h at 20–22 °C in NaCl or choline chloride experimental media containing 50 µM ß-methyl-[U-¹⁴C]glucopyranoside (Amersham Corp.) or 50 µM 2-(methylamino)-[¹-¹⁴C]isobutyrate (Du Pont NEN). Uptake was terminated by rapidly aspirating the experimental medium and rinsing the oocytes, which were then solubilized with 5% SDS; radioactivity was assayed by liquid scintillation counting.

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† Research Fellow of the Human Frontier Science Program. To whom correspondence should be addressed: Dept. of Physiology, Center for the Health Sciences, UCLA School of Medicine, 10533 Le Conte Ave., Los Angeles, CA 90024-1751. Tel.: 310-825-6905; Fax: 310-206-6661; E-mail: bryan@physiology.medsch.ucla.edu.

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Electrophysiology Methods—All the electrophysiological data presented were determined in a single oocyte at 20–22 °C, 6 days after injecting with cRNA; however, each result was confirmed in another 2–4 oocytes. A two-microelectrode voltage-clamp system (5) was used to measure currents evoked by a range of sugars and amino acids; the oocyte was held at −50 mV (V) and step changes in membrane potential (V,−) were applied, each for a duration of 100 ms (±50 mV to −150 mV in 20-mV increments), first in Na+ medium, then after superfusing 2–3 min with the test solution. The currents were averaged over three sweeps and low pass-filtered at 500 Hz by a nine-pole Bessel filter. Additionally, current and voltage were continuously monitored on a chart recorder. Test solutions were always washed out by superfusing the oocyte with Na+-free (choline chloride) medium. Data were fitted to Equation 1, for which I is the evoked current, I succ is the derived current maximum, [S] is the substrate concentration, nH is the Hill coefficient, and K is the substrate concentration at which current is half-maximal (error bars represent the error in the estimates).

\[ I = \frac{I_{\text{succ}}[S]^n}{K^n + [S]^n} \]

(Eq. 1)

RESULTS AND DISCUSSION

The uptake of 50 μM α-methyl-D-[14C]glucopyranoside was increased 20-fold in oocytes injected with pBME1-cRNA (mean ± S.E., 55 ± 6 pmol·h⁻¹/oocyte, n = 9) when compared to water-injected oocytes (2 ± 1 pmol·h⁻¹/oocyte, n = 9). However, the uptake of the amino acid analogue 2-(methylamino)fluorescein, a paradigm substrate for the mammalian amino acid transporter System A, was unchanged (cRNA, 0.4 ± 0.2 pmol·h⁻¹/oocyte, n = 9; water, 1.0 ± 0.3 pmol·h⁻¹/oocyte, n = 8). These results were replicated in two other batches of oocytes.

The electrophysiological data presented are all derived from a single oocyte injected with pBME1-cRNA. The findings have been confirmed in subsequent experiments with cRNA-injected oocytes; sugar-evoked currents are absent in water-injected oocytes. In the cRNA-injected oocyte, clamped at V = −50 mV (Fig. 1), α-galactose evoked a much smaller current (~10 nA). The transporter appears to exclude 3-O-methyl-D-glucopyranose and the L-isomer of glucose. 5 mM MeAIB failed to evoke a current; we have also found, in subsequent experiments, that 5 mM β-alanine, L-alanine, L-arginine, L-glutamate, and L-tryptophan each evoked no current (data not shown).

We proceeded to perform a kinetic characterization of this sugar transporter using αMG as the substrate. The αMG-evoked currents showed a non-linear dependence upon voltage (test potential, V) between −150 mV and +50 mV. The I/V relationships for αMG and D-glucose (at 20 mM sugar and 100 mM Na+) and the I/V relation (see below and Fig. 2B) were each virtually superimposable. The sugar-dependent I/V curves showed no reversal within the range of V tested (upper limit +50 mV) and did not saturate at hyperpolarizing potentials (~150 mV).

The Hill coefficient (nH) for sugar (calculated using Equation 1) was 1 over the entire voltage range. The αMG concentrations (K;αMG) at which current was maximal and the current maximum (I max) evoked by αMG (at 100 mM Na+) were determined for each V. K;αMG was independent of voltage between V = −150 mV and +50 mV (Fig. 2A); at −50 mV, K;αMG is 1.7 ± 0.1 mM. From −10 mV to +30 mV, however, K;αMG rose sharply; this feature is reminiscent of SGLT1 at low Na+ concentrations (3) and is likely a reflection of the voltage dependence for Na+ binding (Fig. 3B). Preliminary data (not shown) from additional cRNA-injected oocytes suggested that SAA1 transports α-glucose with affinity similar to αMG but α-galactose is transported with a very low affinity (K;αM > 20 mM). This differs from the substrate specificity of SGLT1, which transports α-glucose, α-galactose, and αMG each with similar, very high affinities and 3-O-methyl-D-glucopyranose with lower affinity (4).

The Hill coefficient (nH) for α-glucose activation of the αMG-evoked currents was calculated for each V (see Fig. 3); at all voltages...
from -150 to -50 mV, $n_H$ did not deviate significantly from 1, indicating an apparent stoichiometry of 1 Na\+:1 aMG, in contrast to SGLT1, which operates with transport stoichiometry 2 Na\+:1 glucose (3–5).

The apparent $K_{p}^{c}$ exhibited a non-linear dependence upon voltage from -150 mV to -90 mV (Fig. 3B), $K_{p}^{c}$ affinity increasing with hyperpolarization. The apparent $K_{p}^{c}$ varied with $V_m$ in a similar fashion to SGLT1 (3) but over a narrower range of values than SGLT1 and lower at any given $V_m$.

The $\beta$-glycoside phlorizin (Pz) reversibly blocked the aMG-evoked current (Fig. 4). The voltage dependence of the apparent half-maximal phlorizin inhibition constant ($K_{p}^{c}$) was estimated by measuring the phlorizin-inhibitable current evoked by 2 mM aMG in Na\+ medium at 1, 10, and 100 mM Na\+. The relationship between the maximum aMG-evoked current determined as a function of [aMG], $I_{aMG}$, and the test potential ($V_m$) resembled that of SGLT1 (3, 5, 6, 7).

We have presented data that demonstrate that cRNA synthesized from the SAAT1 cDNA encodes a Na\+-dependent, low affinity sugar transporter, and we now propose the renaming of SAAT1 in view of its evident transport function. Since its amino acid sequence is 76% identical (and 88% similar) to that of pig renal SGLT1 (8), we propose that the porcine SAAT1 (GenBank accession number L02900) be renamed pSGLT2 (porcine Na\+-glucose transporter 2).

A human kidney cortex clone, Hu14 (9) has been claimed to be SGLT2 (10) but this designation is disputable since (i) there was very low expression of Hu14 in both oocytes and COS-7 cells, e.g. sugar-evoked currents in oocytes were <1 nA, in contrast to those of 1000–2000 nA observed for SGLT1 and pSGLT2; and (ii) the amino acid sequence of Hu14 is 91% identical to a rabbit renal Na\+-nucleoside cotransporter (11) but only 59% identical to human SGLT1. Further work is required to substantiate the assertion that Hu14 is in fact the human SGLT2.

Failure to detect an increase in aMG uptake in the earlier transient COS-7 cell expression study (1) appears to be a result of impaired expression of the recombinant due to the lack of a poly(A) tail of sufficient length in the SAAT1-pMAMneo construct. Up-regulation of endogenous System A activity could account for the modest increase in MeAIB transport observed after transfection.

Turner and Moran (12–14) classically attributed low and high affinity glucose transport activities to two distinct transporters and molecular evidence exists for two renal Na\+-dependent glucose transporters; Northern blots showed that SGLT1 was located predominantly in outer medulla (S1 segment of the tubule), whereas brush-border membrane vesicles from outer cortex (S2) had a greater capacity for Na\+-dependent glucose uptake (15). The functional characteristics of pSGLT2 are reminiscent of the high capacity system described for rabbit renal outer cortical (S1) brush-border membrane vesicles (12, 14), with regard to its low affinity ($K_{p}^{c}$ for glucose, 6 mM), substrate specificity, and 1 Na\+:1 glucose stoichiometry (facilitating the re-uptake of the bulk of luminal glucose at low energy cost). In contrast, the high affinity SGLT1 in the S2 segment appears to serve a scavenging role, with glucose re-uptake coupled to the transport of 2 Na\+ (13).
The expression of both high affinity (SGLT1) and low affinity (pSGLT2) glucose transporters in LLC-PK cells is consistent with earlier findings previously discussed (16), suggesting that this cell line contains cell types from both S₁ and S₂ segments of the kidney tubule.

The characterization of pSGLT2 may provide more focus to structure/function studies of Na+-dependent glucose transporters; only 24% of the amino acid sequence of pSGLT2 differs from that of pig SGLT1, and this presumably must account for the functional diversity observed between pSGLT2 and SGLT1, most notably (i) 1 Na+:1 αMG transport stoichiometry for pSGLT2 as opposed to 2:1 for SGLT1, (ii) a 10-fold higher \( K_{\text{m}}^{\alpha MG} \) than rabbit SGLT1 (3), (iii) discrete sugar selectivities, and (iv) lower phlorizin affinity than SGLT1.

Further electrophysiological characterization of pSGLT2 will include measurement of \( K_{\text{m}}^{\alpha MG} \) as a function of external Na⁺ concentration, to assess the nature of substrate binding (17), and investigation of pre-steady-state currents as for SGLT1 (18), permitting the development of a testable kinetic model for the operation of pSGLT2.

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REFERENCES

SAAT1 is a low affinity Na+/glucose cotransporter and not an amino acid transporter. A reinterpretation.

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