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Regulation of Amino Acid Availability to Brain: Selective Control Mechanisms for Glutamate

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The transport of circulating amino acids into brain is of much importance to brain function since many pathways of cerebral amino acid metabolism are influenced by precursor availability (29). The rate of synthesis of several putative neurotransmitters (serotonin, catecholamines, histamine, or carnosine) is affected by the level in brain cells of precursor neutral amino acids (tryptophan, tyrosine, histidine) (6,9,43,47). In addition, when brain levels of essential neutral or basic amino acids fall to very low levels, e.g., due to a hyperaminoacidemia, cerebral protein synthesis may become substrate limited. [Protein synthesis in the CNS (35) proceeds at rates independent of precursor amino acid supply at normal brain levels of amino acids.] Unlike the essential neutral or basic amino acids, the acidic amino acids, glutamate and aspartate, can be synthesized in brain cells at rates commensurate with the metabolic demands for these compounds. Consequently, the rate of transport of the acidic amino acids from blood to brain is much lower than for the neutral or basic amino acids (22). Despite the relative independence of the brain on the circulating acidic amino acids, the mechanisms controlling the flux into the CNS of glutamate and aspartate are of much interest; these compounds are putative excitatory neurotransmitters (16) and are neurotoxic when plasma levels are elevated by the administration of large doses (25). This chapter will review the mechanisms regulating the transport of amino acids from the blood into the brain, and emphasis will be placed on the fundamental differences between the factors regulating the brain uptake of glutamate and aspartate versus the neutral and basic amino acids. The three factors (29) controlling the rate of amino acid transport from the blood into the brain are (a) cerebral blood flow, (b) plasma concentration, and (c) the blood-brain barrier (BBB) permeability.

CEREBRAL BLOOD FLOW

The rate of influx of a substrate into brain will be proportional to the rate of cerebral blood flow as long as the BBB permeability constant (PS in ml/min/g) is within an order of magnitude of the rate of flow (F in ml/min/g). The relationship (4) between PS and F is given by the fractional extraction (E) of the unidirectional

influx of the substrate into brain (see Appendix). When E is greater than approximately 15% (4), the influx will vary with changes in flow. Although at normal plasma levels the E value for phenylalanine or leucine is approximately 15% (22), the E value for glutamate or aspartate is generally not higher than 2 or 3% (22); therefore, cerebral blood flow does not normally influence the brain uptake of glutamate or aspartate. However, there are several circumventricular organs (CVO) of the brain that lack a BBB (46). Conceivably, the regional E value for glutamate influx into the CVOs is greater than 15%. Therefore, conditions that increase F, e.g., seizures (which are induced by high levels of glutamate), may accelerate the rate of brain uptake of circulating glutamate within the CVOs on the basis of increased cerebral blood flow (39).

PLASMA CONCENTRATIONS

As long as the K_m (half-saturation constant) of BBB amino acid transport is greater than or equal to the plasma level, then the rate of brain uptake of amino acid will be proportional to the plasma concentration. Postprandial plasma amino acid levels are a function of (a) the dietary amino acid composition and (b) the transport of amino acids across the splanchnic (gut and liver) barriers. Unlike the large neutral or basic amino acids, physiologic doses of glutamate or aspartate are readily metabolized to alanine via transamination by gut epithelial cells (41). However, pharmacologic doses of glutamate or aspartate, or even doses found in a 50% casein diet (41), exceed the capacity of the gut transamination sites, and the acidic amino acids gain access to the portal circulation.

Amino acids in the portal plasma must clear the hepatocyte bed before entering the systemic circulation. Neutral amino acids are transported into liver cells by specific transport systems of very high capacity (high V_{max}) and low affinity (high K_m). Although more quantitative studies are needed, preliminary investigations indicate the K_m of neutral amino acid transport into liver cells *in vivo* is greater than 10 mM (31). Therefore, the neutral amino acid transport systems are probably never saturated during the absorption of even pharmacologic doses of amino acids. The very high capacity of the liver neutral amino acid transport system may explain why the oral administration of cysteine (a neutral amino acid that is oxidized by tissues to form a neurotoxic sulfonic amino acid, cysteic acid) is ineffective in producing brain lesions, whereas doses of this amino acid administered parenterally are effective (25).

Acidic amino acids are commonly believed to enter liver cells poorly. This misconception is due largely to a study of glutamate transport into liver slices (11); glutamate was shown to rapidly equilibrate with liver cells (half-time less than 1 min), but to reach a tissue/medium distribution ratio of only 0.5. Although the short half-time of equilibration suggests glutamate readily enters liver cells, the authors concluded acidic amino acids penetrate liver cells poorly based on the lack of complete equilibration with liver water (11). However, the latter function may be ATP-dependent and Krebs has since shown liver ATP is rapidly depleted, within

minutes of preparing the liver slices (14). Data obtained *in vivo* indicate acidic amino acids readily penetrate the liver cell membrane via a class-specific transport system (31). The transport data for glutamate is shown in Fig. 1 and was obtained with a tissue sampling-single injection technique using a ^{3}H-water internal standard

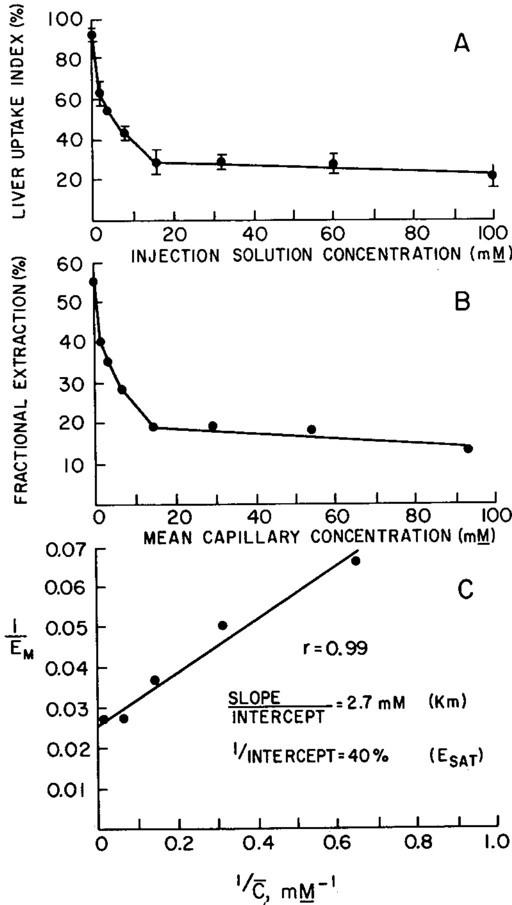

FIG. 1. The transport of ^{14}C-L-glutamate vs a ^{3}H-water reference into rat liver *in vivo* (31). **A:** The LUI for ^{14}C-L-glutamate is plotted against the portal injection solution concentration (C_p) of unlabeled glutamate. Data are means ± SEM (n = 4 to 6). **B:** The fractional extraction (F) of the unidirectional influx of glutamate into liver is plotted against the mean capillary concentration ($\bar{C}$) of glutamate; E = (LUI) (E_{HOH}), where E_{HOH} = 0.64 is the fractional extraction of influx into liver of the ^{3}H-water reference at 18 sec following portal injection (30); $\bar{C}$ is estimated from C_p (see Appendix). **C:** A double-reciprocal plot of $1/E_m$ vs $1/\bar{C}$, where $E_m = E_o - E_s$; E_o and E_s are the fractional extraction of glutamate influx at a tracer concentration and at an inhibiting concentration, respectively. The K_m of glutamate transport (2.7 mM) is equal to the slope/intercept ratio; the fractional extraction of saturable transport (E_{sat} = 40%) is equal to the reciprocal of the intercept. Since E = 55%, the fractional extraction of nonsaturable uptake is 15%. (See Appendix for details of the kinetic analysis.)

in anesthetized rats. A complete kinetic analysis of the original data (31) was not possible due to lack of information on the hepatic clearance of the ^{3}H-water reference, as well as portal blood flow. This information is now available (30) and permits computation of the K_m, V_{max}, and K_d (constant of nonsaturable transport) of glutamate and aspartate transport into liver cells. As shown in Table 1, the K_m of aspartate and glutamate transport is 1.9 mM and 2.7 mM, respectively. Since normal portal plasma levels of the acidic amino acids, up to 0.3 mM for glutamate (41), are less than 10% of the transport system K_m, the liver cell membrane is never saturated by physiologic doses of acidic amino acids. However, since peripheral glutamate levels reach 2.5 mM (18) at 30 min following an oral dose of 2 g/kg of glutamate, portal concentrations of glutamate under these conditions are probably above the K_m of the transport system (Table 1). Therefore, toxic doses of glutamate (1 to 4 g/kg p.o.) achieve portal amino acid levels that approach the maximal capacity of the liver to take up glutamate. Since the capacity of the splanchnic barriers to clear toxic doses of glutamate is somewhat limited, the major factor protecting brain cells from toxic levels of glutamate is the BBB.

BLOOD-BRAIN BARRIER

The BBB [and the blood-retina barrier (BRB)] are the products of a unique capillary structure. The brain capillary endothelial cells are fused together by tight junctions, which convert the brain capillary wall into an epithelial barrier (5,44). The BBB segregates the cerebral and systematic extracellular fluids and is effectively a plasma membrane (with regional specializations) for the entire brain. Due to the presence of the BBB, circulating compounds enter brain via either (a) lipid mediation (lipid-soluble compounds, e.g., drugs) or (b) carrier mediation (water-soluble compounds, e.g., metabolic substrates). The rate at which metabolic substrates penetrate the BBB is a function of the kinetic characteristics (K_m, V_{max}, K_d) of the specific carrier systems (Table 2) that transport the respective substrates (34). Although it has been known for some time that the neutral and basic amino acids readily penetrate the BBB via class-specific transport systems (22), Oldendorf has recently documented the presence of an acidic amino acid carrier for glutamate and aspartate (23). The BBB glutamate carrier is of particular interest in that the failure

TABLE 1. *Kinetics of glutamate and aspartate transport into liver cells* in vivo[a]

Amino acid	K_m (mM)	V_{max} (μmoles/min/g)	K_D (ml/min/g)
Glutamate	2.7	1.3	0.15
Aspartate	1.9	1.2	0.16

[a] Calculated (see Appendix) from previously reported data (30,31). The K_d represents nonsaturable uptake by both the intracellular and extracellular (sucrose) spaces of liver. Since the K_d for sucrose is 0.08 to 0.14 ml/min/g, more than 90% of the nonsaturable uptake of glutamate or aspartate is due to distribution in the extracellular space.

TABLE 2. *The BBB transport systems*[a]

Transport system	Representative substrate	K_m (mM)	V_{max} (nmoles/min/g)	K_d (ml/min/g)
Hexose	Glucose	9	1,600	0.023
Monocarboxylic acid	Lactate	1.9	120	0.028
Neutral amino acid	Phenylalanine	0.12	30	0.018
Basic amino acid	Lysine	0.10	6	0.007
Amine	Choline	0.22	6	0.003
Purine	Adenine	0.027	1	0.006
Nucleoside	Adenosine	0.018	0.7	0.001
Acidic amino acid	Glutamate	0.04	0.4	0.002

[a] From Pardridge and Oldendorf, ref. 34; except values for glutamate calculated from data of Oldendorf and Szabo, ref. 23. The K_d represents nonsaturable transport into both the intracellular and extracellular spaces of the brain. In the case of choline, adenine, adenosine, and glutamate, the component of K_d due to extracellular uptake has been subtracted (^{113m}indium-EDTA was used as an extracellular space reference), and the K_d reported here represents only nonsaturable transport into brain cells. The K_d for glucose, lactate, phenylalanine, and lysine includes the extracellular component and, therefore, overestimates the K_d of nonsaturable transport into brain cells by approximately 0.006 ml/min/g. The latter value is based on the fractional extraction, 0.01, of ^{113m}indium-EDTA uptake by brain.

to raise whole brain glutamate levels after systemic administration has been attributed to the impermeability of the BBB to glutamate (15).

The neutral and basic amino acids penetrate the BBB via their respective transport systems. The K_m and V_{max} for each amino acid are listed in Table 3, as well as their respective plasma concentrations for the rat. Two important points in regard to BBB amino acid transport are to be emphasized: (a) the close approximation of the transport K_m and plasma levels and (b) the bidirectional nature of amino acid transport across the BBB. As shown in Table 3, the K_m of BBB transport of the neutral and basic amino acids approximates the plasma level (29). Since the effect of competition on transport rates is directly related to the ratio of plasma level to K_m (28), it can be seen that when the transport K_m exceeds the plasma level by 10-fold or more, the effect of competition among similar amino acids will be negligible. Since the K_m of neutral amino acid transport in mammalian erythrocytes, renal tubule, liver cells, gut epithelia, and probably skeletal muscle is in the 1- to 10-mM range or greater (29), competition for transport under physiologic conditions does not occur in most tissues. However, amino acid competition does normally occur in brain under day-to-day conditions, e.g., the elevation of brain tryptophan after a carbohydrate meal is due to the insulin-mediated hypoaminoacidemia (9). The fundamental basis for the sensitivity of the CNS to amino acid competition (both physiologic and pathologic, e.g., the hyperaminoacidemias) is the close approximation of the BBB transport K_m and plasma amino acid levels (28,29).

The second important point about BBB transport of the neutral and basic amino

TABLE 3. *Kinetics of the BBB amino acid transport systems*

Amino acid	Plasma level[a] (mM)	K_m[b] (mM)	V_{max} (nmoles/min/g)
Neutral amino acids			
Phenylalanine	0.05	0.12	30
Leucine	0.10	0.15	33
Tyrosine	0.09	0.16	46
Tryptophan	0.10	0.19	33
Methionine	0.04	0.19	33
Histidine	0.05	0.28	38
Isoleucine	0.07	0.33	57
Valine	0.14	0.63	49
Threonine	0.19	0.73	37
Cycloleucine	—	0.75	55
Basic amino acids			
Arginine	0.10	0.09	9
Lysine	0.30	0.10	6
Ornithine	0.09	0.23	11

[a] From Pratt, ref. 40.
[b] From Pardridge and Oldendorf, ref. 33.

acids is that the rate of net uptake (i.e., influx-efflux, determined by multiplying the arteriovenous difference × cerebral blood flow) is much less than the rate of unidirectional influx (determined by either single-injection or constant-infusion isotope dilution techniques). As shown in Fig. 2, the rate of unidirectional influx of the large neutral or basic amino acids is on the order of 1 to 5 nmoles/min/g (28,40). This value approximates the rate at which essential amino acids are incorporated into proteins in the adult rat brain *in vivo* (17). However, the rate of *net* amino acid utilization by brain is small compared to rates of influx. [For example, aromatic amino acids are converted to neurotransmitters at rates (20) less than 50 pmoles/min/g, which are too low to be detected by arteriovenous differences.] In fact, the rate of net uptake of amino acids is not statistically different from zero for most neutral or basic amino acids (8). One exception to this rule is the branched-chain amino acids, leucine and isoleucine, for which positive net uptakes up to 40% of the rate of influx are observed (8). The branched-chain amino acids may be serving as precursors to sterol synthesis in the brain (45).

The relationships between (a) the K_m to plasma level and (b) the rate of influx to net uptake across the BBB for glutamate contrasts with the mechanisms mediating the transport of the basic or neutral amino acids. Although the K_m of glutamate transport across the BBB has not been reported, estimates can be made from data reported by Oldendorf and Szabo (23); linear transformation of their data according to previously reported methods (28) indicates the K_m and V_{max} values for glutamate transport are approximately 0.04 mM and 0.4 nmole/min/g, respectively (Table 2). Since the normal plasma glutamate level, 0.15 mM (40), is nearly fourfold the glutamate K_m, the glutamate carrier is virtually saturated by physiologic plasma

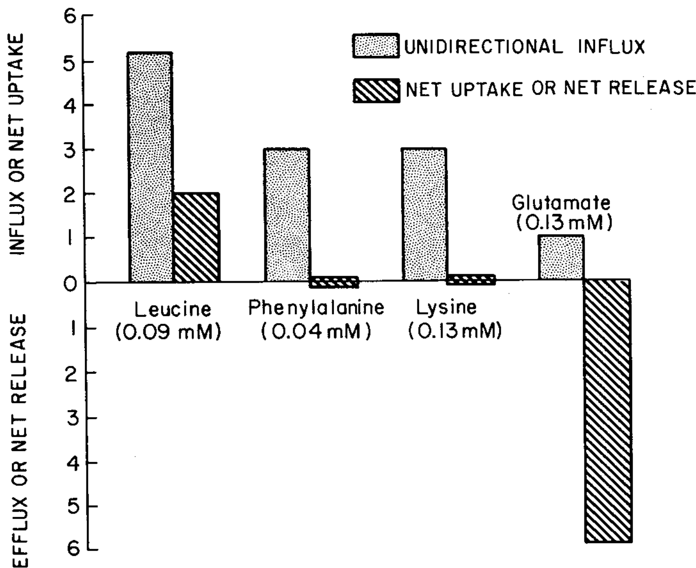

FIG. 2. The rates of unidirectional influx and net uptake for four amino acids—leucine (branched-chain neutral), phenylalanine (aromatic neutral), lysine (basic), and glutamate (acidic)—are shown for a given plasma concentration. Rates of leucine and phenylalanine influx (28) and lysine and glutamate influx (40) are shown for the barbiturate-anesthetized rat. Rates of net amino acid uptake (or output) are calculated from arteriovenous differences across the isolated perfused dog brain (8). The latter preparation provides the most accurate measure of arteriovenous differences across the brain because there is no extracerebral contamination of the venous drainage. Extracerebral contamination is particularly likely in studies with small animals, such as the rat (see ref. 12). The rate of net glutamate efflux from brain shown here is calculated from arteriovenous differences of plasma amino acid concentrations. If analyses of whole blood, i.e., plasma plus erythrocytes, are made, net glutamate release from brain is as high as 10 nmoles/min/g (8), indicating a considerable fraction of glutamate that is transported out of brain is rapidly taken up by circulating red cells.

levels of the amino acid. Therefore, brain glutamate does not rise or fall in parallel with changes in plasma levels, as is the case with the neutral or basic amino acids.

Although the rate of influx of the neutral or basic amino acids is much greater than the respective rates of net uptake, the reverse is true for glutamate. While the rate of glutamate influx into brain is 1.0 nmoles/min/g (40), the rate of *net* flux of glutamate across the BBB is 5.9 nmoles/min/g in favor of production (8). Therefore, glutamate *leaves* the brain and does so faster than any of the amino acids. Since glutamate influx into brain is 1 nmoles/min/g (40) and the net release of glutamate by brain is 6 nmoles/min/g (8), the rate of unidirectional efflux of glutamate, 7 nmoles/min/g, is sevenfold greater than the rate of influx. If the BBB glutamate carrier were symmetric, i.e., equal V_{max}/K_m ratio on both the blood and the brain sides of the BBB, as is the neutral amino acid carrier (33), then the glutamate concentration in the brain interstitial space would have to be over 1 mM to account for the sevenfold disparity between influx and efflux rates. It is unlikely that the

brain interstitial space contains 1 mM glutamate, a putative excitatory transmitter (16), particularly since the CSF level of glutamate is less than 10 μM (36). Therefore, it seems probable that the sevenfold disparity between glutamate influx and efflux across the BBB is due to an asymmetry of the BBB glutamate carrier, i.e., the V_{max}/K_m ratio of the glutamate carrier on the *brain* side of the BBB is several-fold greater than the V_{max}/K_m ratio on the *blood* side of the BBB. Such a condition defines the glutamate carrier as an *active efflux* system, actively transporting glutamate from the brain interstitium to the blood against a concentration gradient.

In addition to the saturable route of glutamate influx into brain, there is also a nonsaturable route of transport, as is the case with the seven other classes of compounds that penetrate the BBB via carrier systems (Table 2). Although the nonsaturable mechanism is generally ascribed to free diffusion, it is unlikely that any polar metabolic substrate "freely" diffuses through the BBB. The nonsaturable systems are probably very low affinity ($K_m \geq 100$ mM) carrier systems. Since the nonsaturable mechanism of glutamate transport across the BBB is characterized by a K_d of 0.002 ml/min/g (Table 2), the rate of glutamate influx via this mechanism is only 0.3 nmoles/min/g at a plasma level of 0.15 mM. However, when plasma glutamate is raised to 40 mM by the parenteral administration of 2 g/kg glutamate (37), the rate of glutamate influx into the brain might be as high as 80 nmoles/min/g (plasma level × K_d). The latter value for glutamate influx exceeds the V_{max} of the neutral amino acid carrier. The observation that brain glutamate is not increased with the administration of toxic doses of glutamate (37), despite the presence of the nonsaturable route of glutamate transport through the BBB, indicates the capacity of the BBB active efflux system for glutamate is relatively high.

Certain regions of brain, however, take up glutamate under conditions of very high plasma levels (37). These regions are the CVO, i.e., periventricular areas that lack a BBB (Table 4). The capillaries in these regions lack tight junctions and have large interendothelial pores and active pinocytosis (5). The neuronal necrosis that occurs when large doses of glutamate (0.5 to 4 g/kg) are administered is typically in areas contiguous with a CVO (26), e.g., the arcuate nucleus (near the median eminence) or the preoptic area [near the organum vasculosum of the lamina terminales (OVLT)]. The selective vulnerability of regions such as the arcuate nucleus to toxic doses of glutamate may be due to one or both of two possible mechanisms.

TABLE 4. *The CVOs of the brain*[a]

CVO	Contiguous brain region
Median eminence	Arcuate nucleus, hypothalamus
OVLT	Preoptic area, hypothalamus
Subfornical organ	Roof of third ventricle
Subcommissural organ	Dorsum of third ventricle
Area postrema	Base of fourth ventricle

[a] These periventricular areas of brain lack a BBB; other areas of brain that do not have a BBB are the choroid plexus, the pineal gland, and the neurohypophysis (see ref. 46).

First, these areas may be exposed to high levels of glutamate simply because of radial diffusion of glutamate from the extracellular space of the CVO to contiguous brain areas. A second possible mechanism is the retrograde axoplasmic flow of glutamate from the nerve ending in the CVO, e.g., the median eminence, to the body of neurosecretory cells within the BBB proper, e.g., the arcuate nucleus. The retrograde axoplasmic flow mechanism explains why certain neurosecretory regions of the hypothalamus, e.g., the supraoptic, paraventricular, and arcuate nuclei, selectively take up horseradish peroxidase (a protein marker that does not cross the BBB) at 8 to 12 hr after the intravenous injection of the protein (5).

When very high doses of glutamate are administered (2 to 4 g/kg), even non-CVO areas of brain may be affected (25). This signifies a breakdown of the BBB and may be due to the seizure activity induced by large doses of glutamate (21). Convulsions cause a breakdown of the BBB (38) due to the acute hypertensive response (e.g., chordotomy prevents the hypertension of seizures and also the breakdown of the BBB).

One non-CVO area of brain that appears to be affected by toxic doses of glutamate, via a mechanism that is apparently not related to convlusive activity, is the retina (25). The retina is protected from the systemic circulation by the BRB, which is formed by tight junctions (44) between capillary endothelial cells analogous to the brain capillary. Retinal capillaries mediate the active efflux of many amino acids from the vitreous humor to the retinal capillary lumen (3). In this way, a concentration gradient of amino acids is maintained from the posterior chamber, where amino acids are actively taken up across the ciliary body, through the vitreous to the retina, where amino acids are actively removed across retinal capillaries (3). Conceivably, the selective vulnerability of the retina to high plasma levels of glutamate is due to a relative decrease in the activity of the BRB active efflux system for acidic amino acids or to a relatively high activity of the active influx system for glutamate transport across the ciliary body.

In addition to the selective vulnerability of the retina and certain regions of brain (CVOs) to toxic doses of glutamate, there are also developmental and species selectivities. Younger animals (less than 10 days old) are more susceptible to the amino acid as compared to older animals (25). This increased sensitivity is often attributed to an "immature or leaky BBB" in the young animal. The historical background leading to the present-day misconception of a leaky BBB in the young has recently been reviewed (42). Actually, the brain endothelial tight junctions are formed in the first trimester of human fetal life (19); the BBB to proteins is complete by the 15th day of fetal life in the rat (24). An anatomically intact BBB in the newborn, however, does not rule out developmental modulations in specific carrier systems (34). For example, the active efflux system for organic acids (e.g., 5-HIAA) does not develop in the rat until the period between 5 to 30 days (2). Conceivably, a low activity of the BBB active efflux system for glutamate in the young animals may explain their greater sensitivity to toxic doses of glutamate. In addition to developmental differences, the active efflux system for organic acids demonstrates a marked species specificity, e.g., this system is probenecid sensitive

(presumably due to a lower transport K_m) in rats and mice, but is probenecid insensitive in rabbits and rhesus monkeys (13). The greater sensitivity of rodents to toxic doses of glutamate, as compared to the rhesus monkey (10), may be due to a lower capacity of the rodent BBB active efflux system for glutamate.

In conclusion, comparison of rates of glutamate influx and net release in the brain suggests an active efflux system for this amino acid exists within the BBB. However, the evidence for regional, developmental, and species selectivity of this transport system is at present only indirect, and future quantitative studies will be needed to adequately characterize the BBB transport of the acidic amino acids.

APPENDIX

The liver uptake index (LUI) is a ratio of the fractional extraction (E) of the unidirectional influx of a ^{14}C-labeled compound (at tracer concentrations) relative to the fractional extraction of a ^{3}H-water reference (E_{HOH}) at 18 sec following a single portal vein injection (31), i.e.,

$$LUI = \frac{E}{E_{HOH}} \quad (1)$$

Given E_{HOH} (0.64 at 18 sec), the LUI may be converted to E (30). E represents the sum of both saturable (E_{sat}) and nonsaturable (E_{ns}) routes of transport; E_{sat} may be computed from a double-reciprocal plot (Fig. 1).

Extraction values may be converted to permeability-surface constants (PS) with the use of Crone's equation (7),

$$PS = (F) \ln \frac{1}{1\text{-}E} \quad (2)$$

which has been shown to be applicable to liver transport studies (1); F is the rate of portal blood flow (0.93 ml/min/g) in the barbiturate-anesthetized, hepatic artery-ligated rat (30). The PS value is related to Michaelis-Menten kinetics (32) according to

$$PS = \frac{(V_{max}}{K_m)} + K_d \quad (3)$$

where $PS_{sat} = V_{max}/K_m$ and $PS_{ns} = K_d$. Since PS_{sat} and K_m may be calculated from the data in Fig. 1, V_{max} may be estimated. It should be emphasized that an accurate estimate of K_d is essential in accurately calculating the transport K_m and V_{max}, particularly when K_d is large, as in liver transport studies. The method described here calculates K_d by extrapolation from data over the entire curve, which provides the best estimate of K_d. The analysis used here for liver transport studies is analogous to that used for BBB transport (28).

One modification of the above analysis that must be made for liver, but is not necessary for most studies in brain, is the estimation of the mean capillary concentration ($\overline{C}$) from the arterial or portal plasma concentration (C_P). Assuming the capillary concentration falls logarithmically as the bolus traverses the capillary bed (27), then

$$\overline{C} = \frac{(E)}{-\ln(1-E)} (C_P) \quad (4)$$

When E is small, $\overline{C}$ approximates C_P; however, when E is large, C_P overestimates $\overline{C}$; e.g., if $E = 25\%$, $\overline{C}$ is 87% of C_P; but when $E = 0.75$, $\overline{C}$ is only 54% of C_P (33).

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