Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Plasma Membrane Transporters for Arginine[1,2]

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ABSTRACT   The supply of arginine may become rate limiting for enzymatic reactions that use this semiessential amino acid as a substrate (e.g., nitric oxide, agmatine, creatine, and urea synthesis), particularly under conditions of high demand such as growth, sepsis, or wound healing. In addition, arginine acts as a signaling molecule that regulates essential cellular functions such as protein synthesis, apoptosis, and growth. In the past decade, a number of carrier proteins for amino acids have been identified on the molecular level. They belong to different gene families, exhibit overlapping but distinctive substrate specificities, and can further be distinguished by their requirement for the cotransport or countertransport of inorganic ions. A number of these transporters function as exchangers rather than uniporters. Uptake of amino acids by these transporters therefore depends largely on the intracellular substrate composition. Hence, there is a complex crosstalk between transporters for cationic and neutral amino acids as well as for peptides. This article briefly reviews current knowledge regarding mammalian plasma membrane transporters that accept arginine as a substrate.   J. Nutr. 134: 2752S–2759S, 2004.

KEY WORDS: • *cationic amino acid transporter* • *hetero(di)meric amino acid transporter*
  • *acid transporter B*$^{0,+}$ • *peptide transporter*

Arginine is the substrate for important metabolic pathways such as nitric oxide (NO), agmatine, creatine, and urea synthesis [for review, see Morris (1)]. Its supply may become rate limiting for any of these enzymatic reactions. In addition, arginine was recently shown to act as a signaling molecule that regulates essential cellular functions such as protein synthesis, apoptosis, and growth (2–5). Although arginine is, strictly speaking, not an essential amino acid (AA),[4] de novo arginine synthesis by the kidney (the major producer of plasma arginine) does not always seem to sustain an adequate supply. Particularly under conditions of high demand, such as growth, sepsis, or wound healing, the endogenous arginine supply may become limiting [for review, see Refs. (6–9)]. Therefore, arginine is considered a semiessential AA in humans (and most mammals), with a recommended dietary intake of 1–3 g/d. Arginine needs to be exchanged between different tissues, irrespective of whether nutrition or biosynthesis is the primary source. Consequently, basically every cell needs to transport arginine across the plasma membrane. However, the lipid bilayer of biological membranes is impermeable for hydrophobic solutes such as AAs. Specialized carrier proteins are therefore necessary to provide adequate import and export routes. In addition, there is accumulating evidence for an intracellular compartmentalization and channeling of arginine to distinct metabolic pathways within the cytoplasm (10). This aspect will be discussed in a separate forthcoming article (Closs, E. I., Rotmann, A., Vékony, N., and Simon, A., unpublished results). This article briefly reviews the current knowledge of mammalian plasma membrane transporters that accept arginine as a substrate.

Distinction of different transport systems for arginine and other AAs

Pioneering work initiated in the 1960s in the laboratory of Halvor Christensen demonstrated that arginine shares the same transport system with other cationic AAs (CAAs) such as lysine and ornithine [for review, see White (11)]. In addition, in most cells, arginine transport through the plasma membrane is not energized by coupling to the Na$^+$ gradient. Thus, a single Na$^+$-independent transport system termed system y$^+$ is postulated to be the major entry route for CAAs to most cells. (The letter y is derived from lysine, the first substrate described for this system, and the + signifies the positive charge of the AA substrates.) Additional features of

[4] Abbreviations used: AA, amino acid; AT, amino acid transporter; BHK, baby hamster kidney; CAA, cationic amino acid; CAT, cationic amino acid transporter; 4F2hc, 4F2 heavy chain; GFP, green fluorescent protein; HAT, hetero(di)meric amino acid transporter; hCAT, human cationic amino acid transporter; HUGO, Human Genome Organization; iNOS, inducible nitric oxide synthase; IRES, internal ribosomal entry sequence; K_m, Michaelis-Menten constant; LPI, lysinuric protein intolerance; mCAT, mouse cationic amino acid transporter; NAA, neutral amino acid; NO, nitric oxide; NOS, nitric oxide synthase; PAEC, porcine pulmonary artery endothelial cells; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; rBAT, related to system b$^{0,+}$; SLC, solute carrier.

system y^+ are pH insensitivity and strong stimulation by hyperpolarization and by substrate at the opposite membrane side (transstimulation). More recently, several additional Na^+-independent transport systems and 1 Na^+-dependent system for CAAs were discovered (**Table 1**) [for review, see Deves and Boyd (19)]. The Na^+-independent systems are further distinguished by their interaction with neutral AAs (NAAs). System y^+L, first defined by Deves et al. (20), transports both CAAs and large NAAs, the latter in a Na^+-dependent manner. (The pattern of NAA substrates resembles the NAA transport system L; the letter *L* stands for the model substrate leucine.) van Winkle and coauthors (21) distinguished several transport systems that accept CAA in mouse preimplantation conceptuses and blastocytes. System $b^{0,+}$ transports both CAA and NAA in a Na^+-independent manner. It prefers large NAAs that are not branched at the α and β carbon (21). (The exponents 0 and + indicate the neutral and positive charges, respectively, of the amino acid substrates.) System $B^{0,+}$-mediated transport of CAAs and NAAs is coupled to Na^+ (indicated by the letter *B*). This system accepts a wide range of substrates, including branched and small NAAs (21). Exceptions to the rule of common transport systems for all CAAs are systems b_1 and b_2 (and further subtypes), which exhibit a strong preference for arginine (e.g., system b_2) and have so far exclusively been described in preimplantation conceptuses and blastocytes (22).

In general, the term *transport system* calls attention to the concept that a complex of different proteins rather than a single carrier protein may mediate a distinct transport activity. In fact, systems y^+L and $b^{0,+}$ turned out to be heteromeric AA transporters (HATs), consisting of 2 unrelated proteins, a glycoprotein and the actual carrier protein (see below). In contrast, systems y^+ and $B^{0,+}$ seem to be encoded by single carrier proteins. However, interactions with other, as yet unidentified, cellular proteins that may modulate the function and subcellular targeting of these proteins cannot be excluded.

In line with this notion is the proposal that the activities of systems b_1 and b_2 are mediated by the same carrier proteins as system y^+ and that differences in substrate recognition are due to the interaction with cellular factors (23). It should also be noted that the transport proteins identified today often differ somewhat in their substrate affinity and pattern from the originally described transport systems. Therefore, even with the discovery of individual carrier proteins involved in arginine transport, the classification into transport systems is still useful in characterizing a specific transport activity in a given cell membrane.

The molecular identification of individual carrier proteins revealed an even greater diversity than had been anticipated from the radiotracer flux experiments used for the original classification of the transport systems. System y^+ activity can thus be mediated by at least 3 different CAA transporter (CAT) proteins: CAT-1, -2B, and -3. In addition, another CAT protein, CAT-2A (an alternative splice variant of the CAT-2 transcripts), exhibits transport properties formerly not defined for a transport system. Likewise, system y^+L comprises at least 2 distinct HATs [the glycoprotein 4F2 heavy chain (4F2hc) combined with either AA transporter (AT) carrier y^+LAT1 or y^+LAT2], and there is evidence for additional unidentified isoforms of systems y^+L and $b^{0,+}$ (24,25). Table 1 lists the proteins involved in arginine transport known to date. They belong to 3 different gene families, called *solute carrier* (SLC) families by the Human Genome Organization (HUGO). The CATs and the carrier proteins of the HATs (the latter also referred to as *light chains*) build the SLC7 family. The HAT glycoproteins (also called *heavy chains*) make up the SLC3 family. $ATB^{0,+}$, the protein that mediates system $B^{0,+}$ activity, belongs to the SLC6 family of Na^+- and Cl^--dependent transporters. The following section summarizes important features of these transporters and refers to excellent recent reviews for further details and references.

TABLE 1

Plasma membrane transporters that accept arginine as a substrate[1,2]

Protein[3]	Gene[3]	Transport system	Arginine transport Na+-dependent	Arginine transport Apparent K_m	Leucine transport Na+-dependent	Leucine transport Apparent K_m	Transstimulation	Ref.
				mmol/L		*mmol/L*		
CAT-1[3,4]	SLC7A1	y^+	No	0.10–0.16	—	—	Yes	12
CAT-2A[3]	SLC7A2	ND	No	3.40–3.90	—	—	No	12
CAT-2B[3,4]	SLC7A2	y^+	No	0.25–0.70	—	—	Moderate	12
CAT-3[3]	SLC7A3	y^+	No	0.20–0.50	—	—	Moderate	13
CAT-4[5]	SLC7A4	—	—	—	—	—	—	
y^+LAT1 + 4F2hc	SLC7A7 + SLC3A2	y^+L	No	0.34[6]	Yes	0.02	Yes	14
y^+LAT2 + 4F2hc	SLC7A6 + SLC3A2	y^+L	No	0.12–0.14[7]	Yes	0.20–0.30	Yes	15
$b^{0,+}AT$ + rBAT	SLC7A9 + SLC3A1	$b^{0,+}$	No	0.08–0.20	No	0.30	Yes	16, 17
$ATB^{0,+}$	SLC6A14	$B^{0,+}$	Yes + Cl^-	0.10–0.15	Yes + Cl^-	0.01	No	18

[1] Known carrier proteins for arginine are listed; only the most common name for each protein is given. Gene names are per HUGO. Apparent extracellular K_m values are for arginine and, where applicable, the neutral amino acid leucine (for comparison).

[2] Abbreviations: AT, amino acid transporter; ND, not defined.

[3] Values are for human CAT proteins. Note that experimental K_m values vary considerably.

[4] CAT-1 and CAT-2 may also mediate activities of systems b_1 and b_2 (23).

[5] No transport activity has been detected.

[6] Value for arginine is 93 μmol/L in the absence of Na^+.

[7] K_m is independent of Na^+ concentration.

Cationic amino acid transporters

Properties of individual CAT isoforms. CATs are integral membrane glycoproteins with 14 putative transmembrane domains and intracellular N- and C-termini [for review, see Closs and Mann (26)]. Among the members of this SLC7 subfamily, 4 bona fide carrier proteins are recognized: CAT-1, -2A, -2B, and -3 (HUGO SLC7A1–SLC7A3). CAT-2A and -2B are splice variants that differ only in a stretch of 42 amino acids. The product of another related gene (SLC7A4), expressed in brain, testis, and placenta, is only 40% identical to CAT-1 to -3 (27). Expression of this protein in the plasma membrane of *Xenopus laevis* oocytes or mammalian cells is not sufficient to confer a transport activity for AAs (28). Its function therefore remains elusive.

As evidenced by transport studies in *Xenopus laevis* oocytes and mammalian cells, CAT-1 to -3 all mediate Na^+-independent transport of CAAs, but differ in their substrate affinities and sensitivities to transstimulation. In good agreement with system y^+, human CAT-1 exhibits an apparent K_m of 0.10 to 0.16 mmol/L for CAA, is strongly transstimulated, and is mainly pH independent. Human CAT-2B and -3 both exhibit apparent K_m values of 0.25 to 0.70 mmol/L for arginine and 0.2 to 0.5 mmol/L for lysine, and their affinities to ornithine are slightly lower. Both carriers are only moderately transstimulated. Like CAT-1, CAT-3 is pH independent in a range of 5.5 to 8. In contrast, CAT-2B shows only 50% activity at pH 5.5, compared with pH 7.5. At low pH, when it is largely protonated, histidine becomes a good substrate of CAT-1, but not of CAT-3. CAT-2A is clearly distinguishable from system y^+, because of its low affinity for CAA (apparent K_m 2–5 mmol/L), insensitivity to transstimulation, and moderate pH dependence.

The differences in transstimulation between the individual CAT isoforms have important implications. First, the strong transstimulation of CAT-1 indicates that it works much better in the exchange than the uniport mode. Cells expressing this isoform will therefore be readily depleted of intracellular arginine only when another CAA is provided at the extracellular side. Arginase present either in the extracellular region or in neighboring cells should therefore deplete CAT-1–expressing cells very efficiently, not only by consuming the extracellular arginine but also by providing extracellular ornithine for exchange. This might be an important mechanism for the regulation of arginine-dependent pathways. Second, once depleted of intracellular CAA, CAT-1–expressing cells take up extracellular arginine only very slowly. This might explain why the expression of CAT-2B is necessary in cells with a high consumption of CAA, e.g., to sustain NO production by the inducible NO synthase (iNOS) (29). Third, because transstimulation is a major determinant of CAT-1 activity, observed changes in arginine uptake in a given experiment may not necessarily be due to changes in CAT-1 expression, but may be due to changes in intracellular CAA levels. To exclude a transstimulation effect, cells must be preequilibrated in buffer containing the same concentration of unlabeled arginine as that used during the uptake measurements (30).

The divergent transport properties of CAT-2A and -2B imply that the stretch of 42 AAs where the 2 carriers differ from each other is a crucial determinant of transport properties. In fact, replacement of the corresponding region of mouse CAT (mCAT)-1 by that of mCAT-2A or -2B (and vice versa) leads to chimeric proteins with the apparent substrate affinity and sensitivity to transstimulation of the respective donor of that region. The 3 isoforms exhibiting similar transport properties (CAT-1, -2B, and -3) also show the highest percentage of AA sequence identity in the region that is located in the fourth intracellular loop (according to the 14-transmembrane model). Replacement of 2 AA residues within this loop of human CAT (hCAT)-2A by the corresponding residues of hCAT-1 confers high substrate affinity to CAT-2A (31).

All CAT proteins mediate influx as well as efflux of CAA. Although the affinities at the intracellular substrate binding side have not been measured directly, indirect evidence suggests that each transporter binds substrate with similar affinities at both sides of the membrane. When allowed to equilibrate in solutions with high arginine concentrations, CAT-2A–expressing oocytes gather substantially more substrate than CAT-1– or -2B–expressing oocytes. Thus, the low-affinity transporter CAT-2A reaches maximum efflux only at much higher intracellular arginine concentrations than do the low-affinity isoforms. These differences also occur in the absence of membrane potential and thus can be explained only by differences in the intracellular affinity of the individual transporters (32).

CAT-mediated transport of CAA is voltage dependent, with hyperpolarization increasing influx rates (33,34). Changes in the membrane potential may thus affect the transport rate for arginine, as shown by numerous radiotracer flux studies in cultured cells [for review, see Refs. (19,35)]. There is a leak conductance associated with the hCAT proteins (32) as previously described for other AA transporters (36,37). In hCAT-2B-expressing oocytes, K^+ is the main charge carrier for this leak conductance. Studies of Rb^+ uptake indicate that it can be inhibited by arginine. However, the physiological relevance of this leak conductance remains elusive.

CAT expression, regulation, and putative physiological function. CATs are widely expressed. In fact, there is probably not a single cell type that does not express at least 1 of the CAT isoforms. Most studies have relied on the detection of CAT mRNA. Much less is known about CAT protein expression, primarily because of the lack of good antibodies. A discussion of all aspects of CAT expression and regulation is beyond the scope of this review; thus, we refer primarily to recent publications [for reviews and further references, see Refs. (38–42)].

To date, the most extensive studies are available for CAT-1. Briefly, CAT-1 exhibits a broad expression pattern; adult liver hepatocytes are the only cell type that does not express this carrier. In spite of its almost ubiquitous presence, CAT-1 expression is highly regulated on the transcriptional and posttranscriptional level. Fast-dividing cells generally exhibit greater CAT-1 mRNA expression than quiescent cells. Among the factors reported to increase CAT-1 mRNA (and system y^+ activity) are interleukin-1, insulin, glucocorticoids, angiotensin II, platelet-derived growth factor, and tumor necrosis factor-α. Upregulation of CAT-1 by tumor growth factor-β may increase polyamine and proline synthesis in vascular smooth muscle cells and contribute to arterial remodeling at sites of vascular damage (43). Bacterial LPS and interferon-γ either upregulate or downregulate CAT-1. Changes in CAT-1 mRNA levels do not necessarily affect CAT-1 protein levels. For example, treatment of endothelial cells with the protein kinase C (PKC)-stimulating phorbol ester phorbol-12-myristate-13-acetate (PMA) causes a large increase in CAT-1 mRNA levels, but CAT-1 protein levels do not subsequently increase (44).

Interestingly, limitation of essential nutritional components such as glucose and AAs (e.g., at a time when global protein synthesis decreases) greatly enhances the stability and translation of rat CAT-1 mRNA. Elegant work by Hatzoglou et al. (45) elucidates the crucial mechanisms underlying this

control. Enhancement of the stability of rat CAT-1 mRNA is mediated by an adenosine-uracil-rich element within the 3′-untranslated region that binds the cytoplasmic shuttling protein HuR (46). Increased translation of the rat CAT-1 mRNA is brought about by an internal ribosomal entry sequence (IRES) within the 5′-untranslated region of the CAT-1 mRNA that is activated by AA starvation [see Yaman et al. (2) and references therein]. Induction of this IRES requires the translation of a small upstream open reading frame within the IRES and phosphorylation of the translation initiation factor eIF2α. In contrast, Kakoki et al. (47) report a downregulation of CAT-1 protein expression in rat renal medulla in vivo in response to arginine depletion. The difference may be due to a difference in the depletion period employed by the 2 groups (5 d vs. 9–16 h).

Associations between CAT-1 and cytoskeletal proteins and caveolin are reported. These protein interactions, as well as the subcellular localization of CAT-1, seem to depend on the cell type (Table 2). Colocalization of CAT-1 with caveolin and endothelial NOS in endothelial cells was not found in 1 study (48), but was found in another (49). Furthermore, in baby hamster kidney (BHK) cells, the majority of fusion proteins between CAT-1 and the green fluorescence protein (GFP) localizes to caveoli (54). In contrast, only a small portion of CAT-1.GFP is found in the triton-insoluble fraction (containing caveoli membranes) of polarized epithelial cells (53). In the latter, expression of CAT-1.GFP is restricted to the basolateral membrane (53,55), whereas the majority of CAT-1.GFP in human U373 glioblastoma cells is found in intracellular vesicles (28). Deletion of AAs 4 through 33 leads to retention of the majority of CAT-1.GFP in the endoplasmic reticulum in BHK cells, but not in human embryonic kidney HEK 293 cells, indicating that the N-terminus directs the subcellular localization of CAT-1 in a cell-type-dependent

manner (56). Interaction with cytoskeletal proteins seems to modulate CAT-1 activity. Arginine uptake in porcine pulmonary artery endothelial cells (PAECs) is inhibited upon exposure to longtime hypoxia, concomitant with an activation of calpain and subsequent degradation of fodrin, but with no effect on the total CAT-1 protein content (50). Calpain inhibitors prevent this hypoxia-induced inhibiton of the arginine transport. The authors thus conclude that association of fodrin with CAT-1 is necessary for full CAT-1 activity. The same group found ~25% induction of arginine transport in PAEC by the actin-stabilizing toxin jasplakinolide and ~25% reduction of arginine transport by the actin-disrupting toxin swinholide (51), again without effect on the total CAT-1 protein content. It is tempting to speculate that association with cytoskeletal proteins (or caveolin) might influence not only the transport activity of CAT-1 but also its substrate affinity and selectivity.

The subcellular localization of CAT-1 is also subject to regulation by PKC. Activation of PKC causes a pronounced downregulation of CAT-1 activity, expressed either endogenously in mammalian cells or exogenously in oocytes of X. laevis (44,57). This downregulation is not accompanied by a reduction in total CAT-1 protein, but is due to a decrease in the cell surface expression of the transporter (Rotmann, A., Strand, D., Martiné, U., and Closs, E. I., unpublished results). In contrast, several authors report stimulation of system y^+-activity by PMA. This increase in arginine transport occurs only after several hours and might thus be due to translation of the PMA-induced upregulated CAT-1 mRNA [for review, see Closs (41)]. How efficiently the CAT-1 mRNA is translated could depend on the cell type and experimental conditions, particularly because of the extensive control of CAT-1 translation by nutrient supply.

Taken together, the effect on arginine transport of a change

TABLE 2

Subcellular localization and association of CAT-1 with caveolin and cytoskeletal proteins[1]

Cell type	Finding	Method	Ref.
PAEC; human fibroblast; rat hepatoma	Localized in clusters (not in caveoli)	Endogenous CAT-1 Immunostaining Clusters disrupted by nocodazole (disrupts microtubules) Soluble in triton	48
PAEC	Localized in caveoli	Endogenous CAT-1 Coimmunostaining eNOS and caveolin Immunodepletion with anti-eNOSAB → reconstitution	49
PAEC	Associated with fodrin (but not ankyrin)	Endogenous CAT-1 Hypoxia → calpain↑ → fodrin↓ → arg transport↓ Immunodepletion with anti-fodrin AB → reconstitution and Western blot	50
PAEC	Associated with actin cytoskeleton	Endogenous CAT-1 Swinh → arg tra↓ → eNOS activity↓ Jasp → arg transport↑ → eNOS activity↑ (CAT-1 and eNOS protein ↔) Immunodepletion with anti-fodrin AB → reconstitution	51
BHK	Localized in caveoli	CAT-1.GFP Coimmunostaining caveolin Co-IP caveolin	52
MDCK; HEK 293	Associated with actin (not in caveoli); localized in basolateral membrane	CAT-1.GFP, CAT-1.HA Confocal Triton solubility Cytochalasin D (actin disruption) → triton solubility↑ Coimmunostaining caveolin	53
U 373	Intracellular vesicles, plasma membrane; ruffled border	CAT-1.GFP Confocal	28

[1] Abbreviations: BHK, baby hamster kidney; CAT, cationic amino acid transporter; eNOS, endothelial nitric oxide synthase; GFP, green fluorescent protein; HA, influenza A virus hemagglutinin; HEK, human embryonic kidney; Jasp, jasplakinolide (stabilizes actin filaments and promotes actin polymerization); MDCK, Madin-Darby canine kidney; PAEC, porcine pulmonary artery endothelial cell; Swinh, swinholide A (severs actin microfilaments); U, human glioblastoma.

in CAT-1 mRNA expression depends on multiple factors. Alterations in CAT-1 mRNA might not be reflected in corresponding changes in CAT-1 protein, or these changes might not involve the surface expressed (active) CAT-1. Furthermore, expression-independent factors such as membrane potential, transstimulation, and subcellular distribution of CAT-1 may cause pronounced changes in CAT-1 activity.

CAT-2B expression can be induced in many cell types by treatment with cytokines or LPS, often together with iNOS [for review, see Refs. (26,41,42)]. However, the signaling pathways leading to induction of CAT-2B and iNOS differ, at least in part. Hence, the 2 proteins are not always coexpressed. Because CAT-2B is generally coexpressed with CAT-1, the question of whether it has specific functions (e.g., for the substrate supply of iNOS) arises. In mouse CAT-2$^{-/-}$ cells, iNOS activity is reduced in macrophages and glia cells, but not in fibroblasts (29,58,59). This indicates a cell-type-dependent interaction of the 2 proteins. As discussed above, CAT-2B might be necessary for efficient arginine transport in cells with low intracellular levels of CAAs (e.g., cells with high activity of arginase and downstream enzymes that together convert arginine into NAAs or polyamines). Alternatively, distinct intracellular arginine pools may exist in different cells. If true, iNOS in macrophages and glia cells should have access to the arginine pool nourished by CAT-2B, but not the pool nourished by CAT-1. In line with the second hypothesis is our observation that iNOS activity is virtually abolished in macrophages when intracellular arginine levels drop below 160 μmol/L and iNOS should still be saturated (35). In addition, overall arginine levels in macrophages from CAT-2$^{-/-}$ mice are not reduced (29). Interestingly, in experimental studies of asthma, CAT-2 is upregulated together with arginase I and II, but not iNOS, pointing to a function of CAT-2 in allergic airway responses independent of iNOS (60). However, the CAT-2 isoform involved was not identified in this study.

The low-affinity splice variant, CAT-2A, exhibits a quite distinct expression pattern. By far the highest expression occurs in the liver, where it most likely serves to clear plasma of excess arginine. Surprisingly, urea production in CAT-2$^{-/-}$ mice is apparently not reduced. However, impairment of the urea cycle might become apparent only with a protein-rich diet. Substantial CAT-2A expression is induced by surgical trauma (hepatectomy and spleenectomy) as well as by food deprivation (61). In this catabolic condition, CAT-2A probably serves as an export route for arginine derived from the breakdown of muscle proteins. It has not been determined whether recovery from surgical trauma is impaired in CAT-2$^{-/-}$ mice. The function of CAT-2A in other tissues where it is moderately expressed (pancreas, skeletal muscle, cardiomyocytes, and vascular smooth muscle cells) remains largely unknown.

CAT-3 is widely expressed during embryonic development (62), but seems to be confined to central neurons in adult mice and rats (62–65). In humans, we observed strong CAT-3 expression in the thymus; moderate expression in the uterus, testis, mammary gland, and brain; and weak expression in the ovary and stomach (13). The specific function of CAT-3 in these tissues is not understood. Although a role for CAT-3 in the substrate supply of neuronal NOS has been postulated, we did not detect any correlation between CAT-3 expression and neuronal NOS in human tissue (13).

Arginine transport by HATs

Heterodimeric AA transporters are comprised of a glycosylated type II membrane protein with a single transmembrane helix and a large extracellular domain (heavy chain) and an associated protein with 12 putative transmembrane helices (light chain). The latter proteins are distantly related to the CATs and together comprise a subfamily of SLC7. Other than the CATs, members of this subfamily are not glycosylated and localize to the plasma membrane only when coexpressed with the respective glycoprotein. Eight different HATs have been identified to date (and there is evidence for the existence of additional isoforms). In all but 1, the heavy chain 4F2hc associates with different light chains to form AA transporters with diverse substrate selectivity. Two of these accept arginine as a substrate: association of 4F2hc with the light chains y$^+$LAT1 and y$^+$LAT2 gives rise to y$^+$L-like transporters (Table 1). Arginine is also transported by the eighth HAT, composed of the heavy and light chain rBAT (related to b$^{0,+}$ AA transporter) and b$^{0,+}$AT, respectively. rBAT/b$^{0,+}$AT mediates system b$^{0,+}$ activity. There are several comprehensive reviews by experts in the HAT field (66–70). The discussion here is limited to arginine transport mediated by HATs.

HAT-mediated renal and intestinal absorption of arginine. All arginine-accepting HATs function as obligatory exchangers (i.e., uptake or efflux of arginine by these transporters can occur only in exchange with another CAA or NAA). Their physiological function is best understood in the absorbing epithelia of the small intestine and renal tubule, where the combined action of rBAT/b$^{0,+}$AT and 4F2hc/y$^+$LAT1 causes transepithelial CAA flux (**Fig. 1**). In addition, rBAT/b$^{0,+}$AT in the apical membrane is essential for the (re)absorption of cystine. Mutations in the human rBAT and b$^{0,+}$AT genes cause cystinuria, a recessive inherited disease involving a defect in the renal and intestinal absorption of cystine and CAAs [for review, see Refs. (67–70)]. Missense mutations in rBAT generate type I cystinuria (i.e., without phenotype in heterozygotes) whereas most mutations in b$^{0,+}$AT give rise to non–type I cystinuria, a more severe phenotype in which hyperexcretion of cystine and CAAs may also occur in heterozygotes. Mutations in the human y$^+$LAT-1 gene (SLC7A7) give rise to lysinuric protein intolerance (LPI), a rare heritable disorder associated with a defect of CAA transport in the basolateral membrane of epithelial cells in the small intestine and renal tubules [for review, see Refs. (67–69)]. Unlike cystinuria, plasma concentrations of arginine (and other CAAs) are reduced in LPI. Apparently CAT-1, which is also present in the basolateral membrane, cannot compensate for the efflux defect (Fig. 1).

After reconstitution in liposomes, the light chain b$^{0,+}$ functions alone (71). It thus represents the catalytic unit of the holotransporter, and this most likely also holds true for other light chains. Expression of b$^{0,+}$ is also necessary for stabilization and complete glycosylation of the partner protein rBAT (72). The glycoproteins, in contrast, function mainly to target the light chains to the plasma membrane. However, a recent study provides direct evidence that mutations in rBAT may modify transport properties of the holotransporter (73). In addition to its role as an anchoring protein for light chains, 4F2hc (which is also called CD98) is involved in a number of other cellular processes such as integrin activation, cell adhesion, differentiation, proliferation, and membrane fusion [for review, see Refs. (24,67)].

The vectorial transport of substrate across the epithelium is brought about by an asymmetry of substrate binding at extra- and intracellular binding sites of the apical (rBAT/b$^{0,+}$AT) and basolateral (4F2hc/y$^+$LAT1) transporters. Thus, uptake of CAAs by rBAT/b$^{0,+}$AT is favored because of its extracellular high affinity for these AAs and the negative membrane potential. In return, NAAs are preferentially exported owing to
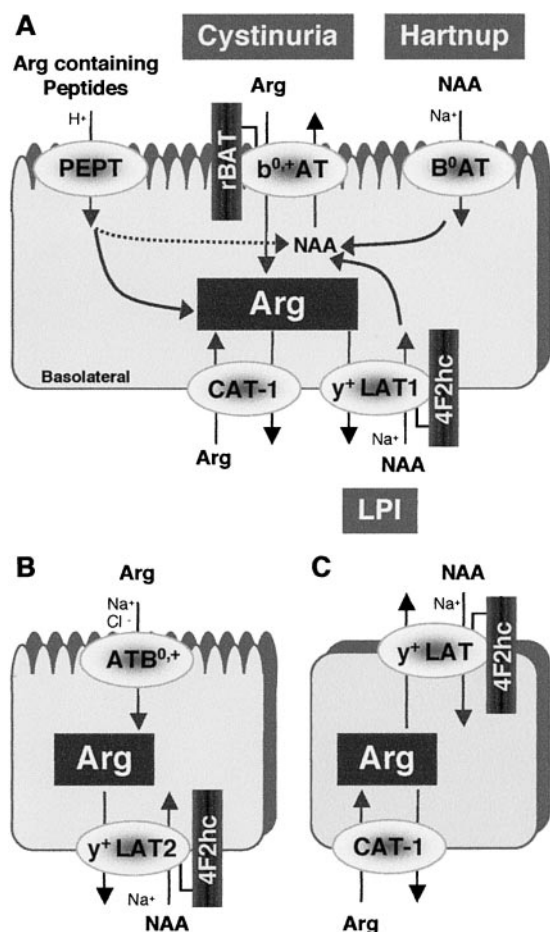
FIGURE 1 Arginine transport in different cell types: flux through the major arginine transporter (for details see text). (*A*) Epithelial cell of small intestine or renal proximal tubule: Arginine absorption in the apical membrane is mediated by rBAT/b$^{0,+}$AT and (in the form of arginine-containing di- and tripeptides) PEPT1 or PEPT2. Arginine efflux at the basolateral membrane occurs via 4F2hc/y$^+$LAT1. In addition, CAT-1, also localized at the basolateral membrane, mediates an exchange of CAAs. NAAs, necessary for exchange with arginine at the apical membrane, are provided by PEPT1 or PEPT2 and B^{0}AT and, at the basolateral membrane, y$^+$LAT1. Defects in rBAT/b$^{0,+}$AT and y$^+$LAT1 cause cystinuria and LPI, respectively. Defects in B^{0}AT cause Hartnup disorder. (*B*) Colon epithelial cell: Arginine import occurs via the Na$^+$-dependent ATB$^{0,+}$, efflux via 4F2hc/y$^+$LAT2. (In lung epithelial cells, ATB$^{0,+}$ is coexpressed with PEPT2 and rBAT/b$^{0,+}$AT in the apical membrane, whereas both 4F2hc/y$^+$LAT1 and 4F2hc/y$^+$LAT2 are expressed in the basolateral membrane.) CAT-1 is probably also expressed in these epithelial cells. (*C*) Nonpolarized cell: In most cells, uptake of arginine is mediated by CAT-1, whereas y$^+$LAT1 or y$^+$LAT2 is responsible for arginine efflux.

their high intracellular concentration. The concentrative uptake of CAAs by rBAT/b$^{0,+}$AT can be considered tertiary active transport because it uses the AA gradient established by a Na$^+$-dependent carrier for NAAs (secondary active transport; see discussion of B^{0}AT1 below) as the driving force (74,75). The basolateral 4F2hc/y$^+$LAT1 has a higher extracellular affinity for NAAs, which are therefore the preferred uptake substrates. Because their transport by 4F2hc/y$^+$LAT1 is coupled to the cotransport of Na$^+$, they cannot exit cells by this route (against the Na$^+$ gradient). CAAs are thus the exclusive export substrates for 4F2hc/y$^+$LAT1. The affinity of 4F2hc/y$^+$LAT1 for CAAs is reciprocal to the Na$^+$ concentration. The resulting low extracellular affinity combined with

substantial competition by NAAs make this an inefficient entry pathway for CAAs. Taken together, rBAT/b$^{0,+}$AT and 4F2hc/y$^+$LAT1 serve as the import and export routes, respectively, for arginine in epithelial cells of the kidney and small intestine.

The coordinate action of rBAT/b$^{0,+}$AT and 4F2hc/y$^+$LAT1 for the transepithelial flux of arginine was recently demonstrated by individual or combined overexpression in polarized Madin-Darby canine kidney cells (76). Interestingly, these studies also showed that leucine (present in equimolar concentration as arginine) is secreted when the 2 transporters are coexpressed. This may be explained by the absence of system B^0, an apical Na$^+$-dependent carrier system for NAAs. Mutations of the system B^0 transporter were assumed to underlie autosomal recessive Hartnup disorder, characterized by hyperexcretion of NAAs (and in 1 case also CAAs) (77). This transporter was recently identified by Stefan Broer's group and named B^{0}AT1 (78). Further work may demonstrate that it is mutated in Hartnup disease (79). B^{0}AT1 belongs to the SLC6 family of neurotransmitters and AA acid transporters and recognizes a wide range of NAAs. Interestingly, leucine, the preferred efflux substrate for rBAT/b$^{0,+}$AT (71), is the preferred uptake substrate for mouse B^{0}AT1.

Arginine transport by HATs in other cell types. The arginine-accepting HATs are expressed in a number of other cell types. 4F2hc/y$^+$LAT expression is strong in lung (y$^+$LAT1) and stomach and colon (y$^+$LAT2) cells, where they may serve as an export route for AAs accumulated due to the action of apical Na$^+$-dependent carriers such as ATB$^{0,+}$ (see below). In organs with blood–tissue barriers (e.g., brain, placenta, and testis), these transporters may contribute to arginine transport across the barrier. However, HAT expression is also found in a number of nonpolarized cells, where their function is less well understood. It can be generally assumed that in these cells, rBAT/b$^{0,+}$AT and 4F2hc/y$^+$LAT1 + 2 also serve as influx and efflux routes, respectively, for arginine. It is therefore highly unlikely that 4F2hc/y$^+$LAT1 provides arginine to intracellular enzymes such as NOS. This view is supported by the finding that arginine infusion normalized decreased NO production in a patient with LPI (80). This indicates that the endothelial dysfunction was due to the low plasma arginine concentration in this patient and not to the reduced flux of arginine through 4F2hc/y$^+$LAT1 in endothelial cells.

ATB$^{0,+}$

ATB$^{0,+}$ is the only known Na$^+$-dependent transporter for arginine. Like B^{0}AT, it belongs to the SLC6 family (18). It is a glycoprotein with 12 putative transmembrane helices and recognizes a wide range of CAAs and NAAs with high affinity. Transport by ATB$^{0,+}$ is coupled to Na$^+$ and Cl$^-$. Its expression is most abundant in lung and salivary gland tissue but can also be found in mammary gland, pituitary gland, stomach, and colon tissue. In mice, ATB$^{0,+}$ is expressed on the apical membrane of epithelial cells of the trachea and bronchi (predominantly in ciliated cells), but also in the bronchioles and alveoli (81). It has therefore been suggested that the transporter contributes to protein clearance by removing AAs from the airway lumen and thus plays a role in lung defense by maintaining a low-nutrient environment. In the gastrointestinal tract, the expression pattern of ATB^0 is similar to that of the basolateral 4F2hc/y$^+$LAT2 (18,82). In the pituitary gland, human ATB^0 has been proposed to play a role in hormone secretion induced by AAs (e.g., arginine and leucine). Hor-

mone secretion may thereby be a direct result of Na^+, Cl^-, or AA influx or due to depolarization of the cell membrane (81).

Peptide transporters

Other important contributors to arginine movement across the plasma membrane are peptide transporters [for review, see Refs. (83,84)]. Four isoforms are known in mammalian cells. They catalyze the H^+-dependent transport of di- and tripeptides. Epithelial cells in the small intestine and kidney express peptide transporters PEPT1 and PEPT2 in the apical membrane (small intestine: PEPT1; kidney proximal tubule: S1, PEPT1; S2 + S3, PEPT2) (Fig 1). The physiological function of these peptide transporters in other tissues is less well understood. The peptide and histidine transporter PHT1, expressed in the brain and eye, additionally accepts histidine as substrate. PHT2 was identified through its sequence homology to PHT1 and may localize to lysosomal membranes.

Conclusions

Analysis of arginine transport in different cell types and tissues in combination with the molecular identification and characterization of individual transport proteins reveals a complex cross-talk between transporters for CAA and NAAs as well as for peptides. Although arginine transport is quantified in many cell types under the influence of various stimuli, a thorough characterization of the respective transport systems involved is often missing. The next important assignment is the correlation of measured transport activities with the expression and subcellular localization of known transporters and, most importantly, the evaluation of net arginine fluxes under physiological (or pathophysiological) conditions. Finally, changes in arginine transport and transporter expression may occur only in specific subcellular compartments. Defining these compartments and deciphering the interplay between their individual constituents will largely improve our understanding of the arginine availability for a given enzymatic reaction.

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