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Cloning and Expression of a Novel Na^+-dependent Neutral Amino Acid Transporter Structurally Related to Mammalian Na^+/Glutamate Cotransporters*

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A cDNA has been isolated from human hippocampus that appears to encode a novel Na^+-dependent, Cl^--independent, neutral amino acid transporter. The putative protein, designated SATT, is 529 amino acids long and exhibits significant amino acid sequence identity (39–44%) with mammalian L-glutamate transporters. Expression of SATT cDNA in HeLa cells induced stereospecific uptake of L-serine, L-alanine, and L-threonine that was not inhibited by excess (3 mM) 2-(methylamino)-isobutyric acid, a specific substrate for the System A amino acid transporter. SATT expression in HeLa cells did not induce the transport of radiolabeled L-cysteine, L-glutamate, or related dicarboxylates. Northern blot hybridization revealed high levels of SATT mRNA in human skeletal muscle, pancreas, and brain, intermediate levels in heart, and low levels in liver, placenta, lung, and kidney. SATT transport characteristics are similar to the Na^+-dependent neutral amino acid transport activity designated System ASC, but important differences are noted. These include: 1) SATT's apparent low expression in ASC-containing tissues such as liver or placenta; 2) the lack of mutual inhibition between serine and cysteine; and 3) the lack of trans-stimulation. SATT may represent one of multiple activities that exhibit System ASC-like transport characteristics in diverse tissues and cell lines.

Carrier-mediated amino acid transport has evolved to maintain transmembrane fluxes of amino acids for cellular nutrition and metabolism. In eukaryotic cells, a large number of distinct amino acid transport systems have been distinguished based on differences in substrate specificity, kinetic properties, and ionic dependence (1, 2). Our knowledge of the structural properties of amino acid transporters is limited. However, recent advances in the cloning of amino acid transporters have begun to provide insights into the molecular basis of carrier-mediated amino acid transport. A cDNA clone, SAAT1, has recently been described that exhibits structural and sequence similarity with the Na^+/glucose cotransporter family (3) and codes for a Na^+-dependent neutral amino acid transporter with properties similar to System A (4). A murine ecotropic retroviral receptor, which exhibits sequence homology with yeast permeases for histidine and arginine, has recently been found to induce Na^+-independent transport of cationic amino acids with properties similar to System y^+ (5, 6). cDNAs have also been reported for putative regulatory subunits of a transporter for cystine, dibasic, and neutral amino acids with properties similar to System $b^{0,+}$, which appear to contain a single transmembrane domain and exhibit sequence similarity to glucosidases (7–9). Furthermore, cDNAs have been isolated for plasma membrane transporters for the neurotransmitter/modulator amino acids, including γ-aminobutyric acid (10–13), glycine (14, 15), and proline (16). Recently three distinct, but related, Na^+/glutamate cotransporters have been cloned and functionally characterized (17–19), which appear to comprise a distinct gene family of membrane transport proteins. An intriguing result to emerge from the molecular cloning studies is the extent of structural diversity within the group of amino acid transporter proteins.

To explore the molecular diversity within the family of glutamate transporters, we performed a polymerase chain reaction on human hippocampal poly(A)$^+$ RNA using primers corresponding to a human expressed sequence tag that exhibits distant homology to an *Escherichia coli* glutamate/aspartate carrier (20, 21). We identified a novel PCR[1] product that was used to isolate a full-length cDNA clone from human hippocampus that exhibited 39–44% homology with mammalian glutamate transporters. Surprisingly, despite the sequence relationship with mammalian glutamate transporters, expression of this cDNA clone in HeLa cells did not induce L-glutamate transport but did induce Na^+-dependent neutral amino acid uptake with several properties similar to System ASC. However, important differences were noted indicating that SATT may represent only one of several molecular entities that collectively account for System ASC-like transport.

EXPERIMENTAL PROCEDURES

PCR and Library Screening—Poly(A)$^+$ mRNA (1 μg) purified from human hippocampus was converted to single-stranded cDNA (Superscript, Life Technologies Inc.) using random primers (Pharmacia LKB Biotechnology Inc.) and subjected to PCR (22) with Taq polymerase as described by the manufacturer (Perkin-Elmer). Amplification was conducted with primers 5′-CCCATTTGCGACAGATTT-3′ and 5′-CGCCTGCTGCTCCAACA-3′ corresponding to a previously described human hippocampal expressed sequence tag (21). Amplification conditions

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L19444.

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[1] The abbreviations used are: PCR, polymerase chain reaction; kb, kilobase(s); KRP, Krebs-Ringer-phosphate; MeAIB, 2-(methylamino)-isobutyric acid; bp, base pair(s).

were 94 °C for 1 min, 45 °C for 1 min, and 72 °C for 1 min, with a final extension of 10 min. The resulting PCR product (~0.3 kb) was cloned into the TA cloning vector (Invitrogen, San Diego, CA). The cloned insert was used as a template in a PCR containing 0.25 mCi of [^{32}P]dCTP to generate a probe that was used to screen 7 × 10^5 phage recombinants from a human hippocampal cDNA library in λ-zap (Stratagene, La Jolla, CA) as previously described (16). Bluescript plasmids pBS SKII(−) were rescued from plaque-purified positives by *in vivo* excision as described by the manufacturer (Stratagene). Overlapping clones were aligned by a restriction map based on the enzymes *Bam*HI, *Xba*I, *Eco*RI, and *Xho*I. The nucleotide sequence was determined on both strands for the two largest clones, 5 and 14, from alkaline lysate minipreps of double-stranded DNA, by dideoxynucleotide chain termination using Sequenase (U. S. Biochemical Corp.), with synthetic oligonucleotides as sequencing primers.

Expression and Pharmacological Studies—Clone 5 contained a full-length coding sequence downstream from the T7 promoter region of the plasmid vector pBS SKII(−). HeLa cells, 150,000–300,000/well in 24-well cell culture plates (Becton-Dickinson), were infected with recombinant vaccinia virus strain VTF_{7-3} (15 plaque-forming units/cell) expressing T7 RNA polymerase (23), followed 30 min later by liposome-mediated (3 μg/well; Lipofectin, Life Technologies Inc.) transfection of clone 5 (designated SATT) (1 μg/well). Triplicate control transfections of a non-functional fragment of a rat proline transporter (16) cloned into pBS SKII(−), done under identical conditions, were included on each 24-well plate to determine background transport. Amino acid transport assays were conducted 8–10 h after transfection as described (24). Briefly, HeLa cells were incubated twice, for 10 min each, in 2 ml of choline Krebs-Ringer-phosphate buffer (choline KRP) followed by a 1-min incubation with radioactive amino acid (3 μM; 0.5–1.5 μCi of the radiolabeled substrate) in 250 μl of uptake buffer (sodium KRP or choline KRP) in the presence or absence of unlabeled amino acids. MeAIB (3 mM) was included in the uptake buffer to eliminate endogenous transport attributable to System A (1, 2). Following incubation with the radiolabeled substrate, the uptake buffer was washed away (four rapid changes of 2 ml each of ice-cold choline KRP). After washing, plates were dried in a fume hood, and the cells were lysed by addition of 0.2 ml of 0.2% SDS, 0.2 N NaOH. Lysate counts/min were determined for each well. For determination of the specific activity of the radiolabeled substrate, aliquots of the uptake mixture were counted. The data are expressed as picomoles of radiolabeled substrate transported per well per min.

To examine the chloride dependence of SATT-induced uptake, chloride in the uptake buffer was replaced by thiocyanate or acetate. To test for trans-stimulation, transport was assayed immediately after preloading HeLa cells (twice for 10 min each in choline KRP) with 3 mM L-alanine or 3 mM L-threonine. The cells were rinsed well prior to conducting the transport assay.

Northern Hybridization Analysis—A 1595-bp *Bam*HI-*Xho*I fragment of clone 14 that represented the coding sequence for the COOH-terminal 421 amino acids of SATT and 332 bp of the 3′-untranslated sequence was radiolabeled with [^{32}P]dCTP by random priming (Life Technologies Inc.) and hybridized to a human multiple tissue Northern blot (Clontech, Palo Alto, CA). Hybridization conditions were 5 × SSPE, 10 × Denhardt's solution, 200 μg/ml sheared and denatured salmon sperm DNA, 35% formamide, 2% SDS, and 1.5 × 10^6 cpm/ml radioactive probe at 42 °C for 18 h. After hybridization, the blot was washed with four changes of 2 × SSC, 0.05% SDS at room temperature for 10 min each followed by two changes of 0.1 × SSC, 0.1% SDS at 50 °C for 20 min each. The blot was exposed to x-ray film for 1, 2, or 6 days at −80 °C.

Chromosomal Localization—DNA from somatic cell hybrids for chromosome mapping was obtained from Coriell Cell Repositories (mapping panel II) (Camden, NJ). A 10-μg aliquot of somatic cell hybrid DNA was digested with *Hin*dIII or *Eco*RI and separated on a 0.8% agarose gel. The DNA was transferred to nylon filters by Southern blotting and hybridized with the 1595-bp fragment from clone 14 described for the Northern blot (see above) that had been radiolabeled with [^{32}P]dCTP using random priming. Hybridization was carried out using the Quikhyb hybridization solution (Stratagene) at 65 °C for 12 h. Filters were washed with 5 ×, 1 ×, and 0.5 × SSPE and 0.1% SDS at 65 °C for 30 min each. Blots were exposed to Kodak XAR film at −70 °C for 24–36 h.

RESULTS AND DISCUSSION

Screening of a human hippocampal cDNA library with the PCR-generated probe described under "Experimental Procedures" yielded 10 overlapping cDNA clones. One full-length cDNA, clone 5, was isolated and sequenced to identify an open reading frame that codes for 529 amino acids (Fig. 1). The first ATG present in the cDNA was assigned as the initiation codon on the basis that it was 6 base pairs downstream from a single in-frame stop codon. The 3′-untranslated region of clone 5 ends in a poly(A^+) tail but lacks a classical polyadenylation signal; however, the stretch of six As 27 bases upstream from the start of the poly(A) tail may substitute for the canonical AAUAAA. Kyte-Doolittle hydropathy analysis of the putative protein reveals the presence of multiple regions (6–10) of significantly extended hydrophobicity suitable for the formation of transmembrane α-helical domains (data not shown). Based on the subjective nature of hydropathy analysis, it is not possible to reliably predict the number of transmembrane domains. The amino terminus does not contain a readily identifiable signal sequence (25), suggesting that this domain resides on the cytoplasmic face of the membrane. Two canonical sites for *N*-linked glycosylation are present on a presumably extracellular 72-amino acid hydrophilic loop between putative transmembrane domains 3 and 4. Interestingly, a large, glycosylated hydrophilic extracellular loop is also present between transmembrane domains 3 and 4 in the Na^+- and Cl^--dependent neurotransmitter transporter family (26). As shown in Fig. 1, a number of consensus sequences for protein kinase-mediated phosphorylation are present in the putative cytoplasmic domains of the SATT protein. A leucine heptad repeat motif extends from the fourth transmembrane domain through the second cytosolic loop into the fifth transmembrane domain (amino acid residues 223–258). It remains to be determined whether this motif plays a role in transporter subunit oligomerization.

A search of protein sequence data bases (April, 1993) with the putative SATT protein sequence revealed significant amino acid sequence similarity (39–44% amino acid sequence identity) with a recently described family of mammalian Na^+/glutamate cotransporters including GLTP (17), EAAC1 (19), and GLT1 (18) (Fig. 2). As shown in Fig. 2, the amino acid sequence conservation is approximately 3-fold higher in the carboxyl-terminal half of these proteins. Furthermore, SATT and the cloned mammalian glutamate transporters exhibit very similar hydropathy profiles (data not shown) and share a conserved putative glycosylation site (SATT residue Asn^{203}) and several putative phosphorylation sites (SATT residues Ser^{250}, Thr^{308}, and Ser^{364}) (see Fig. 2). Of particular interest, the sequence motif AA(I,V,L)FIAQ that is conserved throughout the evolutionary diversity of glutamate transporters from prokaryotes to mammals is also conserved in SATT. In contrast, SATT does not share significant structural or amino acid sequence homology with other known ion-coupled cotransporters.

When a human multiple tissue Northern blot was screened at high stringency with the SATT probe (Fig. 3), four prominent transcripts were observed, at 4.8, 3.5, 2.8, and 2.2 kb. Hybridization signals were most prominent in skeletal muscle, pancreas, and brain; a weaker signal was observed in heart. Only low levels of SATT mRNA were detected in liver, kidney, lung, and placenta. The cDNA whose sequence and expression characteristics are described here most likely corresponds to the 2.2-kilobase species seen in Fig. 3.

To examine whether the multiple transcripts observed by Northern blot analysis represent the products of related genes or differentially processed species arising from a single primary transcript, a probe identical to the one employed in Northern blot analysis was used to locate the SATT gene in the human genome by Southern hybridization of a DNA panel from somatic hybrid cell lines containing each of the human chromosomes. The SATT gene was localized to human chromosome 2 in two separate experiments that each employed a different

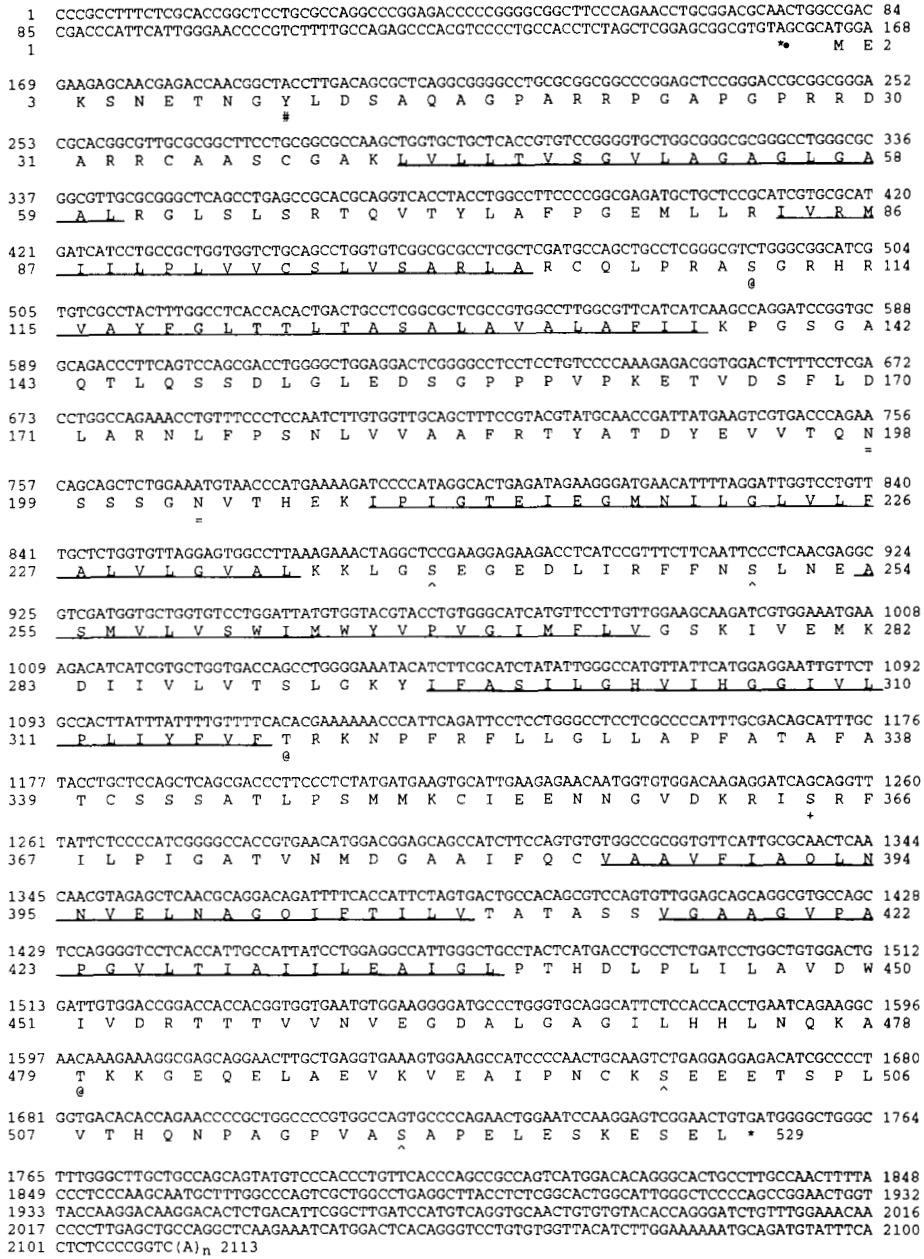

FIG. 1. **Nucleotide and deduced amino acid sequence of SATT.** Putative transmembrane domains are *underlined*. Potential glycosylation and phosphorylation sites have been marked as follows: #, tyrosine kinase site; ^, casein kinase II site; +, protein kinase A site; @, protein kinase C site; =, site for *N*-linked glycosylation. See text for details.

restriction enzyme (*Eco*RI or *Hin*dIII) for cell line DNA digestion. *Eco*RI digestion of control human DNA resulted in hybridization to a single band; hybridization with *Hin*dIII-digested DNA revealed three bands (data not shown). In each case, the corresponding bands were seen only in the human chromosome 2 somatic hybrid cell line. These results are consistent with the hypothesis that the multiple SATT transcripts detected by Northern blotting arise from a single gene (although additional genetic loci on chromosome 2 cannot be excluded). The biological significance of SATT transcripts of different sizes, including the possible generation of functionally distinct isoforms by differential RNA processing within the coding sequence, remains to be determined.

To determine the function of SATT, clone 5 was expressed in HeLa cells, and uptake studies were conducted. Initial studies indicated that expression of SATT in HeLa cells did not induce the transport of radiolabeled L-glutamate or succinate (at pH 7.4). Furthermore, expression of SATT in HeLa cells did not induce the transport of radiolabeled α-ketoglutarate, pyruvate, L-glutamine, β-alanine, taurine, L-histidine, L-ornithine, L-phenylalanine, or L-tyrosine.

HeLa cells transfected with SATT cDNA consistently exhibited a significant increase in 3 μM L-[^{3}H]serine and L-[^{3}H]alanine uptake over basal levels (Table I). Preliminary studies using the System A-specific substrate MeAIB established that ~10% of the basal transport of alanine and serine in our HeLa cell population was mediated by System A. Subsequently, all experiments were performed in the presence of 3 mM MeAIB to minimize System A-mediated transport of neutral amino acids. The presence of 3 mM MeAIB did not inhibit SATT-induced L-[^{3}H]serine uptake. Transfection-induced transport was stereospecific; 3 mM D-serine did not significantly inhibit transport of 3 mM L[^{3}H]serine in transfected cells (Table I). The K_m for L-[^{3}H]serine uptake by SATT-transfected cells was estimated to be 56.5 μM by Lineweaver-Burk analysis of the dependence of initial velocity data on substrate concentration (data not shown). Removal of Na^+ from the uptake buffer reduced basal L-[^{3}H]serine or L-[^{3}H]alanine transport by 90% in untransfected HeLa cells and eliminated the SATT-mediated induction in transport (data not shown). However, SATT-in-

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SATT    MEKSNETNGYLDSA----QAGPARRP--GAPGPRRDARRCAASC--GAKLVLLTVSGVLAGAGLGAALR-GLSLSRTQVTYLAFPGEMLL   81
GLTP    MTKSNGEEPRMGSRMGRFQQGVRKRTLLAKKKVQNITKEDVKSYLFRNAFVLLTVSAVIVGTILGFALR-PYKMSYREVKYFSFPGELLM   89
GLT-1   MASTEGANNMPKQVEVRMHDSHLSSE--EPKHRNLGMRMCDKLG--KNLLLSLTVFGVILGAVCGGLLRLAAPIHPDVVMLIAFPGDILM   86
EAAC    MGKPA-------------RKGC-------------DSKRFLK----NNWLLLSTVVAVVLGIVIGVLVREYSNLSTLDKFYFAFPGEILM   60

SATT    RIVRMIILPLVVCSLVSARLA-RCQLPRASGRHRVAYFGLTTLTASALAVALAFIIKPGSGA--QTL--QSSDLGLEDSGP--PPVPKET  164
GLTP    RMLQMLVLPLIISSLVTGMAALDSKASGKMGMRAVVYYMTTTIIAVVIGIIIVIIIHPGKGT-KENMYREGKIVQVTAADAFLDLIRNMF  178
GLT-1   RMLKMLILPLIISSLITGLSGLDAKASGRLGTRAMVYYMSTTIIAAVLGVILVLAIHPGNPKLKKQLGPGKKNDEVSSLDAFLDLIRNLF  176
EAAC    RMLKLVILPLIVSSMITGVAALDSNVSGKIGLRAVLYYFCTTIIAVILGIVLVVSIKPGVTQKVDEIDRTGSTPEVSTVDAMLDLIRNMF  150

SATT    VDSFLDLARNLFPSNLVVAAFR----TYATDYEVVTQNSSSG-----NVTHEKIPIGTEIEGMNILGLVLFALVLGVALKKLGSEGEDLI  245
GLTP    PPNLVEACFKQFKTSYEKRSFKVPIQANETLLGAVINNVSEAMETLTRIREEMVPVPGSVNGVNALGLVVFSMCFGFVIGNMKEQGQALR  268
GLT-1   PENLVQACFQQIQTVTKKVLVAPPSEEANTTKAVISLLNETMNEAPEETKIVIKKGLEFKDGMNVLGLIGFFIAFGIAMGKMGVAGQADG  266
EAAC    PENLVQACFQQYKTTREEVTASDDTGKNGTEESVTAVMTTAVSE---NRTKEYRVVGLYSDGINVLGLIVFCLVFGLVIGKMGEKGQILV  237

SATT    RFFNSLNEASMVLVSWIMWYVPVGIMFLVGSKIVEMKDIIVLVTSLGKYIFASILGHVIHGGIVLPLIYFVFTRKNPFRFLLGLLAPFAT  335
GLTP    EFFDSLNEAIMRLVAVIMWYAPLGILFLIAGKILEMEDMGVIGGQLAMYTVTVIVGLLIHAVIVLPLLYFLVTRKNPWVFIGGLLQALIT  358
GLT-1   GVLQHSERDCHEVSDHDHVVFPAGIACLICGKIIAIKDLEVVARQLGMYMITVIVGLIIHGGIFLPLIYFVVTRKNPFSFFAGIFQAWIT  356
EAAC    DFFNALSDATMKIVQIIMCYMPLGILFLIAGKIIEVEDWEIF-RKLGLYMVTVLSGLAINSIVILPLIYFIVVRKNPFRFAMGMTQALLT  326

SATT    AFATCSSSATLPSMMKCIEENNGVDKRISRFILPIGATVNMDGAAIFQCVAAVFIAQLNNVELNAGQIFTILVLTATASSVGAAGVPAPGV  425
GLTP    ALGTSSSSATLPITFKCLEENNGVDKRITRFVLPVGATINMDGTALYEALAAIFIAQVNNFDLNFGQIITISITATAASIGAAGIPQAGL  448
GLT-1   ALGTASSAGTLPVTFRCLEDNLGIDKRVTRFVLPVGATINMDGTALYEAVAAIFIAQMNGVILDGGQIVTVSLTATLASIGAASIPSAGL  446
EAAC    ALMISSSSATLPVTFRCAEEKNRVDKRITRFVLPVGATINMDGTALYEAVAAVFIAQLNDMDLSIGQIITISVTATAASIGAAGVPGAGL  416

SATT    LTIAIILEAIGLPTHDLPLILAVDWIVDRTTTVVNVEGDALGAGILHHLNQKAT--------KKGEQEL--AEVKVEAIPNCKSEE----  501
GLTP    VTMVIVLTSVGLPTDDITLIIAVDWFLDRLRTTTNVLGDSLGAGIVEHLSRHEL--------KNRDVEM--GNSVIEE----NEMK----  520
GLT-1   VTMLLILTAVGLPTEDISLLVAVDWLLDRMRTSVNVVGDFSGAGIVYHLSKSELDTIDSQHRMHEDIEMTKTQSVYDDTKNHRESNSNQC  536
EAAC    VTMVIVLSAVGLPAEDVTLIIAVDWLLDRFRTVVNVLGDAFGTGIVEKLSKKELEQMD----VSSEVNIV-NPFALESATLDNEDS----  497

SATT    ---ETSPLVTHQ---NPAGPVASA---PELESKESEL                                                        529
GLTP    ---KPYQLIAQD---N--EPEKPV---ADSETKM---                                                        543
GLT-1   VYAAHNSVVIDECKVTLAANGKSADCSVEEEPWKREK                                                        573
EAAC    ---DTKKSYING---GF-AVDKSD---TISFTQTSQF                                                        524
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FIG. 2. **Multiple sequence alignment depicting relationships of primary structure between the mammalian glutamate transporters and SATT.** *Shaded* residues are conserved across all four proteins.

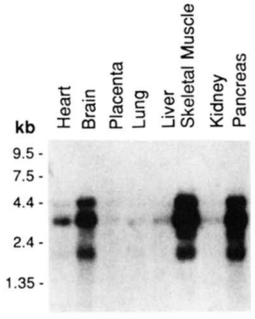

FIG. 3. **Northern hybridization analysis using an SATT probe (see "Experimental Procedures") against mRNA from multiple human tissues.** The main species are the bands at 4.8, 3.5, 2.8, and 2.2 kb in skeletal muscle, pancreas, and brain.

duced transport was independent of extracellular Cl^- in the assay buffer. Substitution of extracellular Cl^- with thiocyanate or acetate anions did not inhibit the SATT-mediated induction of L-[^{3}H]serine transport (Table I). Excess unlabeled L-threonine (3 mM) markedly reduced both the basal and SATT-induced uptake of 3 mM L-[^{3}H]serine (Table I).

These transport properties of SATT are similar to System ASC, a Na^+-dependent neutral amino acid transport activity that does not transport *N*-methylated substrates such as MeAIB (1, 2, 27). Unlike System A, which can be identified by MeAIB uptake, no diagnostic ligands are available to distinguish System ASC. Typically, Na^+-dependent, MeAIB-insensitive uptake of alanine, serine, threonine, or cysteine has been assigned to System ASC. However, subtle differences in System ASC transport properties have been described in different tissues. It is known, for example, that L-cysteine is a better ASC substrate than threonine in rat liver but that the converse is true in the hepatoma cell line HTC (28–31). Indeed, System ASC is usually identified by the exclusion of other transport systems and exhibits significant variability in substrate specificity between tissues (*e.g.* Refs. 28–31).

The transport activity induced by SATT in transfected HeLa cells, although related, cannot account for all the transport properties attributed to System ASC. Thus, although L-cysteine is a substrate for System ASC (1, 2, 27, 28), expression of SATT in HeLa cells did not induce the transport of L-[^{35}S]cysteine (Table I). Furthermore, 3 mM unlabeled L-cysteine did not inhibit L-[^{3}H]serine uptake in SATT-transfected cells (Table I). Substantial Na^+-dependent, MeAIB-insensitive L-cysteine transport is present in both skeletal muscle (32) and pancreas (33) (SATT-rich tissues), indicating additional ASC-like routes for L-cysteine uptake in these tissues. Previous studies have also observed that increased levels of intracellular substrates of System ASC enhance the activity of the system, a process called trans-stimulation (see Ref. 27 for review). However, preloading of HeLa cells with L-alanine or L-threonine did not stimulate SATT-mediated transport (data not shown). Finally, only low levels of SATT mRNA were observed in tissues such as liver and placenta that exhibit prominent ASC transport activity.

The magnitude of SATT-induced transport in our expression system (1.7–2.0-fold, Table I) is comparable with that seen with the System A cDNA, SAAT-1, which produced a 2-fold increase in MeAIB uptake in transiently transfected COS cells (4). The relatively small magnitude of SATT-induced transport is most likely related to high background transport of SATT substrates in HeLa cells. Indeed, a significant proportion of such basal transport is probably occurring through the SATT carrier protein itself, because we observed substantial levels of SATT mRNA (corresponding to the three most abundant transcripts of 2.2, 3.5, and 4.8 kb seen in Fig. 3) in Northern blots of untransfected HeLa cell mRNA (data not shown).

In summary, we have isolated and characterized a cDNA clone, SATT, which induces Na^+-dependent, MeAIB-insensitive uptake of serine, threonine, and alanine in transfected HeLa cells. These SATT transport characteristics are similar to the Na^+-dependent neutral amino acid transport activity designated System ASC. However, several properties of the cloned SATT transporter, as expressed in the HeLa cell, distinguish it from the generically described System ASC. These include: 1) the lack of L-cysteine transport; 2) the lack of trans-stimulation; and 3) the low levels of expression of SATT in ASC-rich tissues such as liver and placenta. Thus, SATT is likely to represent one of multiple activities that collectively account for System ASC-mediated amino acid uptake in diverse tissues and cell lines. Interestingly, SATT does not share significant structural or amino acid sequence homology with SAAT-1, the Na^+-dependent, MeAIB-sensitive "System A" neutral amino acid transporter (4), even though both transporters share several

TABLE I
Transport characteristics of SATT expressed in HeLa cells

Uptake is expressed as pmol of substrate transported per min in one well of a 24-well culture plate. Each value is the mean ± S.D. of triplicate measurements. Transported substrates were present at 3 μM in Na-KRP uptake buffer and 3 mM MeAIB (see "Experimental Procedures"). Cell count per well was identical for a given set of control *versus* transfected uptake measurements but varied from 150,000 to 300,000 among different sets of experiments. The percent increase in transport seen with SATT transfection was consistent for a given substrate; the absolute magnitude of transport varied approximately according to estimated cell density.

Substrate	Uptake		Increase
	Control	SATT-transfected	
	pmol/well/min		%
[³H]L-Serine	3.9 ± 0.48	6.8 ± 0.07	74
[³H]L-Alanine	3.2 ± 0.05	5.4 ± 0.18	69
[³H]L-Serine in presence of 3 mM L-threonine	0.73 ± 0.11	0.92 ± 0.12	26
[³H]L-Serine in presence of 3 mM D-serine	3.7 ± 0.46	6.2 ± 0.36	68
[³H]L-Serine in absence of chloride (replaced by acetate)	5.4[a]	9.4 ± 0.52	72
[³⁵S]L-Cysteine[b]	4.4 ± 1.0	4.0 ± 2.8	−10
[³H]L-Serine in presence of 3 mM L-cysteine[b]	4.2 ± 0.09	8.5 ± 0.81	102

[a] Duplicate measurements were made (5.7 and 5.0 pmol/well/min).
[b] Experiment performed in the presence of 0.1 mM dithiothreitol in uptake buffer.

common substrates and Na^+ dependence (1, 2, 27). Previously, a putative "Na^+-binding domain" has been proposed for a bacterial Na^+/glutamate cotransporter based on a short, loosely defined region of amino acid sequence similarity present in several Na^+-dependent symporters (26, 34), including SAAT1 (4); this sequence motif is not present in SATT. Although SATT does not transport L-glutamate or related dicarboxylates under our assay conditions (sodium KRP, pH 7.4), it does exhibit significant structural and amino acid sequence homology with a recently described family of mammalian Na^+/glutamate cotransporters (17–19). Interestingly, Vadgama and Christensen (30) demonstrated that System ASC does transport anionic amino acids such as cysteate and cysteine sulfinate at pH <6. It will therefore be of interest to characterize the pH dependence of the transport properties of SATT to investigate the functional basis for the structural and sequence similarity between SATT and the mammalian Na^+/glutamate cotransporters.

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