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Amino acid transport of y^+L-type by heterodimers of 4F2hc/CD98 and members of the glycoprotein-associated amino acid transporter family

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Amino acid transport across cellular membranes is mediated by multiple transporters with overlapping specificities. We recently have identified the vertebrate proteins which mediate Na^+-independent exchange of large neutral amino acids corresponding to transport system L. This transporter consists of a novel amino acid permease-related protein (LAT1 or AmAT-L-lc) which for surface expression and function requires formation of disulfide-linked heterodimers with the glycosylated heavy chain of the *h*4F2/CD98 surface antigen. We show that *h*4F2hc also associates with other mammalian light chains, e.g. y^+LAT1 from mouse and human which are ~48% identical with LAT1 and thus belong to the same family of glycoprotein-associated amino acid transporters. The novel heterodimers form exchangers which mediate the cellular efflux of cationic amino acids and the Na^+-dependent uptake of large neutral amino acids. These transport characteristics and kinetic and pharmacological fingerprints identify them as y^+L-type transport systems. The mRNA encoding *m*y^+LAT1 is detectable in most adult tissues and expressed at high levels in kidney cortex and intestine. This suggests that the y^+LAT1–4F2hc heterodimer, besides participating in amino acid uptake/secretion in many cell types, is the basolateral amino acid exchanger involved in trans-epithelial reabsorption of cationic amino acids; hence, its defect might be the cause of the human genetic disease lysinuric protein intolerance.

Keywords: amino acid exchanger/epithelial transport/lysinuric protein intolerance/*Xenopus* oocytes

Introduction

The surface glycoproteins rBAT and *h*4F2hc/CD98 have been shown to induce amino acid transport of the $b^{0,+}$ and L and/or y^+L type, respectively, when expressed in *Xenopus* oocytes or some other heterologous expression systems (Bertran *et al*., 1992; Wells *et al*., 1992; Broer *et al*., 1995, 1997, 1998). For both glycoproteins, it has been proposed that they are not themselves transporters but rather 'activate' endogenous systems (Bertran *et al*., 1992; Wells *et al*., 1992; Broer *et al*., 1998). Both glycoproteins associate covalently with so-called light chains which migrate on SDS–PAGE at ~40 kDa (Haynes *et al*., 1981; Hemler and Strominger, 1982; Lüscher *et al*., 1985; Wang and Tate, 1995). The human heterodimeric surface glycoprotein *h*4F2hc and its murine homologue CD98 were first detected in activated lymphocytes (Haynes *et al*., 1981) and subsequently shown to be expressed widely (Yagita *et al*., 1986; Parmacek *et al*., 1989; Dixon *et al*., 1990), with an exclusively basolateral localization in intestine and kidney epithelia (Quackenbush *et al*., 1986) (B.Sordat, personal communication). However, serious attempts to obtain sequence information from their light chains following biochemical purification have failed (C.Bron, personal communication), a problem compatible with the presence of a mixture of different polypeptides.

Recently, searching for early aldosterone-regulated gene products, we cloned from *Xenopus* A6 cells a cDNA (ASUR4) encoding a highly lipophilic, permease-related protein which migrates at ~40 kDa on SDS–PAGE (theoretical mol. wt 55.5 kDa) (Spindler *et al*., 1997). When ASUR4 is co-expressed together with *h*4F2hc in *Xenopus* oocytes, they form disulfide-linked heterodimers which localize at the cell surface and mediate the Na^+-independent transport of large neutral amino acids, as expected for the L-type amino acid transport system (Mastroberardino *et al*., 1998). Its human homologue E16 has very similar transport properties and, thus, represents the first light chain of *h*4F2hc identified so far. We here term this transporter *h*LAT1 (human L-type amino acid transporter 1), instead of using our former designation *h*AmAT-L-lc, to conform with the nomenclature proposed by Christensen *et al*. (1994) which was also adopted by Kanai *et al*. (1998). Interestingly, a related *Shistosoma mansoni* protein (SPRM1) also bound covalently to *h*4F2hc, but induced an amino acid transport of different specificity and Na^+ dependence, thus representing the first non-mammalian member of a new family of glycoprotein-associated amino acid transporters (gpa-AT).

In 1992, Devés *et al*. first described in human erythrocytes a broad scope amino acid exchange system (y^+L) which, in the absence of Na^+, mediates the exchange of cationic amino acids and, in the presence of Na^+, the exchange of intracellular cationic amino acids with a preference for large extracellular neutral amino acids (Devés *et al*., 1992, 1993; Angelo and Devés, 1994; Fei *et al*., 1995). This complex transport pattern clearly differs from the transport of cationic amino acids produced by the y^+-type transporters of the chloramphenicol acetyltransferase (CAT) family, the first of which had been cloned as a receptor for the ecotropic retrovirus (Albritton *et al*., 1989). Subsequently, the amino acid transport activity detected upon expression of *h*4F2hc in *Xenopus* oocytes has been recognized as being of the y^+L type (Kim *et al*., 1991; Wang *et al*., 1991; Chillaron *et al*., 1996). A y^+L transport system may be involved in the

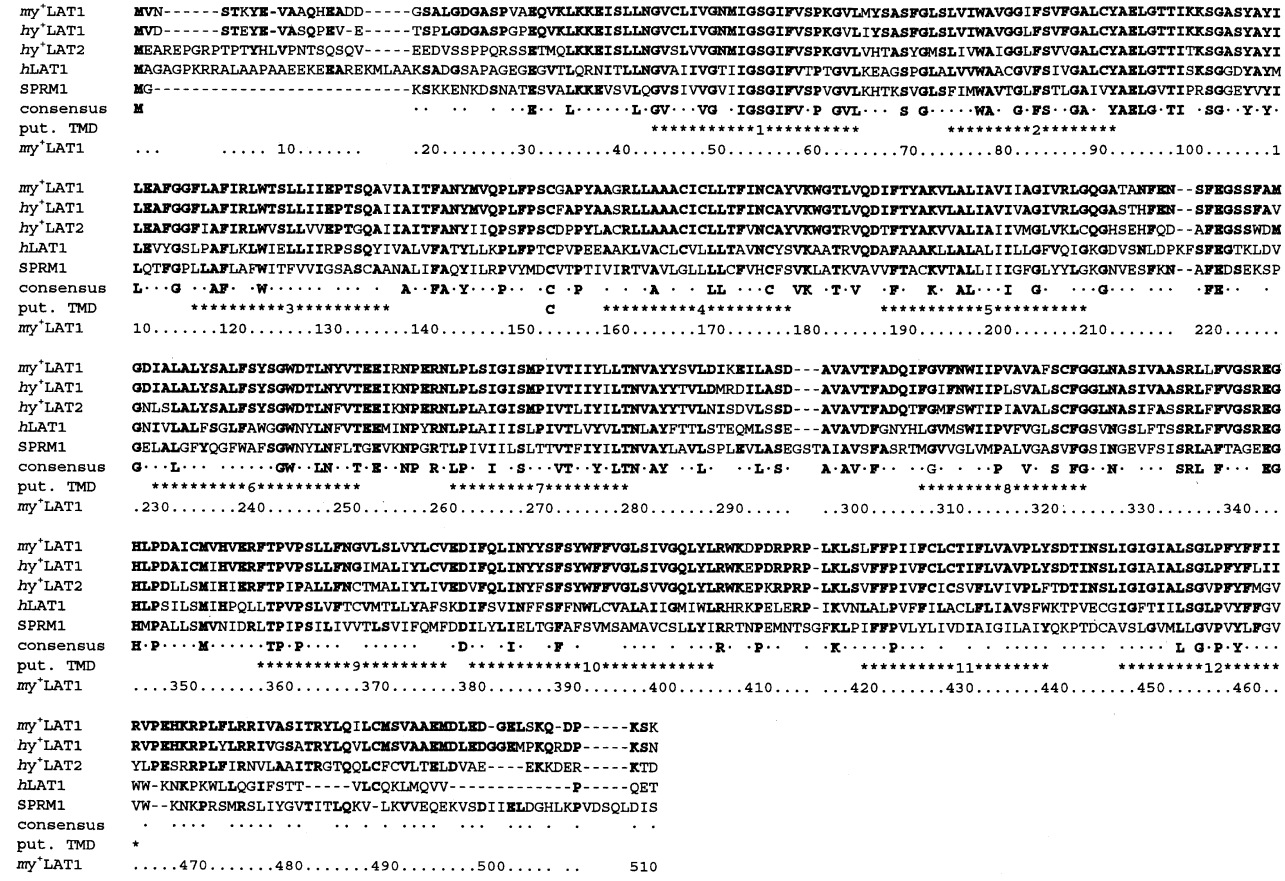

Fig. 1. Alignment of the y^+LAT amino acid sequences with *h*LAT1 and SPRM1. Amino acids identical to those of mouse y^+LAT1 are in bold. Putative transmembrane domains (TMDs), the position of which represents a compromise based on the predictions obtained for the different sequences, are numbered from 1 to 12. The cysteine residue involved in the disulfide bond with *h*4F2hc is indicated by the letter C.

basolateral extrusion step for the (re)absorption of dibasic amino acids across small intestine and kidney proximal tubule epithelia (Angelo and Devés, 1994; Chillaron *et al*., 1996).

In this report, we characterize the mouse and human homologues of a new member of the gpa-AT family which we identified by database screening. When co-expressed with *h*4F2hc in *Xenopus* oocytes, this light chain (y^+LAT1) forms disulfide-linked heterodimers and produces amino acid transport of the y^+L type.

Results

The first identified light chain of *h*4F2hc/CD98, the *h*LAT1 (E16), mediates L-type amino acid transport (Na^+-independent exchange of large neutral amino acids) upon co-expression with *h*4F2hc in *Xenopus* oocytes (Mastroberardino *et al*., 1998). Using its sequence, we performed database searches (Blast programs, NCBI) and identified cDNA sequences corresponding to at least four additional mammalian polypeptides of the same light chain family. The level of identity between their sequences ranges from 42–72%. In this study, we characterize the structure and function of one of the new family members for which we obtained both mouse and human expressed sequence tag (EST) cDNAs from the IMAGE consortium (Lennon *et al*., 1996). The amino acid sequence of the mouse and human proteins are strongly similar (90.4% identity; 98.6% similarity) and show ~48% identity with *h*LAT1 (Figure 1). Upon co-expression with *h*4F2hc, they produce y^+L-type amino acid transport (see below). Thus, we named them *m*- and *h*y^+LAT1. The suffix '1' has been added, since the closest related light chain in the database, the human HA7016 cDNA (product of the *KIAA0245* gene, 72% identity) (Nagase *et al*., 1996), appears to produce a similar type of transport (R.Pfeiffer, unpublished results) and might be named *h*y^+LAT2. We have identified two mouse cDNA clones for y^+LAT1 (a and b), which differ in their 5′-untranslated region and originate from kidney and heart cDNA libraries, respectively. Their encoded proteins differ by a single amino acid (S versus T) at position 4. The functional experiments reported in this study were performed using the kidney cDNA (y^+LAT1a). The closest non-mammalian protein is the *S.mansoni* SPRM1 (~40% identity) (Figure 1). This transporter associates with human 4F2hc and displays an amino acid transport with properties resembling both the mammalian L and y^+L system (Mastroberardino *et al*., 1998). The *m*- and *h*y^+LAT1 are 510 and 511 amino acids long, respectively, with a calculated mol. wt of ~56 kDa. The primary structure of the different gpa-AT family members suggests the conservation of a 12 transmembrane domain (TMD) topology with intracellular N- and C-termini (TMPred Server, ISREC). A conserved cysteine residue in the second putative extracellular loop between TMD 3 and 4 is involved in the disulfide bridging with *h*4F2hc (Pfeiffer *et al*., 1998).

The next most related protein to the light chain family

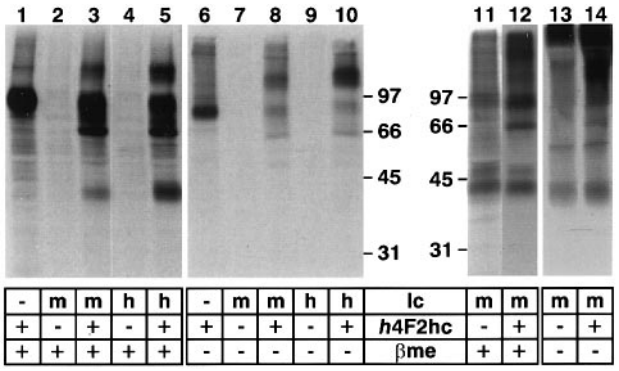

Fig. 2. Co-immunoprecipitation of *h*4F2hc and y+LAT1 using anti-*h*4F2hc antibody (lanes 1–10) or anti-*m*y+LAT1 antibody (lanes 11–14). Oocytes were injected with the indicated cRNA (lc, light chain; m, *m*y+LAT1; h, *h*y+LAT1). Anti-*h*4F2hc antibody (lanes 1–10) precipitates, in oocytes expressing *h*4F2hc alone, a protein migrating at a mol. wt of ~80 kDa (terminally glycosylated *h*4F2hc, lanes 1 and 6). An additional band of 40 kDa is seen when *m*y+LAT1 or *h*y+LAT1 is co-expressed with *h*4F2hc and the samples run in the presence of the reducing agent β-mercaptoethanol (βme) (lanes 3 and 5). The band at 66 kDa, which is much stronger upon co-expression with a light chain than when *h*4F2hc is expressed alone, probably corresponds to the core-glycosylated form of *h*4F2hc. In non-reducing conditions, the proteins remain associated as heterodimers and migrate as a band of ~130 kDa (lanes 8 and 10). Immunoprecipitated *m*y+LAT1 appears as a 40 kDa band (lanes 11 and 13). In oocytes co-expressing *m*y+LAT1 and *h*4F2hc, the heavy chain is co-precipitated and migrates as bands of 80 and 66 kDa in the presence of β-mercaptoethanol (lane 12) and, in its absence, remains associated with the light chain (heterodimer of ~130 kDa) (lane 14).

is the yeast MUP1 methionine permease (~25% identity) which has a hydropathy profile similar to that of the gpa-ATs (not shown). The CAT-type transporters, originally known as ecotropic virus receptors and which produce y+-type transport of cationic amino acids, represent more remote mammalian relatives of the gpa-AT family. The level of identity of *m*y+LAT1 with *r*CAT-1 and *h*CAT-2A is ~21 and 25%, respectively. Sequence alignments (not shown) show several large gaps, and CAT proteins are substantially longer (624 and 657 amino acids) than y+LAT1. They also have a higher number of putative TMDs (14 versus 12).

y+LAT1 forms a covalently linked heterodimer with h4F2hc

We have shown previously that *h*LAT1 is covalently associated with *h*4F2hc (Mastroberardino *et al*., 1998). Using immunoprecipitation of proteins expressed in *Xenopus* oocytes, we tested whether y+LAT1 also associates with *h*4F2hc and thus represents yet another light chain forming a heterodimer with this glycoprotein heavy chain (Figure 2). Immunoprecipitation of *h*4F2hc expressed alone produced, independently of sample reduction, a broad band migrating at ~80 kDa on SDS–PAGE which is typical for the terminally glycosylated form of *h*4F2hc (Figure 2, lanes 1 and 6). In contrast, immunoprecipitation of *m*y+LAT1 revealed a band migrating at ~40 kDa, as expected for a *h*4F2hc light chain (Haynes *et al*., 1981; Hemler and Strominger, 1982) (lanes 11 and 13). The fact that this protein of nearly 56 kDa migrates faster than expected on SDS–PAGE is not unusual for a highly lipophilic membrane protein. Either antibody precipitated both chains when *h*4F2hc and *m- or h*y+LAT1

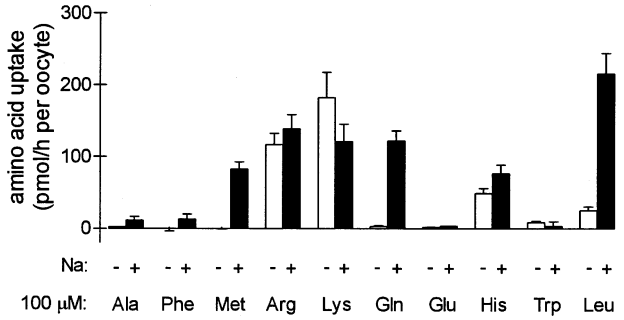

Fig. 3. Specificity and Na^+ dependence of amino acid uptake by *h*4F2hc and *m*y+LAT1 in *Xenopus* oocytes. Uptakes were performed in 100 μM of the tested amino acid with or without Na^+. Background uptake by oocytes injected with *h*4F2hc alone is subtracted. Means of 18 oocytes ± SEMs pooled from three independent experiments are shown.

were co-expressed in the oocytes. In the presence of β-mercaptoethanol, the chains were essentially separated on the gel, while in the absence of reduction, the chains remained associated as heterodimers and migrated as a single band at ~130 kDa. In the presence of a light chain and sample reduction (lanes 3, 5 and 12), an additional band of 66 kDa became prominent which most probably corresponds to the core-glycosylated form of *h*4F2hc. This suggests a retardation of the maturation process of the heavy chain when light chains are co-expressed. The co-immunoprecipitation results demonstrate that *h*4F2hc forms disulfide-linked heterodimers with *m*- and *h*y+LAT1 upon co-expression in *Xenopus* oocytes.

Transport properties of y+LAT1

To test whether *m*y+LAT1 functions as an amino acid transporter, we measured the uptake of radioactively labelled amino acids in *Xenopus* oocytes expressing either *m*y+LAT1 or *h*4F2hc alone or both together. Initial experiments showed that expression of *m*y+LAT1 or *h*4F2hc alone did not increase the amino acid uptake compared with uninjected oocytes when measured 24 h after cRNA injection (Mastroberardino *et al*., 1998; data not shown). However, when both chains were co-expressed, a 2- to 7-fold increase in amino acid transport was observed. All subsequent experiments were performed 24 h after cRNA injection, and control values from oocytes injected with *h*4F2hc alone were subtracted.

Figure 3 shows the uptake of different amino acids added at a concentration of 100 μM. The cationic amino acids L-arginine and L-lysine were transported in the presence of Na^+ as well as in its absence. However, transport of the neutral amino acids L-leucine, L-glutamine and L-methionine was highly Na^+-dependent. L-Alanine and L-phenylalanine, at a concentration of 100 μM, were transported at a much lower rate, but also in an Na^+-dependent manner. L-Histidine was transported similarly in the presence and absence of Na^+ when the experiments were performed at the usual pH of 7.4. However, the ratio of Na^+-dependent to Na^+-independent L-histidine uptake was strongly increased at pH 8.0 (data not shown), indicating that both the neutral and the cationic forms are transported, but that only the transport of the neutral form is Na^+-dependent. L-Glutamic acid and L-tryptophan were not transported above background levels.

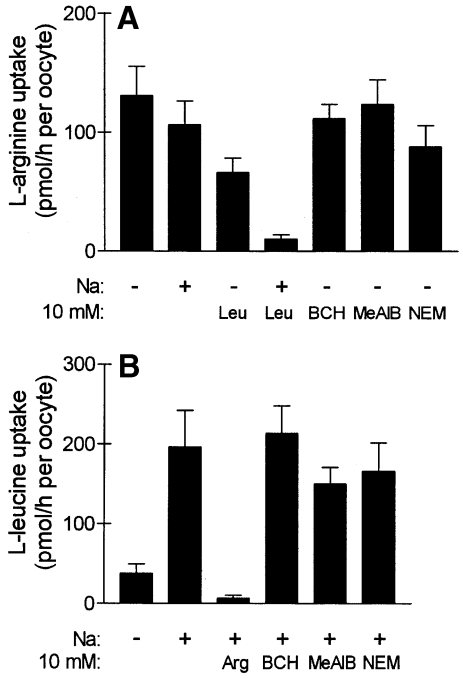

Fig. 4. Inhibition of L-arginine and L-leucine uptake: effect of Na^+ and different inhibitors/competitors. L-Arginine (**A**) and L-leucine (**B**) were added at a concentration of 100 μM to *Xenopus* oocytes co-expressing *h*4F2hc and *m*y$^+$LAT1. The concentration of inhibitors was 10 mM. An excess of L-leucine inhibited L-arginine uptake in the presence of Na^+ and to a lesser extent in its absence. BCH, MeAIB and NEM did not block L-arginine uptake. L-Leucine uptake was inhibited by an excess of L-arginine, but not by BCH, MeAIB and NEM. Background uptake by oocytes injected with *h*4F2hc alone is subtracted. Means of 18 oocytes ± SEMs pooled from three independent experiments are shown.

The observed Na^+-independent uptake of cationic amino acids and the Na^+-dependent uptake of neutral amino acids with a preference for L-leucine compared with L-alanine (higher affinity for L-leucine, see below) is typical for system y$^+$L.

Further evidence that *m*y$^+$LAT1 represents a system y$^+$L-type amino acid transporter is given in Figure 4. The uptake of L-[^{3}H]arginine (100 μM) in the presence of Na^+ was nearly entirely inhibited by 10 mM cold L-leucine. In contrast, L-arginine uptake in the absence of Na^+ was only partially inhibited by the same 100-fold excess of L-leucine. This is compatible with the notion that the apparent affinity of system y$^+$L for neutral amino acids is strongly decreased in the absence of Na^+ (or Li^+, see below). Furthermore, L-arginine uptake was not inhibited by an excess of system L- and A-specific substrates 2-amino-2-norbornane-carboxylic acid (BCH) and methylamino isobutyrate (MeAIB) nor by *N*-ethylmaleimide (NEM) which is known to block system y$^+$ (Devés *et al.*, 1993). Uptake of L-leucine (100 μM) was to a large extent Na^+-dependent and was nearly fully inhibited by 10 mM cold L-arginine. Finally, as expected for system y$^+$L, BCH, MeAIB and NEM had no effect on L-leucine uptake.

Dose–response experiments in oocytes co-injected with *h*4F2hc and mouse or human y$^+$LAT1 were performed for three representative amino acids: L-arginine as a cationic amino acid, L-leucine as a neutral amino acid known to be transported with high apparent affinity by system y$^+$L, and L-alanine as a neutral amino acid with low apparent affinity. Uptake values from oocytes injected with *h*4F2hc alone were subtracted, and curves corresponding to Michaelis–Menten kinetics were fitted to the experimental data by non-linear regression (Figure 5). The apparent K_m of *m*y$^+$LAT1 for L-arginine was 91.5 μM in the absence and 340.8 μM in the presence of Na^+. Such a decrease in apparent affinity due to the presence of Na^+ (10-fold difference versus sucrose) has been reported for system y$^+$L and ascribed to a 'cell surface potential effect' rather than to a direct competition at the level of the binding site (Devés and Angelo, 1996; Devés and Boyd, 1998). In the presence of Na^+, the apparent K_m with the mouse light chain *m*y$^+$LAT1 was 21.7 μM for the large neutral amino acid L-leucine. In contrast, the apparent affinity for the smaller L-alanine was lower by approximately two orders of magnitude (K_m = 1.36 mM). The apparent affinity of the human homologue *h*y$^+$LAT1 for L-leucine in the presence of Na^+ was similar to that of *m*y$^+$LAT1 (31.7 μM). In the absence of Na^+, L-leucine was transported only when present at very high concentrations, whereas no transport of L-alanine was detected in the same conditions. *h*y$^+$LAT1 showed a higher apparent affinity for L-leucine (K_m = 16.2 μM) in the presence of Li^+ than Na^+, a property previously described for system y$^+$L of human erythrocytes by Angelo *et al.* (1996). In contrast, the apparent K_m of *m*y$^+$LAT1 for L-leucine was slightly higher (K_m = 47.9 μM) in the presence of Li^+ than Na^+.

System y$^+$L is known to mediate amino acid exchange (Angelo and Devés, 1994; Chillaron *et al.*, 1996). To test the efflux, oocytes injected with *h*4F2hc alone or co-injected with *h*4F2hc and *m*y$^+$LAT1 were loaded with L-[^{3}H]arginine. After a brief wash, the oocytes were incubated in uptake buffer with or without Na^+ in the absence of any extracellular amino acid (Figure 6A) or in the presence of 1 mM cold L-arginine (Figure 6B) or L-leucine (Figure 6C). Aliquots of the buffer were taken at the indicated time points and radioactivity was counted. No L-[^{3}H]arginine efflux was measured in the absence of extracellular amino acid. However, a notable efflux of L-[^{3}H]arginine was observed in oocytes co-injected with *h*4F2hc and *m*y$^+$LAT1 in the presence of extracellular L-arginine, with a similar rate whether or not Na^+ was present. In control oocytes injected with *h*4F2hc alone, the efflux was much weaker. Extracellular L-leucine stimulated the efflux of L-[^{3}H]arginine as well, but to a lesser extent. This effect showed a partial Na^+ dependence, as expected from the low apparent affinity of the transporter for external L-leucine in the absence of Na^+ (Figure 5A). In similar experiments, we also investigated L-[^{3}H]leucine efflux but, in contrast to L-arginine, no L-leucine efflux mediated by the heterodimer of *h*4F2hc and *m*y$^+$LAT1 was observed. This result parallels a finding of Chillaron *et al.* (1996), who could not detect L-leucine efflux from oocytes injected with *h*4F2hc cRNA alone (several days of expression) and which displayed a weak y$^+$L-type amino acid transport.

Tissue distribution of y$^+$LAT1 mRNA

On Northern blot, *m*y$^+$LAT1 produced a single ~2.3 kb band on mouse kidney poly(A)$^+$ RNA (Figure 7A). A signal corresponding to an mRNA of the same size was also detected in rat kidney and small intestine. Northern

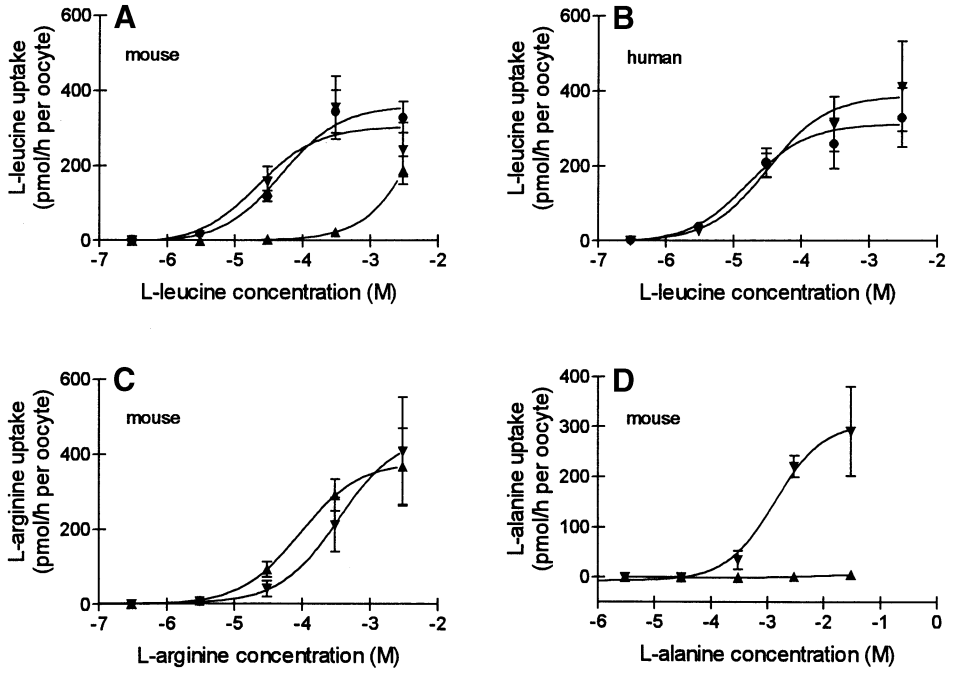

Fig. 5. Concentration dependence of L-leucine (**A**), L-arginine (**C**) and L-alanine (**D**) uptake by *h*4F2hc and *m*y$^+$LAT1 and of L-leucine uptake by *h*4F2hc and *h*y$^+$LAT1 (**B**). Uptake experiments were performed at five different amino acid concentrations, and background uptake by oocytes injected with *h*4F2hc alone was subtracted. Sigmoidal curves were fitted to the experimental curves using a non-linear regression routine. Data from one representative experiment are shown (see Results for apparent K_m values). Error bars represent SEMs of six oocytes. (Symbols: ▲, –Na$^+$; ▼, +Na$^+$; ●, +Li$^+$.)

hybridization on total RNA [lower sensitivity than with poly(A)$^+$] of different rat tissues showed a signal only in kidney cortex. In particular, no signal was detected in kidney medulla and in colon. Hybridization of a commercial mouse RNA dot-blot with a *m*y$^+$LAT1 probe (Figure 7B) confirmed the high level of expression in intestine (RNA from whole intestine) and kidney, further suggesting that this amino acid transporter has a function in transepithelial (re)absorption. Lower levels of *m*y$^+$LAT1 RNA were detected in most tested tissues. The signals in brain, skeletal muscle, heart and uterus were too close to background levels to decide whether *m*y$^+$LAT1 is expressed in these tissues at low levels, possibly in subpopulations of cells. In summary, y$^+$LAT1 appears to be expressed in most tissues, but at the highest level in kidney cortex and the small intestine.

Discussion

The mouse and human y$^+$LAT1 homologues characterized in this study represent a second mammalian 'light chain' belonging to the newly described gpa-AT family of permease-related amino acid transporters which covalently associate with a glycoprotein 'heavy chain' to function at the cell surface (Mastroberardino *et al*., 1998; Figure 2). It is likely that different light chains, which comprise this family, associate with the heavy chain via an equivalent domain. Indeed, they are structurally very similar and display, according to predictions of their 12 TMD topology with intracellular N- and C-termini, a single common cysteine residue (Cys152 for *m*y$^+$LAT1) facing the extracellular space. We have shown for the related light chains ASUR4 (*Xenopus* LAT1) and SPRM1 that this cysteine residue, located between the putative TMDs 3 and 4, forms a disulfide bridge with Cys109 of *h*4F2hc (Pfeiffer *et al*., 1998). Light chains of the same family, which can be expressed in the same cell (Broer *et al*., 1998), might thus compete with each other for binding to the heavy chain. Immunofluorescence experiments performed with the related chain SPRM1 have shown that a function of the heavy chain is to allow surface expression of the light chain. In the absence of heavy chain, the light chain remains in an intracellular compartment, probably the endoplasmic reticulum (Mastroberardino *et al*., 1998). In contrast, surface expression of the heavy chain appears to be independent of co-expression of a light chain (Teixeira *et al*., 1987; Teixeira, 1990; Mastroberardino *et al*., 1998).

The type II glycoprotein heavy chain 4F2hc and the related rBAT display, within their extracellular domain, a region of strong similarity with glycosidases, the function of which has not been elucidated (Wells and Hediger, 1992), and might fulfil other functions besides facilitating expression of amino acid transporters at the correct surface membrane. For instance, a role in integrin activation has been ascribed to CD98 (Fenczik *et al*., 1997). The structural similarity of 4F2hc/CD98 to the rBAT protein, a glycoprotein which induces b$^{0,+}$-type transport when expressed in *Xenopous* oocytes, which is involved in apical amino acid transport and is known to associate with a 40 kDa protein (Wang and Tate, 1995), suggests that rBAT might also associate with light chain(s) of the gpa-AT family. Mutations in the gene encoding this rBAT light chain are expected to explain those cases of cystinuria which are not due to mutations in the rBAT gene. However, this light chain has not been identified as yet and neither y$^+$LAT nor LAT co-precipitated with rBAT (data not shown).

The pattern of amino acid transport via gpa-AT-type

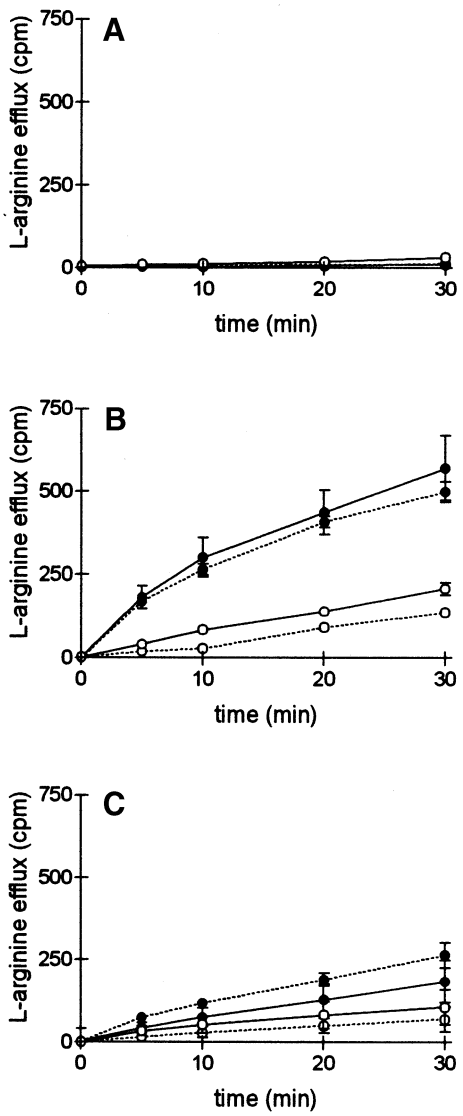

Fig. 6. Stimulation of L-arginine efflux by extracellular L-arginine and L-leucine. Oocytes were loaded with 1 μM L-[^{3}H]arginine and, after a brief wash, incubated in buffer with or without Na^+ (continuous line, $-Na^+$; dotted line, $+Na^+$; ○, oocytes expressing *h*4F2hc alone; ●, oocytes coexpressing *h*4F2hc and *my*$^+$LAT1) without amino acid (**A**) or with 1 mM cold L-arginine (**B**) or L-leucine (**C**). Three aliquots of the incubation buffer were taken at the indicated time points. No efflux was observed in absence of extracellular amino acid. L-[^{3}H]Arginine efflux mediated by *h*4F2hc and *my*$^+$LAT1 was stimulated by extracellular L-arginine in the presence and absence of Na^+ and to a lesser extent and partially Na^+-dependently by L-leucine. Means ± SEMs pooled from two independent experiments are shown.

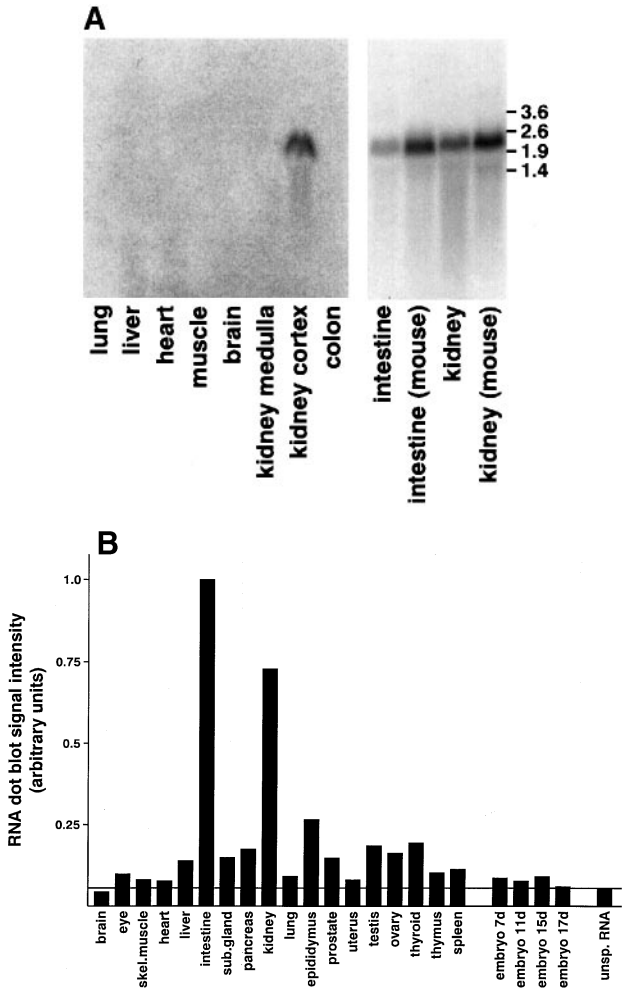

Fig. 7. Tissue distribution of *my*$^+$LAT1 RNA. (**A**) The left panel shows a Northern blot performed on rat total RNA from different tissues using mouse y^+LAT1 as a probe. A signal is observed only in kidney cortex. In the right panel, hybridization of the same probe on rat and mouse poly(A)$^+$ RNA shows that in both species, the mRNA is ~2.3 kb and expressed in (total) kidney and intestine. (**B**) The quantification of the signals obtained on a commercial mouse tissue poly(A)$^+$ RNA dot-blot using *my*$^+$LAT1 as probe shows that high amounts of *my*$^+$LAT1 RNA are expressed in kidney and intestine. Lower amounts of *my*$^+$LAT1 RNA appear to be expressed in most other tissues tested.

exchangers displayed by a cell membrane thus depends on the presence of heavy chain(s) and on the nature of the expressed gpa-AT light chains. The tissue distribution of 4F2hc is broad (Parmacek *et al*., 1989), both in epithelial and non-epithelial cells, while rBAT, which has been shown to be defective in type I cystinuria, is expressed mostly in the apical membrane of kidney proximal tubule and small intestine (Pickel *et al*., 1993). In kidney proximal tubule, 4F2hc has been shown to be localized at the basolateral membrane (Quackenbush *et al*., 1986; Chillaron *et al*., 1996; B.Sordat, personal communication). Based on the mRNA analysis, the tissue distribution of the light chains is different for each one. For instance, y^+LAT1 shows a particularly strong expression in the kidney and intestine compared with y^+LAT2 and LAT1 (Figure 6; B.Spindler and F.Verrey, unpublished results). It will be of interest to test whether the simultaneous expression of different light chains in the same cells is coordinated, and whether structural interactions or a functional network exist.

The analysis of transport properties induced in oocytes by the injection of 4F2hc plus y^+LAT cRNA has to a large extent confirmed expectations based on the description of system y^+L by Devés and colleagues in erythrocytes (Devés *et al*., 1992). There is, however, a major difference in the apparent affinity for extracellular L-arginine in the presence of Na^+ which for y^+LAT1 was two orders of magnitude lower than that reported for the erythrocyte y^+L transport [apparent K_m this study: 341 μM; K_i estimation in Devés *et al*. (1998): 3 μM]. This suggests that y^+LAT1 is not the erythrocyte y^+L transport system

to which the related chain y$^+$LAT2 [HA7016 cDNA, product of the *KIAA0245* gene (Nagase *et al*., 1996)] appears to correspond better (our preliminary results). Qualitatively similar results to those obtained by co-expression of y$^+$LAT1 and *h*4F2hc have been obtained previously by the expression of the heavy chain 4F2hc alone in *Xenopus* ooytes, though only after prolonged incubation and at lower rates (Bertran *et al*., 1992; Wells *et al*., 1992). This suggests that *Xenopus* oocytes express an endogenous y$^+$L-type light chain which can associate with exogenous *h*4F2hc to produce this transport. The fact that *Xenopus* and even *Schistosoma* light chains of the gpa-AT family can associate with human 4F2hc is known for the *Xenopus* type L light chain ASUR4 (*X*LAT1) and SPRM1, respectively (Mastroberardino *et al*., 1998). Furthermore, surface expression of light chains has been shown to depend on association with the glycoprotein heavy chain, a situation which is reminiscent of that of the endogenous Na,K-ATPase α catalytic subunit of *Xenopus* oocytes which can be stabilized and brought to the cell surface upon expression of exogenous Na,K-ATPase β glycoprotein (Geering *et al*., 1989).

The analysis of the transport properties of y$^+$LAT1 reported here can be summarized as follows: in physiological high Na^+ conditions, apparent affinity for neutral amino acids (L-leucine) is an order of magnitude higher than for cationic amino acids (L-arginine) such that this transport system is expected to mediate preferentially the cellular uptake of neutral amino acids. In contrast, cellular efflux of L-leucine is not mediated by this transport system in our experimental conditions, while L-arginine efflux is stimulated readily by extracellular L-arginine or by L-leucine in the presence of Na^+ (though somewhat less efficiently than by L-arginine). Thus, (electroneutral) exchange of intracellular cationic amino acid against extracellular neutral amino acids plus Na^+ ions appears to be the most likely physiological function of this exchanger. It remains to be investigated to what extent the functional asymmetry is due to the different ionic compositions of the cellular and extracellular fluids and/or to a structural asymmetry of the binding sites in the different transporter conformations. The results of the transport experiments support the notion that, in physiological conditions, the y$^+$LAT1 system mediates essentially the efflux of cationic amino acids in exchange for neutral amino acids (plus Na^+).

This pattern of transport corresponds to that expected for the basolateral exchanger of the intestine and kidney proximal tubule which mediates the serosal efflux step of transepithelial cationic amino acid (re)absorption (Angelo and Devés, 1994; Chillaron *et al*., 1996). This step is rate limiting and is known to be defective in lysinuric protein intolerance (LPI) (Rajantie *et al*., 1981; Smith *et al*., 1987). Hence, y$^+$LAT1 is a strong candidate for being the defective transporter in this genetic condition, a hypothesis which is supported by the tissue distribution of its mRNA which we show here to be heavily expressed in mouse/rat small intestine and kidney cortex. The presence of the mRNA of y$^+$LAT1 in many non-epithelial tissues is compatible with the fact that in LPI amino acid transport defects have also been detected in non-epithelial cells such as skin fibroblasts (Smith *et al*., 1987).

Materials and methods

Sequence analysis and cRNA synthesis

The cDNAs for *m*- and *h*y$^+$LAT1 obtained from the IMAGE consortium [EST accession Nos *m*y$^+$LAT1a: AA276085 (mouse kidney library), *h*y$^+$LAT1: AA393488 (human testis library)] were sequenced on both strands (Microsynth, Balgach, Switzerland; ISREC sequencing facility, respectively). Their complete sequences are available under DDBJ/EMBL/GenBank accession Nos AJ012754 and AJ130718. An additional cDNA from a mouse heart library (AA500631) encoding *m*y$^+$LAT1b seems to be a splice variant at the level of the 5′-untranslated sequence (DDBJ/EMBL/GenBank accession No. AJ130943). There are also two differences between the two mouse clones at the level of the nucleotides within the coding sequence leading to a single amino acid change at position 4: Thr for Ser. Sequence alignments were performed using the align program at http://cartan.gmd.de/ToPLign.html. The open reading frames of *m*y$^+$LAT1 and *h*y$^+$LAT1 (flanked at their 5′ ends by 35 nucleotides and a *Cla*I site, and 33 nucleotides and a *Xho*I site, respectively, and on their 3′ ends by 31 nucleotides and a *Bam*HI, site and 35 nucleotides and a *Hin*dIII site, respectively) were amplified by PCR using the Vent^R (New England Biolabs) polymerase and transferred to the pSD5easy vector (Puoti *et al*., 1997). For cRNA synthesis, plasmids containing the cDNAs of *h*4F2hc (vector pSport) and y$^+$LAT1's (pSD5easy) were linearized using the restriction sites *Hin*dIII and *Bgl*II, respectively. cRNA was synthesized with T7 and SP6 RNA polymerase (Promega), respectively, according to standard protocols.

Xenopus laevis oocytes

Oocytes were treated with collagenase A for 2–3 h at room temperature in Ca^{2+}-free buffer containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 10 mM HEPES pH 7.4 to remove follicular cells and then kept at 16°C in ND96 buffer containing 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES pH 7.4.

Amino acid uptake and efflux in Xenopus oocytes

Oocytes were injected with 5 ng of (each) cRNA dissolved in 33 nl of water and kept for 24 h at 16°C in ND96 buffer. Before the experiments, they were washed six times in uptake buffer containing 100 mM NaCl [(+Na)-buffer], choline-Cl [(–Na)-buffer] or LiCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES pH 7.4. Six oocytes per uptake experiment were pre-incubated at 22°C for 2 min. The buffer was then replaced by the respective uptake buffer supplemented with amino acid at the indicated concentration and the corresponding L-[^{3}H]amino acid as a tracer (except for L-[^{14}C]glutamine). Uptake experiments were performed for 1 min because pilot experiments had shown linear amino acid uptake during this time. The oocytes were washed five times with 3 ml of (–Na)-buffer and distributed to individual vials. After oocyte lysis in 2% SDS, radioactivity was counted by liquid scintillation. Uptakes for dose–response curves were performed at five different amino acid concentrations, sigmoidal curves corresponding to Michaelis–Menten kinetics were fitted to the experimental data and amino acid concentrations for half-maximal activation (apparent K_m) were derived.

For efflux experiments, oocytes were loaded for 17 min with 1 μM L-[^{3}H]arginine or L-[^{3}H]leucine in (–Na)- or (+Na)-buffer, respectively. After a brief wash, eight oocytes were transferred to 300 μl of (–Na)- or (+Na)-buffer whithout amino acid or 1 mM cold L-arginine or L-leucine. Three aliquots of 2 μl of the buffer were taken at 0, 5, 10, 20 and 30 min and radioactivity was counted by liquid scintillation.

Labelling of oocytes and immunoprecipitation

Injection of cRNAs in oocytes was as for uptake experiments. After injection, oocytes were incubated for 48 h in ND96 buffer supplemented with 1 mCi/ml [^{35}S]methionine. Then, oocytes were washed twice in ND96 buffer and lysed in oocyte lysis buffer (20 μl/oocyte) containing 120 mM NaCl, 50 mM Tris–HCl pH 8.0, 0.5% NP-40 supplemented with protease inhibitors. Lysates were vortexed for 20 s and incubated briefly on ice. The lysate was then centrifuged for 10 min at 12 000 r.p.m. in an Eppendorf centrifuge at 4°C. The supernatant was frozen in liquid nitrogen. Incorporated radioactivity was determined by scintillation counting of trichloroacetic acid (TCA) precipitates from aliquots.

For *h*4F2hc precipitation, the monoclonal antibody described originally by Haynes *et al*. (1981) was used. A polyclonal antibody against *m*y$^+$LAT1 was raised in rabbits using as antigen a synthetic peptide comprising the amino acids 12–27 of the N-terminal tail coupled to keyhole limpet haemocyanin (Eurogentec, Seraing, Belgium). Antibodies were pre-bound to protein G plus/protein A–agarose (Calbiochem) for

2 h at room temperature in oocyte lysis buffer. The beads were then added to the pre-cleared lysate (equal amounts of counts, incubated twice for 30 min at 4°C with protein G plus/protein A–agarose beads) which was rotated overnight at 4°C. Beads were washed five times in buffer containing 100 mM NaCl, 20 mM Tris–HCl pH 8.0, 1 mM EDTA, 500 mM LiCl, 0.5% NP-40, and five times in buffer containing 100 mM NaCl, 20 mM Tris–HCl pH 8.0, 1 mM EDTA, 0.5% NP-40. They were resuspended in SDS–PAGE sample buffer and heated to 65°C for 15 min. β-Mercaptoethanol was added where indicated and SDS–PAGE analysis was performed. Gels were stained in Coomassie Blue and fixed. After incubation in Amplify (Amersham), the gels were dried and exposed to film at –80°C with an intensifying screen.

Northern blot analysis

Total RNA (10 μg) or poly(A)$^+$ RNA (7 μg) and RNA standards (Promega) were run on 1% agarose/formaldehyde minigels, transferred to Genescreen membranes (NEN Dupont) and immobilized with UV light according to standard protocols. *m*y$^+$LAT1-specific probes labelled with [α-^{32}P]dCTP to a specific activity of 2×10^9 c.p.m./μg DNA were generated by random priming (Oligolabeling kit, Pharmacia). Hybridization and washes of mouse RNA dot-blot (Clontech) and Northern blots were performed according to standard protocols. Blots were exposed, scanned and signals quantified using a PhosphorImager and the Imagequant software (Molecular Dynamics).

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