

# Glutamate and Glutamine in the Brain

## Transport of Glutamate and Other Amino Acids at the Blood-Brain Barrier<sup>1</sup>

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**ABSTRACT** In most regions of the brain, the uptake of glutamate and other anionic excitatory amino acids from the circulation is limited by the blood-brain barrier (BBB). In most animals, the BBB is formed by the brain vascular endothelium, which contains cells that are joined by multiple bands of tight junctions. These junctions effectively close off diffusion through intercellular pores; as a result, most solutes cross the BBB either by diffusing across the lipid endothelial cell membranes or by being transported across by specific carriers. Glutamate transport at the BBB has been studied by both in vitro cell uptake assays and in vivo perfusion methods. The results demonstrate that at physiologic plasma concentrations, glutamate flux from plasma into brain is mediated by a high affinity transport system at the BBB. Efflux from brain back into plasma appears to be driven in large part by a sodium-dependent active transport system at the capillary abluminal membrane. Glutamate concentration in brain interstitial fluid is only a fraction of that of plasma and is maintained fairly independently of small fluctuations in plasma concentration. Restricted brain passage is also observed for several excitatory glutamate analogs, including domoic acid and kynurenic acid. In summary, the BBB is one component of a regulatory system that helps maintain brain interstitial fluid glutamate concentration independently of the circulation. *J. Nutr.* 130: 1016S-1022S, 2000.

**KEY WORDS:** • blood-brain barrier • glutamate • transport • endothelium • aspartate

L-Glutamate is the most abundant free amino acid in brain and is the predominant excitatory neurotransmitter of the vertebrate central nervous system. Among its many functions, L-glutamate plays a critical role in synaptic maintenance and plasticity (McDonald and Johnston 1990); it also contributes to learning and memory through use-dependent changes in synaptic efficacy, such as long-term potentiation and long-term depression. Under pathologic conditions, excess release of L-glutamic acid and other excitatory amino acids can lead to excitotoxic lesions in brain from overexcitation of nerve cells. Excitotoxicity is thought to play an important role in the neural damage that occurs in diseases such as trauma, stroke, epilepsy and hypoglycemia. Comparable damage can be produced by direct administration of L-glutamate to the nervous system and, under select conditions, by peripheral administration of very high doses of L-glutamate to infant animals (Mel-drum 1993).

Under normal conditions, most free L-glutamic acid in brain is derived from local synthesis from L-glutamine and Krebs's cycle intermediates. A considerable fraction is also derived from recycling from brain protein. In synaptic termi-

nals, L-glutamate is stored in vesicles and released via a calcium-dependent mechanism. Once in the synaptic cleft, L-glutamate binds and activates postsynaptic glutamate receptors. Although many different glutamate receptor subtypes have been identified (Nakanishi 1992), the ionotropic glutamate receptors have been studied most extensively and are subdivided into three classes on the basis of sensitivity to their agonists, kainate, quisqualate or N-methyl-D-aspartate (NMDA).<sup>2</sup> The NMDA receptor functions as a gatekeeper for sodium and calcium, and has five separate binding sites, each of which is affected by different substrates capable of altering receptor affinity. The action of L-glutamate is terminated by removal from the synaptic cleft by neuronal presynaptic and glial high affinity reuptake systems, several of which have been cloned (Castagna et al. 1997, Kanai et al. 1994, Kanai 1997). These active sodium-dependent transport systems maintain a very large gradient of L-glutamate from the intracellular to the extracellular space (5000- to 10,000-fold) so that brain extracellular L-glutamate concentrations are normally quite low, with cerebrospinal fluid concentration averaging <0.4  $\mu\text{mol/L}$  (Ferrarese et al. 1993).

Given the critical role of L-glutamate in neural function, it is not surprising that a greater level of regulation is required of brain L-glutamate concentration than that observed in most other tissues. This regulation must include control of both extracellular as well as intracellular free L-glutamate concentrations because L-glutamate acts predominantly at the extra-

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<sup>2</sup> Abbreviations used: BBB, blood-brain barrier; BCH, 2-aminobicyclo-[2,2,1]heptane-2-carboxylic acid; CAT, cationic amino acid transporter; NMDA, N-methyl-D-aspartate.

cellular synaptic cleft. Plasma L-glutamate concentrations fluctuate during the day as a result of changes in diet, metabolism and protein turnover. If these changes were transferred directly to the brain interstitial space, they would have disrupting effects on neuronal synaptic communication.

The isolation and protection of the brain is accomplished in good part through the presence and function of the "blood-brain barrier" (BBB). The BBB is a system of tissue sites, including brain vascular endothelial cells, choroid plexus epithelial cells and arachnoid membrane; together, they restrict and regulate the flux of substrates between the circulation and the central nervous system (Pardridge 1998). The barrier at each site is formed by a single layer of cells that are joined together by multiple bands of tight junctions. These tight junctions seal off the paracellular diffusion space; thus, to cross the barrier, most solutes must either dissolve in and diffuse across the lipid cellular membranes of the barrier cells or be transported across by selected BBB carriers. As a consequence, the passive influx of most polar solutes, such as L-glutamate, is quite limited at the BBB and is <1% of that occurring at the blood vessels of most other tissues. To compensate for the limited passive exchange, the cells of the BBB contain high levels of 20 or more specific transport systems that regulate the flux of key solutes from blood into brain interstitial fluid and cerebrospinal fluid and back out again.

In this paper, we summarize the current status of knowledge of amino acid transport at the BBB. Primary focus will be on the transporters at the brain capillary membranes because the capillaries, due to their large surface area, are the primary site of exchange for most solutes between brain interstitial fluid and the circulation. Further, the brain penetration of other acidic (or anionic) amino acid analogs will be discussed, as well as the changes that occur in brain permeation and transport during development.

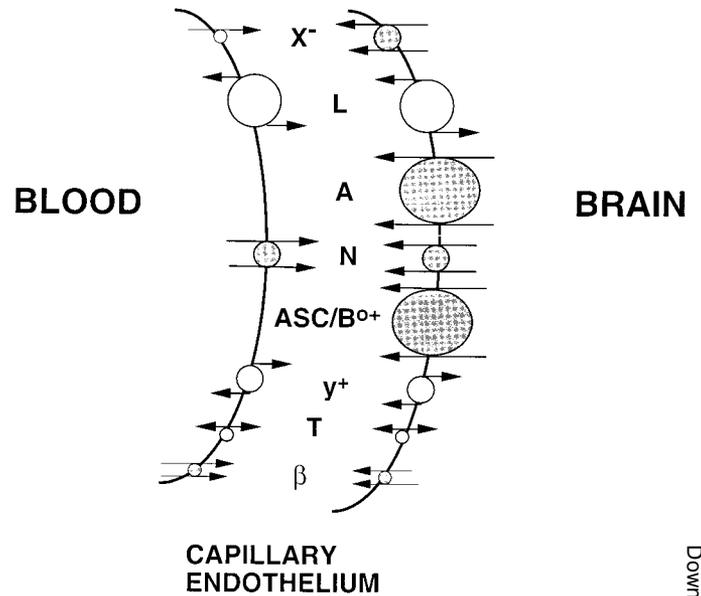
### BBB amino acid transport systems

Currently, nine amino acid transport systems have been reported to be present at the brain capillary endothelium of the BBB. **Figure 1** summarizes the current state of knowledge on their distribution and activity at the capillary luminal and abluminal membranes. These transport systems differ in substrate specificity, inhibition by model ligands (e.g., methylaminoisobutyric acid and 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid [BCH]), and transport dependence on sodium (Smith and Stoll 1998).

The first transport systems to be proposed for the BBB were identified on the basis of results from *in vivo* uptake studies (Oldendorf 1971, Oldendorf and Szabo 1976, Pardridge 1979). These transporters include the following: 1) System L, which mediates high affinity, sodium-independent uptake of zwitterionic amino acids with "large, neutral" side chains, including L-leucine, L-phenylalanine, L-tryptophan, L-tyrosine, L-isoleucine, L-methionine and L-valine; 2) System  $y^+$ , which mediates moderate affinity, sodium-independent uptake of amino acids with cationic side chains, including L-arginine, L-lysine and L-ornithine; 3) System T, which mediates high affinity, low capacity transport of thyroid hormones ( $T_3$  and  $T_4$ ); and 4) System  $x^-$ , which mediates sodium-independent, high affinity uptake of amino acids with anionic side chains, including L-glutamate and L-aspartate.

The System L and  $y^+$  carriers are sodium independent and mediate facilitated exchange at both the capillary luminal and abluminal membranes. Their function is necessary to deliver dietary essential neutral and basic amino acids that cannot be synthesized within the brain (Betz and Goldstein 1978,

## AMINO ACID TRANSPORT SYSTEMS



**FIGURE 1** Diagram of amino acid transport systems at the brain capillary endothelium and their localization to the capillary luminal (plasma-facing) or abluminal (brain-facing) membranes. Shaded systems are sodium dependent, whereas unshaded systems are sodium independent.

Momma et al. 1987, Sánchez del Pino et al. 1992 and 1995, Smith et al. 1987, Stoll et al. 1993). In contrast, L-glutamate and L-aspartate, which can be synthesized readily in brain, show much lower rates of uptake into brain at the BBB (Al-Sarraf et al. 1995 and 1997b, Benrabh and Lefauconnier 1996). For these "dietary nonessential" amino acids, brain supply is governed more by intracerebral synthesis and breakdown.

**Table 1** summarizes transport  $V_{max}$  and  $K_m$  values for amino acid uptake into brain at the BBB as measured with the *in situ* rat brain perfusion technique. Transport rates were determined for each amino acid in the absence of competitors. As shown in Table 1, Systems L,  $y^+$  and  $x^-$  each mediate the uptake of two or more amino acids; thus there is the potential for competition among substrates. Actually, competition is quite important because, as shown in Table 1, the plasma concentration for most of the amino acid substrates equals or exceeds the corresponding transport  $K_m$ . As a consequence, each of the three transport systems is predicted to be nearly saturated with amino acid substrates as a group at normal plasma concentrations. Kinetic calculations for System L reveal a saturation percentage of >95% when all nine or so amino acid substrates are included. Due to transport saturation, individual amino acids must compete for transport, such that the apparent  $K_m$  for uptake from plasma is 3- to 20-fold greater than the true  $K_m$  from saline measured in the absence of competitors. The apparent  $K_m$  is defined as  $K_m(\text{app}) = K_m[1 + \Sigma(C_i/K_{mi})]$ , where  $C_i$  is the plasma concentration of each competing amino acid and  $K_{mi}$  is the corresponding transport  $K_m$  for that amino acid. Transport saturation makes the brain amino acid delivery selectively vulnerable to large imbalances in plasma amino acid concentration such as those that occur in the hyperaminoacidemias, e.g., phenylketonuria and maple syrup disease (Smith and Stoll 1998).

Although L-glutamine shows measurable affinity for System L, Ennis et al. (1998) recently reported that glutamine is

TABLE 1

Blood-brain barrier transport constants for brain amino acid uptake as measured by the *in situ* rat brain perfusion technique<sup>1</sup>

Amino acid	Plasma concentration	$K_m$	$V_{max}$	$K_m(\text{app})$	Influx
	$\mu\text{mol/L}$	$\mu\text{mol/L}$	$\text{nmol}/(\text{m} \cdot \text{g})$	$\mu\text{mol/L}$	$\text{nmol}/(\text{min} \cdot \text{g})$
Neutral amino acids (System L1)					
Phe	81	11	41	170	13.2
Trp	82	15	35	330	8.2 <sup>2</sup>
Leu	175	29	59	500	14.5
Met	64	40	25	860	1.7
Ile	87	56	60	1210	4.0
Tyr	63	64	96	1420	4.1
His	95	100	61	2220	2.5
Val	181	210	49	4690	1.8
Thr	237	220	17	4860	0.8
Gln	485	880	43	19900	1.0
Basic amino acids (System y <sup>+</sup> )					
Arg	117	56	24	302	6.7
Lys	245	70	22	279	10.3
Orn	98	109	26	718	3.1
Thyroid hormones (System T)					
T <sub>3</sub>	$\leq 0.002^3$	0.26	0.16		
Acidic amino acids (System x <sup>-</sup> )					
Glu	30–50	24	0.21		
Asp	5–10	101	0.13		

<sup>1</sup> Values are taken from Smith et al. (1987), Stoll et al. (1993), Al-Sarraf et al. (1997b), Filer et al. (1979), Refetoff (1989) and Hokari and Smith (unpublished data).  $V_{max}$  is the maximal saturable transport capacity;  $K_m$  is the half-saturation concentration in the absence of competitors;  $K_m(\text{app})$  is defined as follows:  $K_m(\text{app}) = K_m[1 + \sum (C_i/K_{m_i})]$  where  $C_i$  is the plasma concentration of each competitor amino acid, and influx is the unidirectional amino acid flux rate from plasma to brain. Apparent  $K_m$  *in vivo* are much greater than true  $K_m$  because of transport saturation and competition (Smith et al. 1987).

<sup>2</sup> Estimated assuming ~70% of albumin-bound Trp contributes to brain uptake.

<sup>3</sup> Estimate of total triiodothyronine (T<sub>3</sub>) concentration in plasma; the free T<sub>3</sub> concentration is ~1% that of total T<sub>3</sub>.

actually taken up into brain by a separate sodium-dependent mechanism at the BBB, which they identified tentatively as System N. In other tissues, System N mediates the sodium-dependent transport of L-glutamine, L-histidine and L-asparagine. Lee et al. (1998) also found evidence for the presence of a separate glutamine transporter distinct from System L at the luminal membrane of the BBB with the use of isolated bovine brain endothelial cell membrane vesicles. However, in that study, the sodium-dependent System N transport was confined to the brain capillary abluminal membrane and was suggested to have a critical role in glutamine efflux from the central nervous system, not uptake. Further research is needed to clarify this discrepancy.

*In vitro* studies have also provided evidence for the presence of five other sodium-dependent, active transport systems for amino acids at the brain capillaries, including System A, System B<sup>+</sup>, System ASC, System  $\beta$ , and System X<sup>-</sup> (Betz and Goldstein 1978, Lee et al. 1998, Sánchez del Pino et al. 1995, Tayarani et al. 1987 and 1989). Systems A, ASC, X<sup>-</sup> and B<sup>+</sup> are proposed to be located primarily at the capillary abluminal membrane (Fig. 1) and actively transport amino acid substrates into the cerebrovascular endothelial cell for efflux from brain extracellular fluid. Systems A and ASC show preference for small neutral amino acids (e.g., L-alanine, L-serine, L-cysteine), whereas System B<sup>+</sup> expresses affinity for both neutral and basic amino acids (Guidotti and Gazzola 1992).  $\beta$ -Amino acids (e.g.,  $\beta$ -alanine and taurine) are shuttled into brain capillaries by a low capacity, Na<sup>+</sup>- and Cl<sup>-</sup>-dependent transport carrier (System  $\beta$ ) (Tamai et al. 1995, Tayarani et al. 1989). System X<sup>-</sup> mediates sodium-dependent transport of anionic amino acids-L-glutamate and L-aspartate. Although the results are preliminary, there is clear evidence of amino

acid transport polarity at the BBB with selective distribution of some carriers on the abluminal membrane.

### Anionic amino acid transport systems

Several *in vivo* studies have demonstrated that L-glutamate and L-aspartate are taken up from plasma into brain by a low capacity, high affinity, sodium-independent transporter, tentatively labeled System x<sup>-</sup>, which shows competitive interaction between glutamate and aspartate (System X<sup>-</sup>;  $K_m = 2\text{--}40 \mu\text{mol/L}$  for L-glutamate) (Al-Sarraf et al. 1995 and 1997b, Benrabh and Lefauconnier 1996, Oldendorf and Szabo 1976). Because transport was measured from plasma into brain, it is presumed that the saturable carrier is located at the BBB capillary luminal membrane. The exact protein that mediates this uptake has not been identified. L-Glutamate uptake at the abluminal membrane of the capillary endothelial cell was shown by Hutchinson et al. (1985) and Lee et al. (1998) to be mediated by a sodium-dependent saturable mechanism similar to that of System X<sup>-</sup>. A family of sodium-dependent anionic amino acid transporters have recently been cloned and identified in brain, but not localized to the BBB (Kanai et al. 1994). The molecular identification of the specific anionic amino acid transporters at the BBB remains to be determined.

The transport capacity for saturable influx of L-glutamate into brain is quite low compared with that of the System L and y<sup>+</sup> carriers (Table 1). This, together with the fact that the glutamate carrier is >80% saturated at normal plasma concentrations, predicts that anionic amino acid flux rates into brain are small (Al-Sarraf et al. 1997a and 1997b, Segal et al. 1990). Hawkins et al. (1995) reported that the brain uptake "permeability-surface area" for L-[<sup>14</sup>C]glutamate from normal

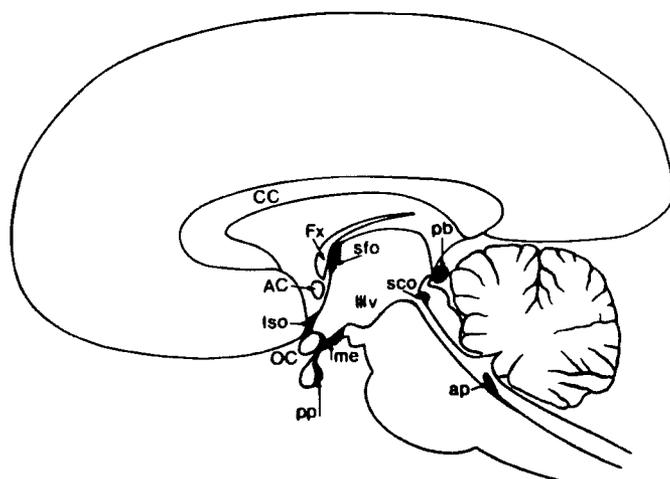
plasma is  $\sim 7 \mu\text{L}/(\text{min} \cdot \text{g})$ , corresponding to an influx rate of  $0.67 \text{ nmol}/(\text{min} \cdot \text{g})$ . This flux rate is 5- to 10-fold less than that of most of the large neutral and basic amino acids listed in Table 1. A portion of glutamate is mediated by a nonsaturable mechanism with a  $K_d$  of  $2 \mu\text{L}/(\text{min} \cdot \text{g})$  (Pardridge 1979).

A number of studies have examined the influence of acute elevations in plasma L-glutamate concentration on brain L-glutamate content. Most have found that in brain regions with an intact BBB, L-glutamate content is fairly independent of plasma L-glutamate concentration (Price et al. 1981 and 1984). The brain intracellular L-glutamate pool, however, is quite large (4–15 mmol/kg wet weight), and may mask changes in brain extracellular glutamate, which is normally in the range of 0.2–5  $\mu\text{mol}/\text{L}$ . With the use of microdialysis, Bogdanov and Wurtman (1994) found significant elevations in brain extracellular fluid L-glutamate concentration after large systemic doses of monosodium glutamate that would be expected to raise plasma L-glutamate concentration into the millimolar level. These results must be interpreted with caution, however, because Westergren et al. (1995) reported that brain microdialysis compromises the integrity of the BBB, allowing greater passive leakage of glutamate into brain. It is likely that with large systemic dosing, some net uptake of L-glutamate occurs in brain. Evaluation of the relationship between cerebrospinal fluid and plasma glutamate may help resolve this controversy.

A second high affinity glutamate transport system has been demonstrated at the choroid plexus epithelium ( $K_m$ , 2–3  $\mu\text{mol}/\text{L}$ ), which may provide an alternate route for glutamate influx into brain (Preston and Segal 1992, Segal et al. 1990). Less is known of glutamate efflux from the central nervous system. Pardridge (1979) has speculated that an active efflux pump for glutamate exists at the blood-brain barrier and may contribute to the regulation of brain extracellular fluid glutamate concentration. Both Hutchinson et al. (1985) and Lee et al. (1998) detected sodium-dependent active components of glutamate transport with the use of *in vitro* brain endothelial preparations that allow evaluation of transport at the capillary abluminal membrane. Both suggested that the sodium-dependent carrier functions *in vivo* to transport glutamate from brain. However, more work on this is required to confirm the issue.

Al-Sarraf et al. (1997b) examined the kinetics of anionic amino acid uptake in 1-wk-old and adult rats and found that for the BBB  $V_{\text{max}}$  for both L-glutamate and L-aspartate is elevated in infant animals. This occurs even though the capillary density in brain is considerably lower in infant animals. No marked alteration was observed in passive BBB permeability, suggesting that if there were age-dependent changes in anionic amino acid transport at the BBB, they were due to alterations in carrier activity. The transport selectivity of the anionic amino acid carriers at the BBB has not been examined closely. BBB transfer rates for several amino acid analogs that have been studied (e.g., domoic acid, kynurenic acid,  $\beta$ -methyl-amino-alanine) are quite low and appear not to use the BBB anionic amino acid carrier (Fukui et al. 1991, Preston and Hynie 1991, Smith et al. 1992).

Although the BBB helps protect most of the brain from changes in circulating plasma L-glutamate, there are a few brain areas that do not contain a BBB (Fig. 2) and do allow rapid L-glutamate uptake from the circulation (Hawkins et al. 1995). These are known collectively as “the circumventricular organs” and include the median eminence, area postrema, subfornical organ, subcommissural organ, pineal gland, neurohypophysis and organum vasculosum of the lamina terminalis



**FIGURE 2** Diagram showing the “nonbarrier” regions of the brain. Nonbarrier regions, shown in black, are as follows: ap, area postrema; pb, pineal body; sfo, subfornical organ; sco, subcommissural organ; me, median eminence; pp, posterior pituitary; and iso, lamina supraoptica. Other regions for reference: Illv, third ventricle; AC, anterior commissure; and CC, corpus callosum (from Landas et al. 1985, with permission).

(Gross and Weindel 1987). Brain uptake rates for small solutes in these areas exceed those of normal brain by 10- to 1000-fold (Gross et al. 1987, Gross 1991, Hawkins et al. 1995, Shaver et al. 1992). Once within brain extracellular fluid, solutes can move into adjacent brain areas via intercellular diffusion or via flow along the Virchow-Robin spaces. Such movement has been documented for glutamate and aspartate in animals after high dose amino acid administration (Price et al. 1981 and 1984). The net result is that certain areas of the brain are vulnerable to acute fluctuations in plasma glutamate concentration of large magnitude as a result of “flooding” from the circumventricular organs.

### System $\gamma^+$

Unlike the anionic amino acids, L-glutamate and L-aspartate, the essential cationic amino acids, L-lysine and L-arginine, cannot be synthesized by brain at adequate rates to meet the needs for brain metabolism and protein synthesis. Thus, the brain requires a steady and balanced supply of these “essential” amino acids from the circulation. In terms of molecular characterization, the cationic amino acid transporter, System  $\gamma^+$ , was the first amino acid transport system at the BBB to be identified at the molecular and genetic level. The cDNA for the first System  $\gamma^+$  cationic amino acid transporter (CAT-1) was cloned serendipitously by Albritton et al. (1989) as part of the search for the host cell protein responsible for infection by the murine ecotropic leukemia virus. The normal physiologic function of this retrovirus receptor was unknown at the time. However, on the basis of a predicted structural homology with yeast histidine and arginine permeases, Kim et al. (1991) and Wang et al. (1991) independently injected the cRNA for the receptor into frog oocytes and demonstrated that it led to enhanced amino acid transport activity with characteristics closely matching those previously reported for System  $\gamma^+$ . The deduced amino acid sequence of the retroviral receptor cDNA clone revealed a 622 amino acid glycoprotein with a predicted molecular mass of 67 kDa. After N-glycosidase treatment, however, the apparent molecular mass was 60–65 kDa. Hydropathy profiling predicted 12–14 transmem-

brane-spanning regions, similar to that of the previously cloned facilitative glucose transporter, GLUT1. Subsequently, the corresponding human and rat genes for CAT-1 were cloned and shown to share >88% sequence identity with the murine sequence (Puppi and Henning 1995, Stoll et al. 1993, Yoshimoto et al. 1992). The human gene maps to chromosome 13q12-q14 and consists of 10 introns and 11 exons (Albritton et al. 1992). The human cDNA sequence predicts a 629 amino acid protein with a comparable 12–14 transmembrane-spanning regions. In rats, the primary CAT-1 mRNA size is 7.9 kb, with a smaller band often seen at 3.4 kb in some tissues (Smith and Stoll 1998).

Stoll et al. (1993) demonstrated by RNase protection assay that CAT-1 is highly expressed at the BBB, with a >40-fold enhancement of mRNA for the protein in brain capillaries compared with whole brain or “capillary-depleted” brain. mRNA for CAT-1 is not ubiquitous among tissues and varies significantly; the highest values are found in bone marrow, intestine, kidney, testes and brain, with essentially no expression in liver (Kakuda et al. 1993, Kim et al. 1991, Puppi and Henning 1995, Smith and Stoll 1998, Stoll et al. 1993). **Figure 3** shows a Northern blot of CAT-1 in brain capillaries, brain and other tissues.

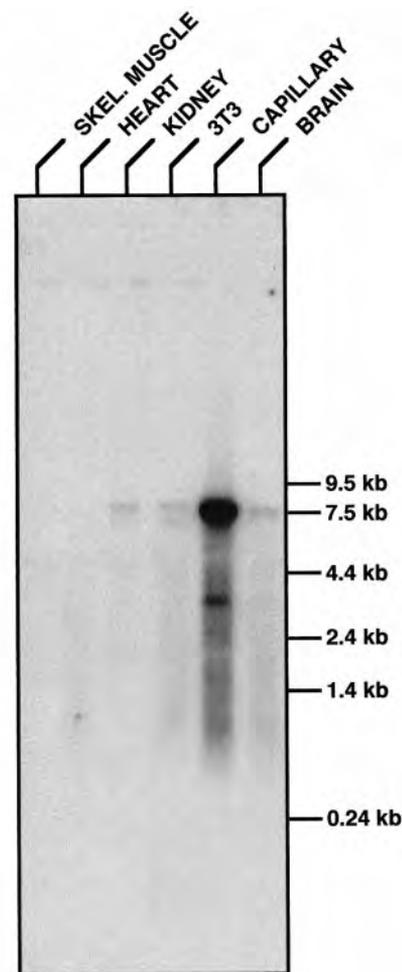
Subsequent work has identified two additional genes for cationic amino acid transporters of the same family (CAT-2 and CAT-3). CAT-2 encodes for two transporter proteins of low and high affinity that are identical with the exception of an alternatively spliced region that differs in 20 amino acids over a 41 amino acid section within the predicted fourth extracellular loop (Closs et al. 1993a and 1993b, Kakuda et al. 1993). CAT-2 is differentially expressed in tissues, including brain, but does not appear to be enriched at the BBB, where message levels are <10% of that of CAT-1 (Smith and Stoll 1999). CAT-3 is highly expressed in brain and readily discriminates between L-lysine and L-arginine on the basis of affinity (difference of approximately twofold).

A CAT-1 knockout mouse model has been developed in which targeted mutagenesis was used to alter the domain of the protein that is critical for virus binding and produce a germ line with null transport activity (Perkins et al. 1997). Homozygous pups with this mutation were 25% smaller than normal, very anemic and died on the day of birth. The results suggest a critical role in hematopoiesis and growth during development.

### System L

The brain also requires a steady stream of essential large neutral amino acids (e.g., L-leucine, L-phenylalanine and L-tryptophan) to maintain metabolic function and protein synthesis. Many of these amino acids are shuttled into brain by the “large neutral amino acid” System L carrier. This carrier was characterized initially in Ehrlich cells by Oxender and Christensen (1963) and later shown by Oldendorf and colleagues to contribute to the brain uptake of 14 of the 16 primary neutral amino acids (Oldendorf 1971, Oldendorf and Szabo 1976, Pardridge 1983). The carrier appears to operate principally via a sodium-independent, substrate-coupled antiport, although it can mediate net influx (Guidotti and Gazzola 1992).

System L at the BBB shares many of the characteristics of the L System transporter in other tissues, including inhibition by BCH (Aoyagi et al. 1988, Hargreaves and Pardridge 1988, Smith et al. 1987). The two carriers differ, however, in apparent transport “affinity” ( $1/K_m$ ) for most substrates. For example, the  $K_m$  for L-phenylalanine uptake into brain (~10–20



### Northern Blot Analysis

**FIGURE 3** Northern blot of cationic amino acid transporter (CAT)-1 mRNA in rat skeletal muscle, heart, kidney, NIH 3T3 cells, brain capillaries (isolated microvessels) and whole brain (from Smith and Stoll 1998, with permission).

$\mu\text{mol/L}$ ; Momma et al. 1987, Sánchez del Pino et al. 1995, Shulkin et al. 1995) is 100–1000 times less than that in other tissues. On the basis of this difference, it has been proposed that the blood-brain barrier L System represents a separate isoform, designated System L1.

Several genes have been identified that enhance neutral amino acid uptake when their mRNA is injected into cells. One encodes for a cell surface protein, 4F2hc, also known as CD 98, and has been demonstrated to enhance System L activity in cells (Bertran et al. 1992, Bröer et al. 1995 and 1997, Palacín, 1994). Recently, a System L cDNA was cloned by both Kanai et al. (1998) and Mastroberardino et al. (1998) and shown to encode for a ~41-kDa protein (LAT) with multiple transmembrane-spanning regions, which operates in conjunction with 4F2hc. The two proteins are reportedly linked by a disulfide bridge. The 4F2 complex was identified first in a human T-cell line as a heterodimer composed of an 85-kDa glycosylated heavy chain (4F2hc) with a single trans-

membrane-spanning region with a disulfide linkage to a 45-kDa nonglycosylated light chain. This smaller subunit was proposed by Palacín (1994) to be a transporter. The cDNA isolated by Kanai encodes for a protein that mediates BCH-sensitive neutral amino acid transport, when expressed in *Xenopus* oocytes ( $K_m$  for L-phenylalanine is 20  $\mu\text{mol/L}$ ). Message for the protein was found in multiple tissues including brain. The functional relation between the transporter and the 4F2hc is not precisely understood. It has been suggested, however, that 4F2hc is a required regulatory or modulator subunit for the transporter complex.

Consistent with the proposed role of 4F2hc in System L transport into brain, message for 4F2hc was found in mRNA from isolated rat brain capillaries, as well as in whole rat brain (Smith and Stoll 1999). Similarly, Boado et al. (1999) have demonstrated high level expression of the mRNA for the light chain of the L transporter (LAT) at the blood-brain barrier. Expression of 4F2hc also decreased during postnatal development in the rat, as expected from L-System transport studies.

### SUMMARY

Over the past 25 years, significant progress has been made in the identification and characterization of blood-brain barrier amino acid transport systems, primarily with the use of physiologic methods. However, with the advent of the cloning and identification of the first blood-brain barrier amino acid transport protein and gene (System  $\gamma^+$ ) in 1993, a new era has been ushered in for the investigation of barrier amino acid transport systems. It is hoped that these new approaches will provide novel, more selective tools and probes with which to study the transport systems and evaluate their regulation. With these new molecular biological approaches, it may be possible ultimately to modify blood-brain barrier amino acid transport expression to treat human disease.

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