Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 337 (2005) 892–900
BBRC
www.elsevier.com/locate/ybbrc

Characterization of a branched-chain amino-acid transporter SBAT1 (*SLC6A15*) that is expressed in human brain ☆

Hitomi Takanaga [a,b], Bryan Mackenzie [a,c], Ji-Bin Peng [a,d], Matthias A. Hediger [a,e,*]

[a] *Membrane Biology Program and Renal Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA*
[b] *Carnegie Institution of Washington, Division of Plant Biology, 260 Panama Street, Stanford, CA 94305-4101, USA*
[c] *Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, P.O. Box 670576, Cincinnati, OH 45267-0576, USA*
[d] *Division of Nephrology, University of Alabama at Birmingham, 1530 3rd Avenue South, Birmingham, AL 35294-0006, USA*
[e] *Institute for Biochemistry and Molecular Biology, University of Berne, Bühlstrasse 28, CH-3012 Bern, Switzerland*

Received 8 September 2005
Available online 30 September 2005

Abstract

The *SLC6* gene family comprises membrane proteins that transport neurotransmitters, amino acids, or osmolytes. We report the first functional characterization of the human *SLC6A15* gene, which codes for a sodium-coupled branched-chain amino-acid transporter 1 (SBAT1). SBAT1 expression is specific to the brain. When expressed in *Xenopus* oocytes, SBAT1 mediated Na^+-coupled transport of hydrophobic, zwitterionic α-amino and imino acids. SBAT1 exhibited a strong preference for branched-chain amino acids (BCAA) and methionine ($K_{0.5}$ 80–160 μM). SBAT1 excluded aromatic or charged amino acids, β-amino acids, glycine, and GABA. SBAT1-mediated transport of amino or imino acids was extremely temperature-dependent ($Q_{10} = 9$) and was inhibited at acidic pH. PKC activation reduced the plasma-membrane population of SBAT1 protein. SBAT1-mediated transport of BCAA, particularly leucine, may be an important source of amino nitrogen for neurotransmitter synthesis in glutamatergic and GABAergic neurons.

Keywords: Amino acid transport; Cotransporters; Neurotransmitter synthesis; Sodium-dependent transport; *Xenopus* oocyte

Neurotransmitter reuptake was proposed in the 1960s as a means of terminating synaptic transmission and regulating the release of neurotransmitter. The observation of high-affinity, saturable transport suggested specific, carrier-mediated transport. In the 1970s and 1980s, functional assays using synaptosomal membrane preparations identified which of the neurotransmitters were cleared by reuptake transporters and identified the driving ions associated with these transporters.

The original members of the *SLC6* gene family of solute carriers were the Na^+- and Cl^--dependent neurotransmitter transporters [1] (see http://www.bioparadigms.org/slc/SLC06.htm). First among these was the rat GABA transporter, GAT1 (SLC6A1) [2]. Expression cloning of the human norepinephrine transporter NET (SLC6A2) was reported soon thereafter [3]. Several other transporters—those serving the monoamine neurotransmitters (norepinephrine, serotonin, and dopamine), the amino-acid neurotransmitters (e.g., glycine), and the osmolytes (e.g., betaine, taurine)—were cloned in the 1990s, based on their close homology (Fig. 1) (see [1] for review).

More recently, the characterization of "orphan" members of this family has revealed a fourth branch, transporters serving a broader range of neutral amino acids (Fig. 1). Proteins of this branch differ in structure from those of the other branches in having two large potentially glycosylated

☆ *Abbreviations:* BCAA, branched-chain amino acid(s); BCH, 2-amino-bicyclo-[2,2,1]-heptane-2-carboxylic-acid; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; ChoCl, choline chloride; DOG, 1,2-dioctanoyl-*sn*-glycerol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HO-Pro, hydroxy-proline; MeAIB, 2-methylaminoisobutyrate; NMDGCl, *N*-methyl-D-glucamine chloride; NaGlc, sodium gluconate.

* Corresponding author. Fax: +41 31 631 3410.
E-mail address: matthias.hediger@mci.unibe.ch (M.A. Hediger).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.09.128

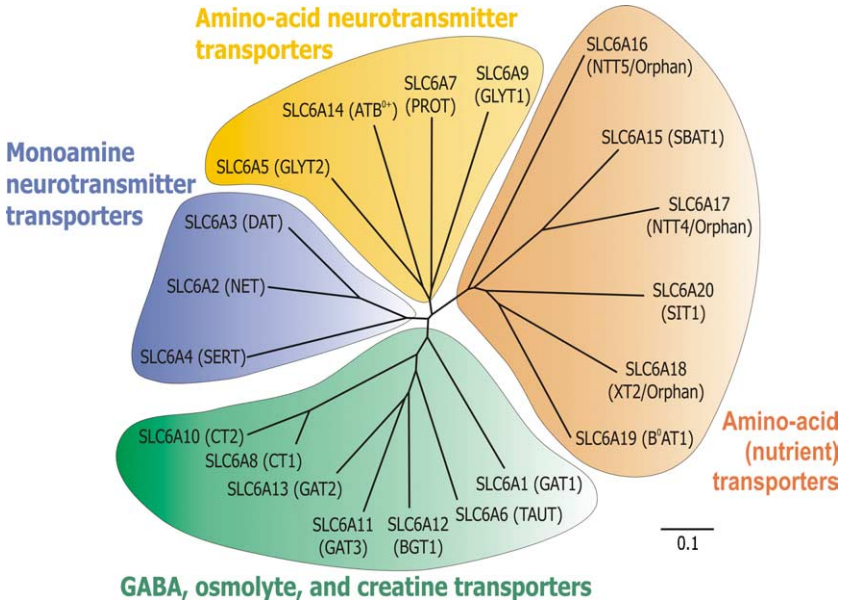

Fig. 1. Unrooted phylogenetic tree of the 17 members of the human SLC6 transporter family. Multiple sequence alignment was performed using ClustalW [34] at http://www.ebi.ac.uk/clustalw/ and the tree was displayed using TreeView [35] (see http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). The bar indicates the fraction of amino acid residues substituted.

extracellular loops. This new SLC6 branch includes the broad-range neutral amino acid transporter B^0AT1 (SLC6A19) known to be defective in Hartnup's disorder [4–6], and a proline transporter SIT1 (SLC6A20) with properties of classic System Imino [7,8]. The SLC6 family is part of a superfamily that extends from mammals to insects and is composed of proteins mediating transport of neurotransmitters or other amino acids required for neurotransmitter synthesis [9].

We report here the functional characterization of SLC6A15, a member of the novel, fourth branch of the human SLC6 family. Uhl et al. [10] first isolated the SBAT1 cDNA in 1992 but its function remained unknown. With the aid of voltage clamp and radiotracer assays in cRNA-injected oocytes, the present study reveals that the *SLC6A15* gene codes for a sodium-coupled amino acid transporter that we have named SBAT1. SBAT1 transports several large, hydrophobic, zwitterionic (net neutral) amino acids, with a preference for the branched-chain amino acids (BCAA; leucine, valine, and isoleucine) and methionine.

Methods

Isolation of SBAT1 cDNA. To clone human SBAT1, we added 100 ng of random hexamers and 1 μl dNTPs (10 mM) to 0.5 μg of human brain poly(A)$^+$ RNA (Invitrogen) in a total volume of 10 μl, and incubated the mixture for 10 min at 65 °C before it was chilled on ice. We then added 2 μl dithiothreitol (0.1 M), 2 μl of 10× reverse transcriptase buffer, 4 μl $MgCl_2$ (25 mM), and 40 U of RNaseOUT (Invitrogen) before incubating the mixture at 25 °C for 2 min. To initiate cDNA synthesis, we added 200 U of Superscript II-RT (Invitrogen) and incubated the reaction at 25 °C for 10 min and then at 42 °C for 1 h. The full coding sequence for SBAT1 was amplified using AccuPrime Pfx DNA polymerase (Invitrogen) with proofreading activity. Amplification conditions were as follows: 95 °C, 2 min, 30 cycles of denaturing (95 °C, 20 s), annealing (58 °C, 30 s), and extension (68 °C, 2 min 30 s), 95 °C, 2 min, 35 cycles of denaturing (95 °C, 20 s), annealing (60 °C, 30 s), and extension (68 °C, 2 min 30 s) for the nested PCR. The PCR product was inserted into the pOX(+) vector (see [7] for details). Human SBAT1 was amplified (bp −429–2767) using primers 5′-GTATCCCGTGCTGTTTCCCTGG-3′ and 5′-TCATCC CAGCGTTAGTGCCTTCTC-3′, and, for the nested PCR (bp 1–2193), using primers 5′-TTTGGTACCATGCCCAAAAATAGCAAGGT-3′ (containing a *Kpn*I restriction site) and 5′-GGGTCTAGACTA CAAATCAGATTCTGGCA-3′ (containing an *Xba*I restriction site) and inserted into the polylinker of pOX(+). We performed sequencing in both directions and found no differences between the coding sequence of the SBAT1 cDNA that we isolated and that of a clone with Accession No. NM_182767in the NCBI database.

Heterologous expression of human SBAT1 in Xenopus oocytes. The pOX(+) vector containing human SBAT1 cDNA under the SP6 promoter was linearized with *Swa*I. cRNA was synthesized in vitro with the use of the mMESSAGE mMACHINE kit with SP6 RNA polymerase (Ambion). Oocytes were isolated from *Xenopus laevis* (under 2-aminoethylbenzoate anesthesia), treated with collagenase A (Roche Diagnostics), and stored at 18 °C in modified Barths' medium [11,12]. Oocytes were injected with ≈50 ng cRNA and incubated for 2–3 days before functional assays were performed at 22–23 °C (except where noted in Fig. 7I). Functional assays were performed using a standard Na^+ uptake medium containing 100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.5 with Tris base). For low-pH medium, 5 mM Mes was used in place of 5 mM Hepes. For Na^+-free or low-Na^+ medium, NaCl was replaced by equimolar choline chloride (ChoCl) or *N*-methyl-D-glucamine chloride (NMDGCl). For low-Cl^- medium, NaCl was replaced by equimolar sodium gluconate (NaGlc).

Voltage-clamp experiments. A two-microelectrode voltage clamp (Dagan CA-1B) was used to measure currents associated with SBAT1 in oocytes. Microelectrodes (resistance 1–7 MΩ) were filled with 3 M KCl. When we superfused chloride-free solution, the bath (reference) electrode was placed in a 3-M KCl well connected to the main bath via a 3-M KCl/agar bridge. Voltage-clamp experiments included two protocols: (i) continuous current recordings were obtained at a holding potential (V_h) of −70 mV, low-pass filtered at 1 Hz, and digitized at 10 Hz. (ii) Oocytes were clamped at $V_h = -70$ mV, and step-changes in membrane potential (V_m) were applied (from +50 to −150 mV in 20-mV increments), each for a duration of 100 ms, before and after the addition of substrate. Current was low-pass filtered at 500 Hz and digitized at 5 kHz. Steady-state data were obtained by averaging the points over the final 16.7 ms at each V_m