

Rapid report

## Evidence for the transport of neutral as well as cationic amino acids by ATA3, a novel and liver-specific subtype of amino acid transport system A

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Received 5 December 2000; accepted 12 December 2000

### Abstract

We report here on the cloning and functional characterization of the third subtype of amino acid transport system A, designated ATA3 (amino acid transporter A3), from a human liver cell line. This transporter consists of 547 amino acids and is structurally related to the members of the glutamine transporter family. The human ATA3 (hATA3) exhibits 88% identity in amino acid sequence with rat ATA3. The gene coding for hATA3 contains 16 exons and is located on human chromosome 12q13. It is expressed almost exclusively in the liver. hATA3 mediates the transport of neutral amino acids including  $\alpha$ -(methylamino)isobutyric acid (MeAIB), the model substrate for system A, in a Na<sup>+</sup>-coupled manner and the transport of cationic amino acids in a Na<sup>+</sup>-independent manner. The affinity of hATA3 for cationic amino acids is higher than for neutral amino acids. The transport function of hATA3 is thus similar to that of system y<sup>+</sup>L. The ability of hATA3 to transport cationic amino acids with high affinity is unique among the members of the glutamine transporter family. hATA1 and hATA2, the other two known members of the system A subfamily, show little affinity toward cationic amino acids. hATA3 also differs from hATA1 and hATA2 in exhibiting low affinity for MeAIB. Since liver does not express any of the previously known high-affinity cationic amino acid transporters, ATA3 is likely to provide the major route for the uptake of arginine in this tissue. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** System A; Amino acid transporter A3; Glutamine transporter family; Neutral amino acid; Cationic amino acid; Liver; Human

### Transport of cationic amino acids in mammalian

Abbreviations: MeAIB,  $\alpha$ -(methylamino)isobutyric acid; ATA, amino acid transporter A; hATA, human ATA; SN, system N; hSN, human SN; NMDG, *N*-methyl-D-glucamine; HRPE, human retinal pigment epithelial; MES, 4-morpholino-ethanesulfonic acid; bp, base pair(s)

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cells is mediated by a multitude of transport systems [1–4]. Several of these transport systems have been cloned and functionally characterized. Based on functional characteristics, these transport systems fall into four distinct categories: (a) the members of the cationic amino acid transporter family CAT1–4, (b) the transporter b<sup>0</sup>,+ that is known to be associated with the genetic disorder cystinuria, (c) the transporter y<sup>+</sup>L that is related to the genetic

disorder lysinuric protein intolerance, and (d) the transporter  $B^{0,+}$ . The cationic amino acid transporters CAT1–4 mediate  $Na^+$ -independent transport of cationic amino acids. These transporters, with the exception of CAT1, have little or no affinity for neutral amino acids [1–4]. CAT1 is capable of interacting with certain neutral amino acids in the presence of  $Na^+$  [5].  $b^{0,+}$  is a heterodimeric transporter consisting of  $b^{0,+}AT$  and rBAT [6–9] or  $b^{0,+}AT$  and 4F2hc [6,10] that mediates the transport of cationic amino acids as well as neutral amino acids in the absence of  $Na^+$ .  $y^+L$  is also a heterodimeric transporter consisting of  $y^+LAT1$  and 4F2hc or  $y^+LAT2$  and 4F2hc, but it transports cationic amino acids in a  $Na^+$ -independent manner and neutral amino acids in a  $Na^+$ -dependent manner [11–14].  $B^{0,+}$  belongs to the neurotransmitter transporter family and it transports cationic amino acids as well as neutral amino acids in a  $Na^+$ - and  $Cl^-$ -coupled manner [15].

Recent cloning studies have identified a new amino acid transporter family, the members of which mediate  $Na^+$ -coupled transport of glutamine. This glutamine transporter family consists of two subtypes, system N and system A. Two members of the system N subtype have been identified. They are system SN1 [16–18] and system SN2 (T. Nakanishi et al., unpublished). There are also three members in the system A subfamily: ATA1 [19,20], ATA2 [21–24] and ATA3 [25]. SN1 and SN2 mediate the  $Na^+$ - and  $H^+$ -dependent transport of neutral amino acids. The transport mechanism involves the movement of  $Na^+$  and the amino acid substrate in one direction in exchange for  $H^+$  on the *trans*-side and the  $Na^+$ :amino acid: $H^+$  stoichiometry is 2:1:1. ATA1, ATA2 and ATA3 also mediate the  $Na^+$ -dependent transport of neutral amino acids. Though the transport function of ATA1, ATA2 and ATA3 is strongly influenced by intracellular pH, there is no movement of  $H^+$  associated with the transport process. The  $Na^+$ :amino acid stoichiometry is 1:1. These two subfamilies can be differentiated not only on the basis of the involvement of  $H^+$  in the transport mechanism but also on the basis of interaction with the model amino acid substrate  $\alpha$ -(methylamino)isobutyric acid (MeAIB) [26]. SN1 and SN2 do not recognize MeAIB as a substrate whereas ATA1, ATA2 and ATA3 do. In fact, the  $Na^+$ -dependent transport of MeAIB is a distinguishing characteristic of the sys-

tem A subfamily. There is no evidence in the literature for the interaction of system N or system A with cationic amino acids [2–4]. This is supported by recent studies with cloned SN1, SN2, ATA1, and ATA2 [16–24]. There is no information available currently on whether or not ATA3, the newest member of the glutamine transporter family to be identified, interacts with cationic amino acids. Here we report on the cloning of human ATA3 and on the ability of the transporter to interact with cationic amino acids.

The HepG2 cell cDNA library [22] was screened under high stringency conditions using  $^{32}P$ -labeled rat ATA3 cDNA [25] as the probe. Two positive clones were obtained and they were analyzed for their restriction fragment pattern. Both clones were found to be identical. One of these clones was selected for sequence and functional analyses. Both sense and antisense strands of the cDNA were sequenced using an automated Perkin-Elmer Applied Biosystems 377 Prism DNA sequencer. The sequence was analyzed using the GCG sequence analysis software package GCG version 10 (Genetics Computer Group, Madison, WI, USA).

The functional expression of the cloned transporter was done in human retinal pigment epithelial (HRPE) cells using the vaccinia virus expression system as described previously [6,10,17,20–22]. Transport measurements were made at 37°C for 15 min with [ $^{14}C$ ]MeAIB, [ $^3H$ ]glycine, or [ $^3H$ ]arginine as a substrate. The transport buffer in most experiments was 25 mM Tris/HEPES (pH 8.5) containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM  $CaCl_2$ , 0.8 mM  $MgSO_4$ , and 5 mM glucose. When the influence of pH on transport was studied, buffers of varying pH were prepared by appropriately mixing the following two buffers: 25 mM Tris/HEPES (pH 9.0) and 25 mM MES/Tris (pH 5.5), both containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM  $CaCl_2$ , 0.8 mM  $MgSO_4$ , and 5 mM glucose. When the influence of  $Na^+$  on transport was assessed, a  $Na^+$ -free buffer was prepared by isoosmotically substituting *N*-methyl-D-glucamine (NMDG) chloride for NaCl. Endogenous transport was always determined in parallel using cells transfected with vector alone. cDNA-specific transport was calculated by adjusting for the endogenous activity. The kinetic parameter Michaelis-Menten constant ( $K_t$ ) was calculated by fitting the cDNA-specific transport data to the Michaelis-Men-

ten equation describing a single saturable system. Analysis was done by non-linear regression, and the resultant value was confirmed by linear regression.

A commercially available human multiple tissue blot (Origene, Rockville, MD, USA) was used to determine the tissue expression pattern of the cloned cDNA. The blot was hybridized sequentially under high stringency conditions first with  $^{32}\text{P}$ -labeled hATA3 cDNA and then with  $^{32}\text{P}$ -labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

The hATA3 cDNA is 3965 bp long and contains an open reading frame (nucleotide positions 365–2008) (GenBank accession No. AF305814). The cDNA codes for a protein of 547 amino acids. The amino acid sequence of this protein shows significant homology to that of rat ATA3 [25] (88% identity), human ATA1 [20] (47% identity) and human ATA2 [22] (57% identity). The homology with the other two members of the glutamine transporter family, human SN1 [17] and human SN2 (T. Nakanishi et al., unpublished), is also significant (56% identity with human SN1 and 51% identity with human SN2). hATA3 consists of the highest number of amino acids among the members of the system A subfamily (547 amino acids in hATA3 versus 487 amino acids in hATA1 and 506 amino acids in hATA2). The extra amino acids in hATA3 are located exclusively

in the middle of the protein. The three proteins show marked diversity in amino acid sequence in their amino termini. Hydropathy analysis of the amino acid sequence suggests the presence of 10–11 putative transmembrane domains in hATA3. These structural features of hATA3 are similar to those of rat ATA3 recently reported from our laboratory [25].

A GenBank database search with the nucleotide sequence of hATA3 cDNA revealed that the human gene coding for this protein has been completely sequenced (GenBank accession Nos. AC005854 and AC008014). The gene is located on chromosome 12q13. The gene is approx. 40 kb long. By aligning the nucleotide sequence of the cloned hATA3 cDNA with the genomic sequence, we were able to deduce the exon-intron organization of the gene. The gene consists of 16 exons and 15 introns. The size of each of the exons and introns and the nucleotide sequences of the splice junctions are given in Table 1. The 5'- and 3'-termini of each intron possess the consensus sequence for RNA splicing (gt/ag). The translation start site ATG is located in exon 2 and the translation termination site TAA is located in exon 16. Exon 1 does not code for the protein.

Northern analysis with a multiple human tissue blot shows that ATA3 is expressed almost exclusively in the liver (Fig. 1). ATA3 mRNA is detectable in the kidney, but the levels are severalfold lower com-

Table 1  
Exon-intron organization of the human ata3 gene

Exon			Intron			Exon		
No.	Size (bp)	3' junction	5' junction	Size (bp)	No.	3' junction	5' junction	No.
1	194	...AATAATTAAG	gtacagctgt...	10 409	1	...ctgtttgcag	AACAGAGTGT...	2
2	231	...CAATGAGCAG	gtatggggtt...	4 334	2	...gtgtgttcag	TCAATTTGCT...	3
3	91	...TGATGAACAC	gtaagtgaat...	496	3	...ctttttcaag	CATCCCGGAA...	4
4	116	...TACTTTTAT	gtaagtgaat	2 738	4	...ccctttgcag	AATCATGCTG...	5
5	74	...AAGGAAGGAG	gtatgctacc...	469	5	...ttgattgtag	GGTCTTTGAT...	6
6	93	...AACATTGGAG	gtaaggggat...	4 511	6	...tccattttag	CAATGTCAAG...	7
7	82	...AAAATACTGG	gtatgtctta...	86	7	...tcacttctag	AGAATGGTAC...	8
8	83	...AAAAATTTAG	gtaaagatat...	110	8	...taacttctag	GTTATCTTGG...	9
9	59	...TGTTAGTGTG	gtaagtgatg...	834	9	...ctgagtctag	GTGATTTACA...	10
10	276	...CAACTCCCGG	gtaagtgagc...	132	10	...tgtttttcag	ACGGCCTATG...	11
11	80	...AACTTAAAGA	gtaaggcagc...	1 284	11	...ctttttccag	TCGGTCCCGG...	12
12	101	...ACCTTCTATG	gtaggtcact...	1 730	12	...cctgtttag	GAGAAGTTGA...	13
13	125	...CCTCTCCCA	gtaagtacat...	5 620	13	...cttgtttaag	ATTCGTACAT...	14
14	145	...GGATTCATAG	gtgagtttca...	876	14	...gtcttctcag	GGGCTTCTTC...	15
15	98	...AAAGTCCGGG	gtaagtaaac...	1 507	15	...tgaattacag	GCTTTAATTT...	16
16	2 043	...TTCAAATGAC						

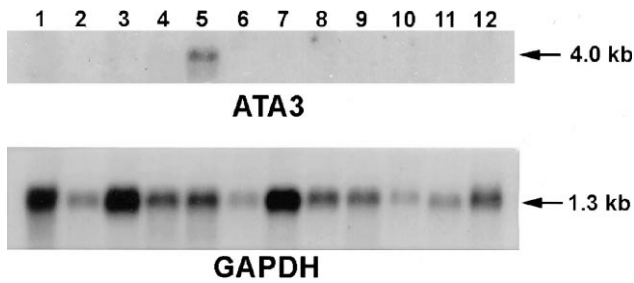


Fig. 1. Northern blot analysis of ATA3-specific mRNA in human tissues. A commercially available hybridization-ready blot containing poly(A)<sup>+</sup>mRNA (2 µg per lane) from 12 different human tissues was sequentially hybridized with <sup>32</sup>P-labeled hATA3 cDNA and then with <sup>32</sup>P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA under high stringency conditions. The sizes of the hybridization-positive signals are indicated. Lanes 1–12 represent brain, colon, heart, kidney, liver, lung, skeletal muscle, placenta, small intestine, spleen, stomach, and testis, respectively.

pared to the levels in the liver. The size of the mRNA is 4 kb. ATA3 mRNA is not detectable in the brain, colon, heart, lung, skeletal muscle, placenta, small intestine, spleen, stomach, and testis.

Since ATA3 is a subtype of system A [25], we first assessed the ability of hATA3 to transport MeAIB, the model substrate for system A. When measured at

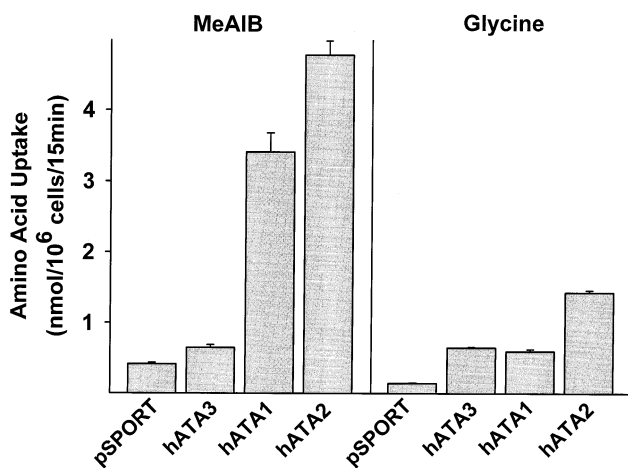


Fig. 2. Transport of MeAIB and glycine by hATA3, hATA1 and hATA2. HRPE cells were transfected with pSPORT alone, hATA3 cDNA, hATA1 cDNA, or hATA2 cDNA. The cDNAs were expressed by vaccinia virus expression system. Transport of [<sup>14</sup>C]MeAIB (20 µM) and [<sup>3</sup>H]glycine (20 µM) was measured in these cells at 37°C for 15 min in the presence of NaCl (pH 8.5). Values are means ± S.E. from four determinations.

pH 8.5 in the presence of NaCl, the uptake of MeAIB was significantly higher in hATA3 cDNA-transfected cells than in vector-transfected cells (Fig. 2). However, the cDNA-induced increase was surprisingly small (approx. 50%). Under identical conditions, MeAIB uptake was increased much more markedly by hATA1 (8-fold) and hATA2 (11-fold). We also compared hATA3 with hATA1 and hATA2 for the ability to transport glycine. Glycine uptake was increased 4-fold by hATA3. The ability of hATA1 to increase glycine uptake was similar to that of hATA3. hATA2 was, however, able to increase glycine uptake to a much higher level

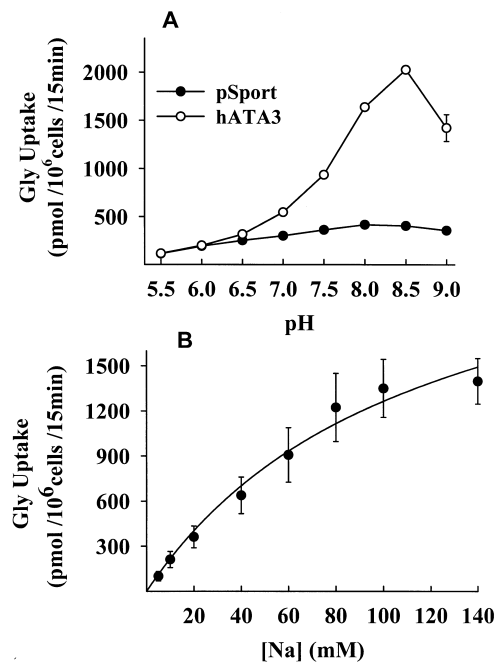


Fig. 3. pH dependence (A) and Na<sup>+</sup> activation kinetics (B) of hATA3-mediated glycine transport. (A) HRPE cells were transfected with either pSPORT alone (●) or hATA3 cDNA (○). Transport of [<sup>3</sup>H]glycine (20 µM) was measured in these cells at 37°C for 15 min in the presence of NaCl. pH of the transport buffer was changed from 5.5 to 9.0 as indicated. Values are means ± S.E. from four determinations. (B) HRPE cells were transfected with either pSPORT alone or hATA3 cDNA. Transport of [<sup>3</sup>H]glycine (20 µM) was measured in these cells at 37°C for 15 min in the presence of varying concentrations of NaCl (5–140 mM). NaCl was replaced isoosmotically with NMDG chloride as required. pH of the transport buffer was 8.5. Data represent only hATA3-specific transport which was calculated by subtracting the transport in pSPORT-transfected cells from the transport in hATA3 cDNA-transfected cells. Values are means ± S.E. from four determinations.

(9-fold). These data show that hATA3 is an authentic member of the system A subfamily due to its ability to mediate MeAIB uptake. However, this newly identified system A transporter is clearly less effective than hATA1 and hATA2 in handling this model substrate.

We used glycine as a substrate for further functional characterization of this transporter. Fig. 3 describes the pH dependence and  $\text{Na}^+$  activation kinetics of hATA3-mediated glycine uptake. The transport function of hATA3 with glycine as the substrate was markedly influenced by the pH of transport buffer. The transport function was negligible at pH 6.5, but it increased dramatically when the pH of transport buffer was increased above 6.5. The hATA3-specific transport was maximal at pH 8.5. There was a 6-fold increase in hATA3-specific glycine transport when pH was changed from 7 to 8.5. The transport function of hATA3 was obligatorily dependent on the presence of  $\text{Na}^+$ . hATA3 was non-functional when  $\text{Na}^+$  was replaced with NMDG (data not shown). The relationship between  $\text{Na}^+$  concentration and hATA3-specific glycine uptake was hyperbolic over the range of 5–140 mM. The transport was saturable with increasing concentra-

tions of glycine (data not shown) and the Michaelis-Menten constant ( $K_t$ ) was  $1.6 \pm 0.3$  mM.

We then investigated the substrate specificity of hATA3 by assessing the ability of various amino acids to inhibit hATA3-specific uptake of [ $^3\text{H}$ ]glycine (20  $\mu\text{M}$ ) (Table 2). At a concentration of 2.5 mM, all neutral amino acids tested showed marked inhibition. Histidine was the most potent inhibitor whereas leucine and phenylalanine were the least potent. Glutamine showed about 50% inhibition. The inhibition by MeAIB was only 30%. The anionic amino acids did not show any inhibition. The most surprising finding was that the cationic amino acids arginine and lysine were very potent inhibitors. The inhibition caused by these two amino acids (60–70%) was much higher than the inhibition caused by MeAIB, glutamine, proline, glycine, asparagine, and serine. This was an unexpected finding because there is no evidence in the literature for any significant interaction of system A with cationic amino acids. Moreover, the previously cloned ATA1 and ATA2 have shown no or little affinity for cationic amino acids. But, the present data show that hATA3 exhibits much higher affinity for cationic amino acids than for most neutral amino acids.

Table 2  
Inhibition of hATA3-specific transport of [ $^3\text{H}$ ]glycine by other amino acids

Unlabeled amino acid	hATA3 cDNA-specific [ $^3\text{H}$ ]Gly transport	
	pmol/ $10^6$ cells/15 min	%
Control	1 701.8 $\pm$ 82.2	100
Serine	809.1 $\pm$ 52.0	48
Alanine	537.5 $\pm$ 16.4	32
MeAIB	1 205.6 $\pm$ 71.2	71
Glycine	737.7 $\pm$ 63.5	43
Glutamine	782.2 $\pm$ 41.1	46
Asparagine	637.0 $\pm$ 38.2	37
Proline	1 228.2 $\pm$ 76.7	72
Histidine	268.1 $\pm$ 24.3	16
Leucine	1 324.1 $\pm$ 71.6	78
Phenylalanine	1 383.1 $\pm$ 34.8	81
Arginine	528.0 $\pm$ 59.3	31
Lysine	665.7 $\pm$ 33.8	39
Glutamate	1 715.8 $\pm$ 115.4	101

HRPE cells were transfected with either pSPORT alone or pSPORT-hATA3 cDNA. Transport of [ $^3\text{H}$ ]glycine (20  $\mu\text{M}$ ) was measured in these cells at 37°C for 15 min in the presence of NaCl (pH 8.5). When present, the concentration of unlabeled amino acids was 2.5 mM. cDNA-specific [ $^3\text{H}$ ]glycine transport was calculated by subtracting the transport in vector-transfected cells from the transport in cDNA-transfected cells. Values are means  $\pm$  S.E. from four determinations.

In order to demonstrate that hATA3 does not simply interact with cationic amino acids but it actually transports these amino acids, we studied directly the ability of hATA3 to mediate the uptake of arginine. When measured in the presence of  $\text{Na}^+$ , the uptake of arginine in cells transfected with hATA3 cDNA was significantly higher than in control cells transfected with vector alone (Fig. 4). Surprisingly, the hATA3-specific uptake was observed at all pH values within the pH range 5.5–9.0. The lack of pH dependence of hATA3-specific arginine uptake is in contrast to the marked pH dependence of glycine uptake mediated by the same transporter. Another interesting difference between arginine uptake and glycine uptake via hATA3 is the involvement of  $\text{Na}^+$  in the transport process. hATA3-specific arginine uptake was not dependent on  $\text{Na}^+$  (Fig. 4). The transport was saturable with increasing concentrations of arginine (data not shown) with a  $K_t$  of  $0.30 \pm 0.04$  mM.

We then investigated the substrate specificity of hATA3 with [ $^3\text{H}$ ]arginine as the substrate. The ability of various amino acids (2.5 mM) to compete with [ $^3\text{H}$ ]arginine (20  $\mu\text{M}$ ) for the hATA3-mediated uptake process was assessed in the presence of NaCl (data not shown). The cationic amino acids arginine and lysine were the most potent inhibitors of [ $^3\text{H}$ ]arginine uptake, followed by neutral amino acids. Again, neutral amino acids such as alanine, serine, histidine, asparagine and glutamine were less effective than cationic amino acids in competing with [ $^3\text{H}$ ]arginine uptake via hATA3. These data show clearly that hATA3 has higher affinity for cationic amino acids than for neutral amino acids.

hATA3 is structurally similar to the members of the system A subfamily and therefore it is expected to interact with MeAIB, the model substrate for system A. ATA1–3 form a subgroup within the glutamine transporter family and therefore hATA3 is also expected to interact with glutamine similar to ATA1 and ATA2. To determine the affinities of hATA3 for MeAIB and glutamine, we assessed the potencies of these amino acids to inhibit hATA3-specific glycine uptake. The uptake was inhibited by both amino acids. The  $\text{IC}_{50}$  values (i.e., concentration of the inhibitor necessary for 50% inhibition) were  $6.7 \pm 0.8$  and  $2.5 \pm 0.5$  mM for MeAIB and glutamine, respectively. Thus, hATA3 does interact with glutamine

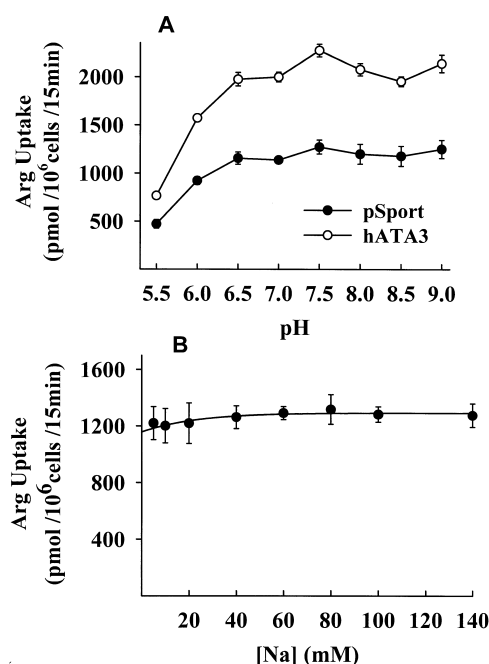


Fig. 4. pH dependence (A) and  $\text{Na}^+$  independence (B) of hATA3-mediated arginine transport. (A) HRPE cells were transfected with either pSPORT alone (●) or hATA3 cDNA (○). Transport of [ $^3\text{H}$ ]arginine (20  $\mu\text{M}$ ) was measured in these cells at 37°C for 15 min in the presence of NaCl. pH of the transport buffer was changed from 5.5 to 9.0 as indicated. Values are means  $\pm$  S.E. from four determinations. (B) HRPE cells were transfected with either pSPORT alone or hATA3 cDNA. Transport of [ $^3\text{H}$ ]arginine (20  $\mu\text{M}$ ) was measured in these cells at 37°C for 15 min in the presence of varying concentrations of NaCl (5–140 mM). NaCl was replaced isoosmotically with NMDG chloride as required. pH of the transport buffer was 8.5. Data represent only hATA3-specific transport which was calculated by subtracting the transport in pSPORT-transfected cells from the transport in hATA3 cDNA-transfected cells. Values are means  $\pm$  S.E. from four determinations.

and MeAIB as expected of a member of the system A subfamily, but the affinity of the transporter for these two neutral amino acids is significantly less than the affinity for arginine ( $0.30 \pm 0.04$  mM).

ATA3 reported in this paper is a novel transporter with functional and physiological significance. This is the first transporter in the glutamine transporter family that interacts with not only neutral amino acids but also with cationic amino acids. Since ATA3 mediates  $\text{Na}^+$ -dependent transport of MeAIB, it is functionally related to amino acid transport system A. The interaction of ATA3 with cationic amino acids with high affinity came as a surprise because

system A has never been shown to mediate the transport of cationic amino acids. The failure to detect this novel subtype of system A in earlier studies may be due to several reasons. This transporter is expressed almost exclusively in the liver and therefore studies of system A in tissues other than the liver would not detect a system A that interacts with cationic amino acids. There have been numerous studies of system A in the liver [27,28]. Since the liver expresses not only ATA3 but also ATA2, it is possible that this co-expression masked the transport function of ATA3 in earlier studies.

Even though ATA3 mediates  $\text{Na}^+$ -dependent transport of MeAIB and is structurally related to ATA1 and ATA2, there are several important functional and structural characteristics that are unique to ATA3. ATA3 does transport MeAIB, but its affinity for this system A model substrate is very low. ATA1 [19,20] and ATA2 [21–24] recognize MeAIB with a  $K_t$  value of 0.2–0.4 mM. In contrast, the  $K_t$  value for hATA3 for interaction with MeAIB is about 20-fold higher (6.7 mM). Because of this marked difference in the affinity between ATA2 and ATA3, it is likely that at concentrations usually employed for transport measurements ( $< 25 \mu\text{M}$ ), MeAIB transport is predominantly mediated by ATA2 in the liver in spite of the co-expression of ATA2 and ATA3 in this tissue. MeAIB is used widely as a model substrate for system A and therefore previous studies with this substrate were unable to detect MeAIB transport in liver cells that was sensitive to inhibition by cationic amino acids. A comparison of data related to the interaction of several neutral amino acids with hATA1 [20], hATA2 [22] and hATA3 (this study) suggests that the affinity of ATA3 for most of the neutral amino acids is lower than the affinities of ATA1 and ATA2 for these amino acids. This includes alanine, glycine, serine, proline, glutamine, and asparagine. The most striking functional difference is in the interaction with cationic amino acids. ATA1 and ATA2 do not interact with arginine and lysine [20,22] whereas ATA3 is able to recognize these amino acids as substrates. Interestingly, the transport of neutral amino acids via ATA3 is a  $\text{Na}^+$ -coupled process as is the case with ATA1 and ATA2, but the transport of arginine via ATA3 is not  $\text{Na}^+$ -dependent. Thus, ATA3 is a  $\text{Na}^+$ -dependent transporter for MeAIB and other

neutral amino acids and a  $\text{Na}^+$ -independent transporter for cationic amino acids. In this respect, ATA3 is similar to CAT1 and  $\text{y}^+\text{L}$ .

The transport function of hATA3 is influenced markedly by extracellular pH, but this effect is seen only with neutral amino acid substrates. The transport of arginine via hATA3 is not influenced within the pH range 6–9. This is in contrast to glycine transport which is undetectable at pH 6 but becomes robust at pH 8.5. We are unable to explain this interesting phenomenon at present. The relative proportion of cationic species and zwitterionic species of arginine is expected to vary to some extent within the pH range 6–9. Even if one assumes that the affinity of hATA3 is much greater for cationic species than for zwitterionic species, the change in the relative proportion of these two ionic species of arginine is expected to be small within the pH range and it is not clear whether this change would suffice to account for the lack of pH influence on arginine transport.

Though hATA3 is able to transport MeAIB and other neutral amino acids, it shows greater affinity for cationic amino acids than for neutral amino acids. Arginine is transported by hATA3 with a  $K_t$  value of  $0.30 \pm 0.4 \text{ mM}$ . A comparison of  $K_t$  values for arginine for various transporters that are capable of arginine transport indicates that the affinity of hATA3 for arginine is similar to the affinities of other transporters. The cationic amino acid transporters CAT1–3 exhibit a  $K_t$  of 50–400  $\mu\text{M}$  for arginine [1,2]. Similarly, the  $K_t$  for arginine for the transporters  $\text{b}^{0,+}$ ,  $\text{y}^+\text{L}$  and  $\text{B}^{0,+}$  is also in the range of 100–300  $\mu\text{M}$  [10,13,15]. The  $K_t$  for arginine for hATA3 in the present study is 300  $\mu\text{M}$ .

The tissue expression pattern of ATA3 is unique among the known members of the glutamine transporter family. ATA3 is the only member of the family that is expressed almost exclusively in the liver. More importantly, ATA3 may be the major transporter responsible for the uptake of cationic amino acids in this tissue. Liver does not express any of the other known cationic amino acid transporters.  $\text{b}^{0,+}$  and  $\text{y}^+\text{L}$  are expressed primarily in the small intestine and kidney [1–4]. The expression of  $\text{B}^{0,+}$  is restricted to the lung and mammary gland [15]. The members of the cationic amino acid transporter gene family, though expressed more widely in mam-

malian tissues, are not present in the liver [1–4]. To date, the only cationic amino acid transporter that has been shown to be expressed in the liver is CAT2a, an alternative splice variant of CAT2 [1,2,29]. It is a low-affinity transporter with a  $K_t$  for arginine in the range of 2–5 mM. ATA3 that is expressed abundantly in the liver has about 10-fold higher affinity for arginine compared to CAT2a. Since ATA3 is expressed abundantly in the liver, we speculate that this transporter provides the major mechanism for the entry of arginine into hepatocytes under physiological conditions.

We thank Ida Walker for expert secretarial assistance. This work was supported by National Institutes of Health Grants DA10045, HD33347 and HL64196.

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