A New Member of the Cationic Amino Acid Transporter Family Is Preferentially Expressed in Adult Mouse Brain*

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We have isolated and characterized a novel member (CAT3) of the cationic amino acid transporter (CAT) family. In oocyte injection assays, CAT3 cRNA exhibited a saturable, sodium ion-independent transport activity with high affinity for L-arginine and L-lysine (Kₘ = 40–60 and 115–165 μM, respectively). Transport of L-arginine was effectively competed only by cationic amino acids in L-form: arginine, lysine, ornithine, and 2,4-diamino-n-butyrlic acid but not by 2,3-diaminopropionic acid. The presence of L-arginine in the incubation medium stimulated the efflux rate of L-arginine, indicating that CAT3 is subject to trans-stimulation. All these results are consistent with the idea that CAT3, along with CAT1 and CAT2, constitutes the transport activity originally assigned to system y⁺. Like CAT2, but unlike CAT1, the expression of CAT3 is regulated in a highly tissue-specific manner; when various adult tissues were examined, significant levels of CAT3 transcript were detectable only in brain. In situ hybridization on brain sections revealed that CAT3 transcripts were localized predominantly along the midbrain-thalamus-hypothalamus axis, whereas neither CAT1 nor CAT2 transcripts demonstrated a similar localization. In contrast to its highly localized expression during the primitive streak stage and in the adult stage, CAT3 expression was detected more widely in 13.5 day post-coitum mouse embryos.

Before the first report of molecular characterization of an amino acid transporter in 1991 (1, 2), amino acid transport activities were physiologically characterized and categorized according to the specificity, affinity, and capacity for the substrates and the dependence on particular ions (3, 4, 43). In a wide variety of tissues, cationic amino acids are transported across the plasma membrane by an activity termed “system y⁺.” System y⁺ is a saturable, sodium ion-independent transport activity with high specificity and affinity for cationic L-amino acids (5). System y⁺ mediates transport of the substrates across the membrane in both directions and is stimulated by the presence of substrates in the opposite side of the membrane (trans-stimulation) (6). A low affinity and high capacity transport activity with no significant degree of trans-stimulation, but otherwise identical to system y⁺, was detected in liver, where system y⁺ was barely detectable (7). Distinct transport activities with broad substrate specificities that include some of the neutral amino acids as well as cationic amino acids have also been reported. For example, system b⁰⁺, and system b⁰⁻, originally detected in mouse blastocysts (8), transport in sodium ion-independent and sodium ion-dependent manners, respectively, both cationic amino acids and neutral amino acids. System y⁻L in erythrocyte and in placenta exhibits sodium ion-independent uptake of cationic amino acids and sodium ion-dependent uptake of neutral amino acids (9, 10). Similar activities have been reported for intestine and kidney as well.

The first amino acid transporter gene to be cloned and characterized (1, 2) was a mouse cationic amino acid transporter (CAT) gene CAT1. CAT1 is widely expressed, and the protein product exhibits the characteristics originally assigned to system y⁺. A subsequently identified cationic amino acid transporter gene, CAT2, shares high sequence homology with CAT1. However, the transcript encoding CAT2 protein is subject to differential tissue-specific splicing, resulting in two distinct proteins, CAT2 and CAT2a.² CAT2 is detectable in activated lymphocytes as well as several other tissues and exhibits the same characteristics assigned to system y⁺ (11). CAT2a is highly specific to liver, with low affinity and high capacity for the cationic amino acid substrates, and does not exhibit significant trans-stimulation; thus CAT2a is likely identical to the activity described previously in liver (12). These three proteins compose the CAT family of cationic amino acid transporters (reviewed in Ref. 13). Members of the CAT family share high sequence homology, each containing 12–14 putative transmembrane domains. Members of the rBAT/4F2hc family of transmembrane proteins (reviewed in Ref. 13) contain only one or four putative transmembrane domains and exhibit system b⁰⁺-like or system y⁻L-like activities when expressed in Xenopus oocytes (14). Members of this family possess less strict substrate specificity than the CAT family members and exhibit substrate-dependent preference in the direction of transport (14).

The present study reports the fourth member of the CAT family, which in the adult mouse is expressed primarily in brain. In primitive streak stage mouse embryos, CAT3 transcripts are detectable only in embryonic mesoderm. CAT3 protein also exhibits the characteristics of system y⁺, indicating that the activity described as system y⁺ is composed of products of multiple genes.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U708859.

1 The abbreviations used are: CAT, cationic amino acid transporter; RT-PCR, reverse transcriptase-polymerase chain reaction; DAB, 1,2,4-diamino-n-butyrlic acid; DAP, L-2,3-diaminopropionic acid; bp, base pair(s).

2 We adapted the terminology of the CAT2 proposed by Malandro and Kilberg (13).

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26780
After the submission of our sequence to GenBank™ and during the preparation of this manuscript, another report of a rat homolog of CAT3 appeared (15). The tissue-specific expression patterns of both CAT3 genes appear to be highly conserved between the species, suggesting functional importance of CAT3 protein in brain.

EXPERIMENTAL PROCEDURES

Isolation of Mouse CAT3 cDNA Clones—Subtractively enriched germ layer-specific cDNA libraries were constructed from a single mid-streak-stage mouse embryo. Differential screening of the libraries with germ layer-specific probes yielded about 100 mouse cDNA clones that hybridized with the germ layer-specific as confirmed by whole mount in situ hybridization. Sequence analysis revealed that one of these clones shared high homology with several members of a cationic amino acid transporter family. Longer cDNA clones of this sequence were isolated from a Agt10 cDNA library that was constructed from mouse embryonic stem cells differentiated in culture for 3.5 days, and the longest cDNA insert was subcloned into the pBluescript II KS plasmid vector(strategante).

Whole-mount In Situ Hybridization—Whole-mount in situ hybridization on primitive streak stage mouse embryos was carried out following the procedure of Rosen and Beddington (16).

Xenopus Oocyte Injection—In vitro transcription by T3 RNA polymerase was carried out in the presence of the RNA cap structure analog G(5)ppp(5)G (New England Biolabs) (17) on a linearized plasmid template with a trimmed 5'-untranslated region. The cRNA (in vitro transcription product from cDNA) thus synthesized contained a 38-nucleotide-long 5'-untranslated sequence and an intact 3' end with a stretch of 31 A residues. Tnt T7 Quick Coupled transcription/translation system (Promega) was used to confirm translational competency of the template sequence. Oocytes were prepared by digestion with 0.2 g/100 ml of either Type II collagenase (Sigma) or Type IA collagenase (Sigma) in calcium-free OR2 medium (44) then incubated overnight at 15.5 °C in supplemented 50% Liebovitz L-15 medium for recovery. After injection of approximately 25–50 nl of either 2 ng of cRNA/nl injection buffer (88 mM NaCl, 5 mM Tris-HCl, pH 7.5) or injection buffer alone, the oocytes were incubated in the same medium for 2 more days either at 15.5 °C or at 22.5 °C. Before transport activity assays, the oocytes were washed extensively with uptake solution (10 mM Hepes, 50 mM Tris, pH 7.5, 2 mM KC1, 1 mM MgCl2, 1 mM CaCl2, 100 mM NaCl; Ref. 1) to remove amino acids carried over from the culture medium. For transport activity assays, oocytes were incubated at room temperature for 1 h in most cases, in uptake solution containing 1 μCi/300 μl of tritiated L-arginine (ICN or Amersham Life Science, Inc.) or L-lysine (ICN) plus 1 mM unlabeled amino acids as controls. For the trans-stimulation assay, 2 μCi/300 μl was used to label oocytes. To examine sodium ion dependence of CAT3 transport activity, the 100 mM sodium chloride in uptake solution was replaced by 100 mM choline chloride. Following incubation, the oocytes were washed extensively, transferred into 1.5-ml microfuge tubes, lysed with the aid of 20 μg/ml proteinase K, for 30 min at 37 °C, and then centrifuged for 3 min at 12,000 × g. The supernatant was mixed with 5% polyacrylamide gels. The primers used for PCR amplification were 5'- GAGCCCTCTGCCTGAAGC and GGATGAGAACGCAAATGGACACC-3' (New England BioLabs) (17) on a linearized plasmid containing the template sequence. The cDNA was inserted at the unique HindIII site of the EcoRI-cleaved vector pBluescript II KS plasmid. The plasmid was isolated and linearized with XhoI. The linearized plasmid was treated with calf intestinal alkaline phosphatase and then subcloned into the pBluescript II KS plasmid vector.

RESULTS

Isolation of an Embryonic Mesoderm-specific Sequence That Shares High Homology with Cationic Amino Acid Transporters—In an attempt to isolate germ layer-specific transcripts, differential screening was carried out on subtractively enriched mouse embryonic germ layer-specific cDNA libraries. Among about a dozen cDNA clones thus isolated, the sequence of one clone was detectable only in the mesoderm of mid-streak stage mouse embryos by whole-mount in situ hybridization (Fig. 1). This mesoderm-specific sequence showed extensive homology with several cationic amino acid transporters (CAT1, CAT2, and their homologs) and, as described below, Xenopus oocyte injection assays revealed that the translational product of this sequence could transport cationic amino acids. Thus, we tentatively named this novel gene CAT3.

CAT3 Protein Is Highly Homologous to, but Different from, CAT1 or CAT2 Proteins—The longest CAT3 cDNA clone from the Agt10 library was 2,370 bp, which contained 1,857 bp of uninterrupted open reading frame preceded by 275 bp of 5'-untranslated sequence and followed by 440 bp of 3'-untranslated sequence plus a stretch of poly(A). The purine A, which is the most conserved and influential base for initiation by eukaryotic ribosomes (19) is located at the third position upstream from the start codon. A variant poly(A) addition signal, AAAAAA, is found at 21–27 nucleotides upstream of the poly(A) stretch. Northern analysis of RNA isolated from in vitro differentiated mouse embryonic stem cells and from brain revealed a single band of approximately 2.5 kilobases (Fig. 2), indicating that this is the only, or at least the major, transcriptional product. In contrast to this single band of <3 kilobases, the major transcripts of CAT1 and CAT2 are 7–9 kilobases and contain long 3'-untranslated regions (Refs. 20–23; see “Discussion”). Southern hybridization of EcoRI- and BamHI-digested mouse genomic DNA yielded only single bands of approximately 5.5 and 3.5 kilobases, respectively (data not shown), thus indicating that CAT3 exists as a single-copy gene. Hydrophilicity analysis indicates the presence of 12–14 potential transmembrane domains, and analysis of CAT1, CAT2, and CAT2a proteins by the same method resulted in nearly identical profiles (data not shown).

A data base search revealed 10 entries of peptide sequences were prepared on 3'-triethoxysilylpropalamine (TESPA) (Sigma)-subbed slides. 5p- or 32p-labeled RNA probes were synthesized by bacteriophage RNA polymerase from linearized plasmids containing cDNA as inserts. The cDNAs of CAT1 and CAT2, 689 and 543 bp, respectively, were isolated by RT-PCR using the primers described above and cloned into the EcoRV site of pBluescript II by blunt-end ligation. For detection of CAT3 transcript, either the 2,371-bp cDNA or 810-bp 3' sequence was used as a template with essentially identical results. Pretreatment, hybridization, and post-hybridization washes were according to the procedure described by Wilkinson and Nieto (18). The slides were directly exposed to x-ray film for autoradiography.

In Situ Hybridization on Sectioned Samples—Paraffin- or cetylpyridine-doped samples (mouse brains and 13.5-day post-coitum mouse embryos) were embedded in Paraplast X-TTRA (Fisher) and 7 micron sections

FIG. 1. Detection of an embryonic mesoderm-specific transcript, CAT3, in primitive streak stage mouse embryos by whole-mount in situ hybridization. Digoxigenin-labeled riboprobe was visualized by alkaline phosphatase-conjugated anti-digoxigenin antibody. A, anterior; P, posterior; D, distal, Pr, proximal. Dashed line indicates the embryonic-extra-embryonic junction.
with significant homology (>70% identity over a stretch of 200 amino acid residues). When minor differences were excluded, these entries actually represented just five distinct sequences. Alignment of the five peptide sequences, as well as that of CAT3, divided the sequences into three groups (Fig. 3). One group included mouse CAT1 and homologous sequences from human and rat, whereas the second group included mouse CAT2, mouse CAT2a, and a homologous human sequence. CAT2 and CAT2a proteins differ within a stretch of 41 amino acid residues (49% identity) located in the putative intracellular loop between the eighth and ninth transmembrane domains; CAT2 (also known as CAT2b, or Tea) is expressed in activated lymphocytes and several other tissues (11, 24), whereas CAT2a is highly expressed in liver (12). Importantly, CAT3 does not belong to either group. In Fig. 3, shades are placed over amino acid residues that are conserved among three or more proteins so that the difference among the three groups is more easily recognizable. Although neither the presence nor the positions of 13th and 14th putative transmembrane domains was agreed upon by different analysis methods, an excellent agreement was obtained for the initial 12 putative transmembrane domains. For this reason, only the first 12 putative transmembrane domains are underlined in Fig. 3.

As seen in Fig. 3, both the CAT1 and the CAT2 groups maintain two conserved potential N-linked glycosylation sites (NXT/S, where X can be any amino acid except proline) between the fifth and sixth putative transmembrane domains. Actual N-linked glycosylation of CAT1 at these two sites has been reported in Xenopus oocytes (25). Interestingly, CAT3 contains only one site in this putative extracellular loop.

The very recently reported rat CAT3 protein (15) shares highly conserved peptide sequence (94% overall) with the mouse CAT3. The three major domains (positions 223–239, 432–454, and 591–618) that distinguish the mouse CAT3 from other members are highly conserved in the rat CAT3 as well. Conservation between the species is further seen in the position as well as the number of potential N-linked glycosylation sites and also in the short length of the 3′-untranslated region of the mRNAs.

**CAT3 cDNA-Injected Oocytes Exhibit Increased Transport Activity for L-Arginine and L-Lysine**—To confirm that CAT3 protein is truly a transporter of cationic amino acids, cRNA was synthesized and injected into Xenopus oocytes. The cRNA contained a 38-nucleotide 5′-untranslated region and an intact 3′-untranslated region including a stretch of 31 A residues at the 3′ terminus. In vitro translation produced a protein of approximately 65 kDa, which is in a good agreement with the expected size of 67 kDa (data not shown).

Incubation of oocytes in the uptake solution containing triitated L-arginine or triitated L-lysine resulted in incorporation of radioactivity into both cRNA-injected and buffer-injected (control) oocytes. As expected, radioactivity incorporated into cRNA-injected oocytes was consistently and significantly higher than control oocytes. Amounts of internalized radioactivity contributed by CAT3 cRNA increased linearly with increasing incubation time, at least up to 80 min (data not shown). Replacement with choline ion for sodium ion did not change the transport profile substantially (data not shown). The transport activities of both native transporters and CAT3 protein were saturable at higher amino acid concentrations. The ratio of transported radioactivities between CAT3 cRNA-injected oocytes and control oocytes (cRNA-injected oocyte/control oocyte) varied significantly (from 1.5/1 to 10/1). However, the affinities of CAT3 protein for L-arginine and for L-lysine, inferred from Michaelis-Menten constant $K_m$, remained essentially constant in different experiments. Fig. 4 shows one example of such results for L-arginine and L-lysine. The $K_m$ values obtained from the three assays all fell within the range of 40–60 μM for L-arginine and 115–165 μM for L-lysine. The clear difference in $K_m$ values between the two substrates is some of interest, since no such difference has been reported regarding the other members of the CAT family (2, 6, 7). Similar to the reported variation observed in CAT1 and CAT2 oocyte injection experiments (1, 11), the $V_{\text{max}}$ values obtained from Eadie-Hofstee plots varied from 16 to 60 pmol of L-arginine/oocyte/h for approximately 50–100 ng of injected cRNA. Very similar variation was observed for L-lysine as well. The largest changes were seen in the $V_{\text{max}}$ values obtained for control oocytes, which ranged from 5 to 125 pmol of L-arginine/oocyte/h (and similar results for L-lysine). These differences are attributable to the types of collagenase used to prepare oocytes. The use of Type II collagenase to prepare oocytes coincided with high $V_{\text{max}}$ for control oocytes and low $V_{\text{max}}$ for translational product of cRNA, whereas the use of Type IA collagenase coincided with low $V_{\text{max}}$ for control oocytes and high $V_{\text{max}}$ for the translational product of cRNA (data not shown).

**Substrate Specificity and Effects of Various Amino Acids on Transport Activity of CAT3**—Substrate specificity of CAT3 was examined by incubating cRNA-injected oocytes and control oocytes in the presence of competitor amino acids at 10 mM concentration (Fig. 5). Transport of triitated L-arginine by CAT3 was effectively competed against by cationic amino acids; L-arginine, L-lysine, L-ornithine, L-2,3-diaminopropionic acid (DAP). L-Ornithine, DAP, and DAP are L-lysine homologs and contain between the α-carbon and the ε-amino group three, two, and one methylene group, respectively. When compared with L-lysine or L-ornithine, DAP reproducibly showed a slightly lower ability to compete L-arginine. The results were similar to those reported for L-arginine homologs, where shorter homologs exhibited lower affinity to system y$^+$ (5, 6). Thus, as in the case of L-arginine homologs (5), the failure of DAP to compete L-arginine could be explained by the short side chain and/or the close proximity of the ε amino group to the α amino group. From our results, DAP appears to be the shortest cationic amino acid that can be transported by CAT3 with ease. D-Arginine, which was tested at a concentration of 0.1 mM to minimize effects of
potentially contaminating l-form, did not compete with l-arginine effectively (Fig. 5), thus confirming the high degree of substrate specificity of CAT3. The mild suppression by L-histidine was an expected result, since a minor portion of the amino acid exists in cationic form in the uptake solution.

One of the characteristics described for system y is the sodium ion-dependent inhibition of the transport activity by some neutral amino acids (for example by L-homoserine) in cultured cells. However, no such inhibition was reported for CAT1 in Xenopus oocytes (1). To determine if such an inhibition might occur on CAT3, we replaced the sodium ion in the uptake solution with choline ion (1) for several amino acids. As shown in Fig. 5, no sodium ion-dependent inhibition by L-homoserine was detectable on CAT3 cRNA-injected oocytes. However, as reported previously (26), the native transport activity of oocytes was significantly suppressed by various neutral amino acids, including L-homoserine, in the presence of sodium ion (data not shown). Whereas the sodium ion-dependent suppression of the native transport activity by neutral amino acids was expected, the CAT3 activity was influenced by several amino acids in unexpected ways. Those that showed mild, but noticeable, effects on CAT3 were the two sulfur-containing amino acids (L-methionine and L-cysteine) and the two anionic amino acids (L-aspartic acid and L-glutamic acid). Effects of these four amino acids on CAT3 activity were reproducible throughout four separate assays.

Efflux of L-Arginine Was Mildly Stimulated by the Presence of Cationic Amino Acids in the Incubation Medium—The system y cationic amino acid transport activity can be stimulated by the presence of cationic amino acids on the opposite side of plasma membrane, a phenomenon called trans-stimulation. This phenomenon has been detected for CAT1 and CAT2 proteins but not for CAT2a, which can transport substantial amounts of substrates even in the absence of cationic amino acids in the opposite side of the membrane (11, 12). We examined CAT3 to see if it was also subject to trans-stimulation. As seen in Fig. 6, the efflux of radioactivity from cRNA-injected oocytes was stimulated by the presence of cationic amino acids in the incubation medium. The degree of trans-stimulation was rather mild when compared with the reported data for CAT1 but was comparable to that for CAT2 (11). The result indicates that CAT3 activity is less affected by the presence or absence of cationic amino acids on the trans side than is CAT1 activity. No significant trans-stimulation was detected for the native oocyte activity (data not shown).

CAT3 Is Expressed Highly Specifically in Adult Mouse Brain—To examine the tissue distribution of CAT3 transcript in adult mice, we used a semi-quantitative RT-PCR. Northern
Northern analysis and an RT-PCR analysis on RNA samples detected in brain by the RT-PCR analysis shown in Fig. 7, a

Although all of the three cationic amino acid transporters were expressed in other tissues as well. Nonetheless, CAT3 expression appears to be far more restricted compared with CAT1 or CAT2. Expression patterns of CAT3 transcripts with probe. Under the PCR conditions used in this analysis, the reaction products are roughly linear to the amounts of input mRNAs. 14 adult tissues and 8-day post-coitum mouse embryos were analyzed (Fig. 7). Surprisingly, CAT3 transcripts were readily detectable only in 8-day post-coitum mouse embryos. In contrast, CAT1 transcripts of 13.5-day post-coitum embryos were also reported for CAT1 (1), whose transcribed CAT3 cRNA are plotted against substrate concentrations. The mean value ± S.E. was obtained from 40 (L-arginine) or 20 (L-lysine) each of cRNA-injected oocytes and buffer-injected oocytes. A transport of L-arginine; B, transport of L-lysine. Eadie-Hofstee plots are shown in the insets. Native transporters of Xenopus oocytes exhibited $V_{max}$ and $K_m$ values of 20 pmol/oocyte/h and 100 $\mu$M for L-arginine and 25 pmol/oocyte/h and 110 $\mu$M for L-lysine in these particular assays.

analysis was not used to determine tissue distribution because the CAT3 transcript partially co-migrates with 18 S RNA in formalddehyde gels, thus preventing the efficient hybridization of CAT3 transcripts with probe. Under the PCR conditions used in this analysis, the reaction products are roughly linear to the amounts of input mRNAs. 14 adult tissues and 8-day post-coitum mouse embryos were analyzed (Fig. 7). Surprisingly, CAT3 transcripts were readily detectable only in 8-day post-coitum embryos and adult brain. In contrast, CAT1 transcripts were detected in most tissues except for liver, as reported previously (1), whereas expression of CAT2 appears to be much more restricted. The primer pair used for detecting CAT2 does not discriminate CAT2 from CAT2a. Expression patterns detected for both CAT1 and CAT2 were consistent with those reported previously (1, 11, 12, 24). As there are many other tissues that have not been examined, it is possible that CAT3 is expressed in other tissues as well. Nonetheless, CAT3 expression appears to be far more restricted compared with CAT1 or CAT2.

CAT3 Is Preferentially Localized in Subregions of Brain—

Although all of the three cationic amino acid transporters were detected in brain by the RT-PCR analysis shown in Fig. 7, a Northern analysis and an RT-PCR analysis on RNA samples prepared from different parts of brain indicated that CAT3 transcripts were further localized within brain (data not shown). To assess distribution patterns of transcripts within brain, we carried out in situ hybridization on sectioned samples. As shown in Fig. 8, CAT3 showed significantly localized expression, with particularly high expression along the mid-brain-thalamus-hypothalamus region. On the other hand, transcripts of CAT1 and CAT2 were readily detectable throughout the brain, with particularly high levels present in the cortex (CAT1) or in the cerebellum and olfactory bulb (CAT2) (data not shown).

CAT3 Is Expressed in a Wide Variety of Tissues in the Mouse Fetus—In early- to mid-stage stage mouse embryos, CAT3 transcripts were detectable only in embryonic mesoderm (Fig. 1). No such high level or tissue-specific expression was observed for CAT1 or CAT2 (data not shown). As embryonic development progressed beyond the mid-stage, the mesoderm-restricted expression of CAT3 disappeared. As shown in Fig. 9, CAT3 transcripts were detectable in a wide variety of tissues of 13.5-day post coitum embryos. Similarly, less restricted expression was observed for CAT1 (e.g. in fetal liver) and CAT2 during this stage (data not shown).

**DISCUSSION**

An attempt to isolate mouse embryonic germ layer-specific transcripts resulted in the isolation of a cationic amino acid transporter gene CAT3. When injected into Xenopus oocytes, CAT3 cRNA exhibited a saturable, sodium ion-independent transport activity with high affinity and specificity for cationic L-amino acids. The CAT3 cRNA-stimulated activity was also subject to a mild degree of trans-stimulation. DAB appears to be the smallest cationic amino acid that CAT3 can transport with ease, since DAP could not effectively inhibit transport of L-arginine, whereas DAB could. All of these characteristics were consistent with the cationic amino acid transport activity termed system y$^+$.

Another characteristic of system y$^+$ is a mild sodium ion-dependent inhibition of the transport activity by neutral amino acids; for example, by L-homoserine, when assayed in cultured cells (6). In our oocyte injection assays, however, no sodium ion-dependent inhibition by L-homoserine or by other neutral L-amino acids of the transport activity of CAT3 was observed. Failure in inhibiting the transport activity in sodium ion-dependent manner by neutral L-amino acids in Xenopus oocytes was also reported for CAT1 (1), whose transport activity is now widely accepted as representing the activity assigned to system y$^+$. Although the lack of sodium ion-dependent inhibition by neutral L-amino acids may lead to an argument that CAT1 does not truly represent the activity assigned to system y$^+$, the apparent inconsistency may originate from the use of Xenopus oocytes instead of cultured cells. For example, it is possible that some properties of the transport activity may be altered by interactions between CAT proteins and other factors that are absent from Xenopus oocytes. A system y$^+$-like activity, which was characterized by sodium ion-independent transport of cationic L-amino acids and sodium ion-independent inhibition of the activity by neutral L-amino acids in Xenopus oocytes, was described for the 4F2hc protein (26). Members of the rBAT/4F2hc family exhibit broader, less strict substrate specificity and asymmetric exchange of cationic and neutral amino acids in oocytes (14). Interactions with other proteins is likely essential for rBAT/4F2hc family of transporter proteins, which possess only one or four putative transmembrane domains (Refs. 26–28; reviewed in Ref. 13). It has been suggested that members of rBAT/4F2hc family function as accessory proteins in multi-subunit transporter complexes (26–29). Thus, interaction or lack of interaction with other factors might be responsible for the discrete effects of various amino acids on the...
transport activity of CAT3 and on the native transport activity of oocytes as well.

It appears that system Y\textsubscript{1}, the principal cationic L-amino acid transporter (30), is composed of multiple constituents. One question regarding the physiological role of CAT3 is why the gene is required in particular tissues where CAT1 or CAT2 might be expected to suffice. As described above, other than the clearly distinct \( K_m \) values between the two substrates and the low degree of trans-stimulation, CAT3 did not appear to differ in function very much from CAT1 or CAT2, at least in the oocyte injection assays. However, the molecular mechanism of cationic amino acid transport is still unknown, and future studies may reveal important differences among CAT family members, such as association with other factors.

A major difference among CAT1, CAT2, and CAT3 might be their responsiveness to various types of signals. In rat vascular muscle cells, angiotensin II stimulated both CAT1 and CAT2 mRNA expression (31), whereas interleukin 1\textbeta and tumor necrosis factor \( \alpha \) stimulated CAT2 but not CAT1 expression (32). Platelet-derived growth factor treatment of smooth muscle cells strongly induced CAT2 mRNA expression but only moderately induced CAT1 mRNA expression (33). In cardiac myocytes, interleukin 1\textbeta and interferon \( \gamma \) co-induced CAT1, CAT2, and CAT2a, whereas insulin increased only CAT1 levels (34). In resting myocytes, only CAT1 was expressed (34). In brain astrocytes, lipopolysaccharide/interferon \( \gamma \) induced CAT2 but...
not CAT1 (20). Various signals that cause transition of cells from the resting state to the proliferating state appear to activate CAT1 expression (35–37).

The regulation of CAT family genes could be at the transcriptional, post-transcriptional (37), translational, or post-translational level. Potential targets for CAT1 and CAT2 regulation include the long 3′-untranslated regions in the mRNAs and N-linked glycosylation sites in the protein, which have been implicated in the regulation of mRNA stability (38) and membrane trafficking (39, 40), respectively. CAT3 differs from the other family members in that its mRNA does not contain a particularly long 3′-untranslated region, and the protein is missing one particular potential N-linked glycosylation site. Of particular interest, the insulin-responsive glucose transporter Glut4 contains a single N-glycosylation site, and the effects of N-glycosylation of this transporter have been shown to be cell-type specific (41). However, at least in Xenopus oocytes, removal of the N-glycosylation sites from CAT1 by amino acid substitution did not change the transport activity of the protein (25). It is possible that the tissue-specific expression of CAT3, as well as CAT2, may reflect important differential responses of the proteins to changes in physiological conditions, as exemplified by the glucose transporter Glut-4, which, upon stimulation by insulin, translocates from an intracellular site to the plasma membrane.

The distinct distribution patterns detected for transcripts of CAT family members in mouse brain suggest potentially different roles played by these transporters. In this context, the induced nitric oxide synthesis observed in brain astrocytes was reported to be partly dependent on induction of CAT2 (20). It has also been reported that cationic amino acid transport activity across the blood-brain barrier had the kinetic properties of system y\textsuperscript{+} and that CAT1 mRNA was 38-fold enriched in microvesicles as compared with wholebrain (42).

The significance of the highly localized distribution of CAT3 transcripts within adult mouse brain or in early stage mouse embryos is not yet clear. In addition, we do not know whether the mRNA distribution actually corresponds to the presence of CAT3 protein at the membrane. However, the embryonic and adult patterns of CAT3 reported here provide a framework for further investigations regarding CAT3 function.

This study, along with studies on CAT2, has suggested that the activity originally assigned to system y\textsuperscript{+} is actually composed of products of multiple genes with distinct tissue distributions. As more cationic amino acid transporter genes are isolated, additional members of the CAT family may be discovered, some of which may exhibit the activity described as system y\textsuperscript{+}. The disagreements observed between system y\textsuperscript{+} and CAT1 or CAT3 may be solved by isolation of new transporters or by isolation of associating factors that confer different characteristics to the transporters.

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