L-Methionine Uptake by Human Cerebral Cortex: Maturation from Infancy to Old Age

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Age-associated changes in amino acid transport from blood to normal frontal cortex were studied using positron emission tomography (PET). Seventeen patients, 1.8–71 yr, were injected intravenously with tracer doses of [^{11}C] L-methionine and a baseline PET scan was obtained. To assess competitive inhibition of [^{11}C]L-methionine uptake, patients received either oral L-phenylalanine or an i.v. infusion of amino acids 1 hr before a second PET study. Uptake of [^{11}C]L-methionine by frontal cortex decreased seven-fold between 1.8 and 71 yr ($r = -0.71$; $p < 0.05$). Blood-to-brain transfer of [^{11}C]L-methionine, at 4.5 yr, exceeded mean adult values by more than five-fold. Competitive inhibition reduced L-methionine uptake in all patients older than 4.6 yr. These developmental changes parallel findings in animals. The neutral amino acid transport system may modulate human brain amino acid levels to meet changing developmental metabolic needs.

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In the developing brain, protein synthesis is greater than that in the adult (*1,2*). For several amino acids, rates of influx are similar to rates of incorporation into cerebral proteins (*3,4*). It would be of interest to know if there is an increase in blood-to-brain transport of amino acids in the developing human brain, which would parallel the need for increased protein production. Studies in rodents suggest that there is enhanced blood-to-brain transfer of amino acids during development (*5,6*).

With the advent of positron-emitting tracers, it has become possible to assess metabolism of amino acids and protein in the human brain (*7,8*). The blood-brain barrier transfer of amino acids involves competitive kinetics and hence can be modified by prior administration of another amino acid sharing the same carrier. This pattern was noted first in rodents (*9–12*) and has been confirmed in humans using positron emission tomography (PET) (*13–15*).

We now report a study of the maturation of L-methionine (L-MET) transport in the human brain from childhood through the seventh decade. Our findings show that transport of the neutral amino acid L-MET is strikingly increased, showing characteristics of competitive inhibition, in the immature human brain. Our data were obtained in brain tumor patients. All regions analyzed appeared free of pathologic changes that could be defined by either structural or functional criteria. We believe that the information obtained in our study can be extrapolated to events in the normal human nervous system.

MATERIALS AND METHODS

Patient Population

Seventeen patients with histologically confirmed brain tumor were studied by [^{11}C]L-methionine positron emission tomography (METPET) (Table 1 A-B). Children and adolescents ranged in age from 1.8 to 15.75 yr (Group A, n = 10). Adults (Group B, n = 7) ranged in age from 21 to 71 yr. Written informed consent was obtained from each patient or guardian, according to a protocol approved by the Johns Hopkins Hospital Joint Committee on Clinical Investigation. All patients fasted for 8–9 hr prior to the study. Two of the patients in Group A received oral sedation in an amount sufficient to minimize movement during the scanning procedure.

PET Method

Studies were obtained using a Neuro-ECAT system (C.T.I., Inc., Knoxville, TN) with intrinsic resolution of 8 mm full width at half maximum (FWHM). A synthetic lightweight face mask was used to aid in positioning and to restrict patient motion. Data were acquired simultaneously from three parallel slices with a center-to-center separation of 1.6 cm. The transverse tomographic planes were oriented parallel to the inferior orbitomeatal line. Imaging began 30 sec after tracer injection. Three sets of images (plane sets) were acquired simultaneously. Images were acquired for 1 min starting at about 1, 3, and 5 min after injection and for 2 min starting at about 8, 11, and 14 min. Imaging began 30 sec after tracer

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injection and was continued for 30–45 min. Analysis of this data allowed construction of a curve that defined the time course of radioactivity in brain. Attenuation correction was carried out by a geometric method. Radioactivity measured in regions of interest placed on the reconstructed images was corrected for sensitivity using a brain phantom.

TABLE 1A
Age and Tumor Types for All Patients

Patient	Age (yr)	Tumor histology	Tumor location
Group A			
1	1.8	Ependymoma	4th ventricle
2	2.9	Ependymoma	Post. fossa
3	4.5	Glioma	Hypothalamus
4	4.6	Glioma	Pons
5	5.6	Glioma	Optic pathway
6	6	Ependymoma	Post. fossa
7	12.5	Astrocytoma	Temporoparietal
8	13.5	Astrocytoma	Cerebellum
9	15	Astrocytoma	Frontal
10	15.75	Medulloblastoma	4th ventricle
Group B			
1	21	Astrocytoma	Frontal
2	27	Astrocytoma	Frontal
3	36	Astrocytoma	Frontoparietal
4	38	Meningioma	Temporal
5	40	Astrocytoma	Frontoparietal
6	68	Meningioma	Temporal
7	71	?Astrocytoma	Post. callosal

METPET: Baseline Study

Assessment of brain uptake of L-MET under basal conditions was made using a tracer dose (6.0–24.44 mCi) of [^{11}C] L-MET given as an i.v. bolus over ~30 sec. The administered dose was estimated by counting the syringe before and after injection in a dose calibrator (Capintec). In 10 patients, the time course of blood radioactivity was estimated so as to allow model-fitting of the data and estimation of blood-to-brain transfer rates of [^{11}C]L-MET. "Arterialized" venous blood was withdrawn from the opposite arm during the first 20 min of the study on a schedule of ~15-sec intervals for the first minute, 30-sec intervals for the next 2 min, 1-min intervals up to 10 min after injection, and 5-10-min intervals thereafter. Vascular access was frequently compromised in younger patients who had received chemotherapy courses; blood radioactivity data could be obtained in only one child under 12 yr. Sample aliquots were assayed for ^{11}C radioactivity in a calibrated well counter. In six patients, plasma amino acids were analyzed by ion exchange chromatography on a Beckman 6300 amino acid analyzer using the standard Beckman protocol.

Competitive Inhibition Studies

Twelve patients received a second METPET study following their baseline study. To alter the plasma amino acid profile so as to favor inhibition of L-MET blood-to-brain transport, patients received either oral L-phenylalanine (L-PHE) (n = 10) or an i.v. infusion of multiple amino acids (MAI) (n = 2). In both of these situations, additional molecules of the same transport class as L-MET would arrive at the blood-brain

TABLE 1B
Treatment Modalities and PET Study Details for All Patients

Patient	Age (yr)	Surgery	Radiotherapy	Chemotherapy	Pet Baseline	PET Com. Inhib.
Group A						
1	1.8	+	+	+	+	–
2	2.9	+	+	+	+	+
3	4.5	+	+	+	+(2)	+(1)
4	4.6	+	+	+	+(2)	+(1)
5	5.6	+	+	+	+	+
6	6	+	+	+	+(2)	+(1)
7	12.5	–	–	–	+	+
8	13.5	+	+	+	+	+
9	15	–	–	–	+	–
10	15.75	+	–	–	+	+
Group B						
1	12	+	–	–	+	+
2	27	+	+	+	+(3)	+(2)
3	36	+	+	–	+	–
4	38	+	–	–	+	+
5	40	+	+	+	+	+
6	68	+	–	–	+	–
7	71	+	–	–	+	+

In Case 7, the CT diagnosis was most consistent with astrocytoma; metastasis from prostatic carcinoma was also possible.
In Case 6, radiotherapy was delivered to the whole brain.
Numbers in parentheses indicate number of PET studies acquired. Com. Inhib. = PET study following administration of competitive inhibitor.

TABLE 2
Plasma Amino Acid Concentrations (μmole/1) in Patients Undergoing [^{11}C]L-Methionine PET

Patient	Age (yr)	Baseline*		Compet. Inhib.†			
				POST-PHE†		POST-MAI‡	
		L-MET‡	L-PHE‡	L-MET	L-PHE	L-MET	L-PHE
−1	12	27	51	16	2211	—	—
2	15	25	49	18	190	—	—
3	13.5	16	55	19	355	—	—
4	40	18	43	—	—	—	—
5	40	46	86	—	—	468	416
6	71	25	61	—	—	1672	818

* Indicates amino acid (L-methionine or L-phenylalanine) concentration observed in plasma samples obtained during baseline METPET. Each value is the mean of two plasma samples: one obtained immediately before tracer injection and one at the conclusion of the study;

† Indicates concentration observed in plasma samples obtained during competitive inhibition METPET.

PHE-L-phenylalanine; MET-L-methionine; and MAI-multiple amino acid infusion.

capillary interface and "compete" with L-MET for entry into brain.

Post-L-PHE Study. The second METPET was obtained 1 hr after the competing amino acid L-PHE was administered orally as a suspension (100 mg/kg) in water. This dose was chosen on the basis of clinical research documenting the blood L-PHE response to different amounts of the ingested amino acid (*16*). By comparing brain uptake of radiotracer in the two scans, we were able to assess the fraction of [^{11}C]L-MET uptake that was affected by competitive inhibition. Plasma amino acids were determined before and after L-PHE administration (n = 3) (Table 2).

Post-MAI Study. An i.v. infusion of multiple amino acids (5.4% Nephramine, McGaw) was given at a rate of 125 ml/hr over 30 min prior to the second METPET study. Plasma amino acid levels were measured before and after MAI (n = 2).

Statistical Analysis. Group values were expressed as the mean ± s.d. Statistical differences were computed with the Student's t-test.

Image Analysis

We analyzed the reconstructed images obtained during the first METPET acquisition period. Rectangular regions of interest were placed by hand over selected areas. The size of the regions was varied according to the individual tissue configuration and ranged from 4 to 81 pixels (0.3–6.2 cm). Selected regions were: (a) located in the anterior frontal cortex contralateral to the side of the tumor; (b) free of abnormal accumulation of L-MET to visual inspection; (c) normal appearing on computed tomographic (CT) and/or magnetic resonance (MR) images obtained shortly before the PET examination; and (d) outside the limits of radiotherapy ports (except in Case 6, who had received whole brain radiation: frontal cortex regions were used in this case). Brain uptake of L-MET was expressed as percentage dose accumulated per cubic centimeter of cortical region. Blood-to-brain transport of [^{11}C]L-MET was also expressed as the initial unidirectional transfer rate (K_{in}) (n = 10) in units of ml/cc/min, using linear graphic analysis (*17,18*). By multiplying K_{in} by the plasma L-MET level (nmole/ml), we were able to compute the unidirectional influx of L-MET in units of nmole/cc/min.

To quantify the fraction of [^{11}C]L-MET uptake that was affected by competitive inhibition, the results of the two-phase METPET studies were expressed as a percentage inhibition of [^{11}C]L-MET uptake after the administration of L-PHE or after MAI. In patients with blood radioactivity data, the percentage difference in the [^{11}C]L-MET transfer rate observed before and after L-PHE administration was determined.

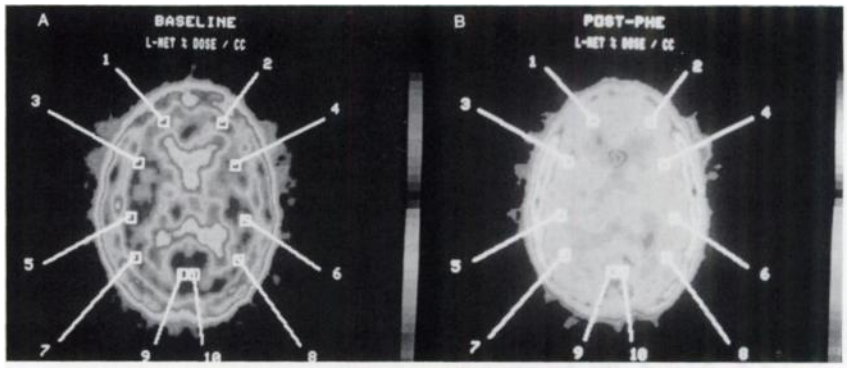
RESULTS

METPET: Baseline Study

The typical normal distribution of [^{11}C]L-MET in cerebral cortex is shown in Figure 1. The uptake of [^{11}C]L-MET by frontal cortex at 1.5 min after tracer injection (Fig. 2), expressed as %dose/cc × 1000, showed a seven-fold, age-dependent decrease for the entire patient population. Uptake of the radiolabeled amino acid was highest in the younger children and adolescents (Group A) (mean ± s.e.: 4.5 ± 0.9; range 1.3–12.1) and showed lower levels in adult patients (Group B) (mean: 1.7 ± 0.3; range 1.1–3.3). The values for uptake in Groups A and B were significantly different ($p < 0.05$). The correlation coefficient for uptake versus age in Group A was −0.71 (n = 10), which was statistically significant ($p < 0.05$). By contrast, the uptake of L-MET in Group B showed no statistically significant variation with age ($r = -0.20$, $p > 0.05$).

Preliminary analysis suggested that if radiation exposure has any influence on [^{11}C]L-MET uptake, the effect is small. In two patients examined before surgery or other treatment, L-MET uptake (%dose/cc × 1000) in normal frontal cortex was 1.4 and 1.3, respectively. Two patients who had received radiotherapy (9000 and 5100 rads) had [^{11}C]L-MET uptakes of 1.3 and 1.9, respectively, in regions distant from the tumor. These

FIGURE 1
Positron emission tomographic scan images following [^{11}C]L-methionine administration in a typical normal study. Each image is an axial section in a plane parallel to and approximately 5 cm above the inferior orbitomeatal line. The front of the head is to the top of the image and the right of the head to the right of the image. The color bar is the scale used to represent the uptake of [^{11}C]L-methionine (percentage dose accumulated per cc of brain) with highest values at the top and lowest values at the bottom of the scale. Placement of regions of interest is shown over the frontal (1,2), anterior temporal (3,4), posterior temporal (5,6), parietal (7,8), and occipital (9,10) cortex. The baseline (A) and post-phenylalanine (B) studies are shown. The gray scale is identical for both studies.



values did not differ significantly from those for normal cortex in non-irradiated patients.

For those patients with blood radioactivity curves, rates of [^{11}C]L-MET uptake into normal frontal cortex were analyzed. The method of Gjedde (*17*) and Patlak (*18*) was used to estimate the initial unidirectional blood-to-brain transfer rate of L-MET. Analysis was confined to measurements obtained between 0.5 and 3 min after tracer injection. [We have shown that L-MET uptake in normal human brain is linear for at least 6 min after tracer injection (*15*).] Age-associated changes in the blood-to-brain transfer rates for L-MET (K_{in}) (n = 10; Fig. 3) showed similar trends to the findings for initial uptake. The rate of uptake of L-MET into normal frontal cortex was 0.077 ml/cc/min for the youngest patient (4.5 yr) in whom it was possible to obtain the time course of blood radioactivity. This estimate exceeded by 2–4-fold the values obtained for all patients older than 10 yr of age. The mean value of K_{in} for this group was more than three standard deviations less than the estimate for the 4.5-yr-old patient. As noted for brain uptake of [^{11}C]L-MET, there was no correlation between the K_{in} values and age within the Group B patients.

For the baseline [^{11}C]METPET study, plasma neutral amino acid (NAA) levels were within normal limits (n = 6) (Table 2).

Competitive Inhibition Studies

The typical distribution of [^{11}C]L-MET in normal cerebral cortex after competitive inhibition of the transport system is shown in Figure 1.

Post-L-PHE Study. In the 12 patients receiving a

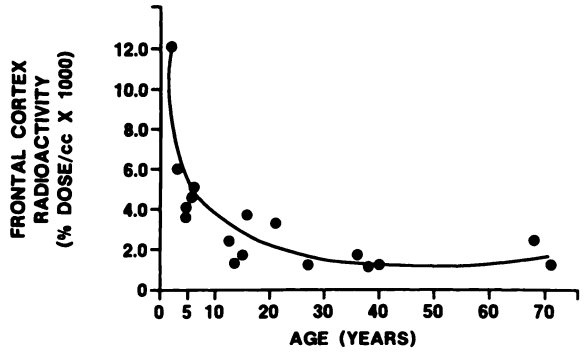


FIGURE 2
The uptake of [^{11}C]L-methionine by normal human frontal cortex (ordinate) plotted as a function of age (abscissa). Tissue uptake of radiotracer is expressed as percent dose administered per cc tissue volume × 1000. These values were assessed on the region of interest placed on the reconstructed METPET image of normal frontal cortex obtained from the first acquisition interval (mid-point 1.5 min after tracer injection). Age is given as years.

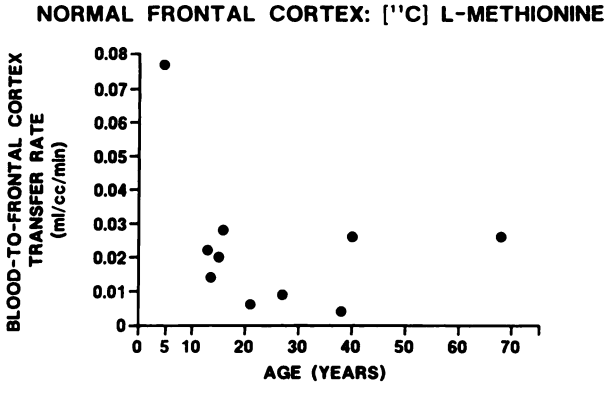


FIGURE 3
The transfer rate (K_{in}) for entry of [^{11}C]L-methionine into human frontal cortex (ordinate) plotted as a function of age (abscissa). The influx is expressed in units of radiolabel cleared from blood per cc of brain volume per min. This estimate is obtained by recalculating the blood and tissue time-radioactivity curves according to the graphic analysis of Gjedde (1982) and of Patlak et al. (1983). Age is given as years.

two-phase METPET study, L-PHE administration was followed by a reduction in the blood-to-brain transport of [^{11}C]L-MET (Fig. 4) ($p < 0.001$). The magnitude of this reduction was variable, ranging from 9% to 77%, with a mean value of 41.0% ± 20.3%. There was no significant correlation between the age of the patients and the percentage decrease in L-MET uptake seen after L-PHE administration; however, values in young patients tended to be less than in patients over the age of 15 yr. In the three post-L-PHE studies (ages 12.9, 15.8, and 38 yr) where blood radioactivity was available, K_{in} decreased by 97.2%, 35.7%, and 50%, respectively.

In the patients receiving oral L-PHE, the plasma level of this amino acid was elevated to a variable extent, ranging from 3.8 to 43-fold (Table 2).

Post-MAI Study. The two patients receiving MAIs also showed reduced [^{11}C]L-MET uptake (45% and 63%, respectively). Plasma L-PHE was raised to greater than five-fold normal values (Table 2), as were plasma levels of several other amino acids including threonine, valine, methionine, isoleucine, leucine, phenylalanine, and tyrosine. Regression analysis of percentage dose uptake versus the plasma level of amino acid competitors for both Group A and Group B patients showed a significant correlation ($r = 0.849$, $p < 0.01$) between [^{11}C]L-MET uptake and the plasma level of amino acid competitors (Fig. 5).

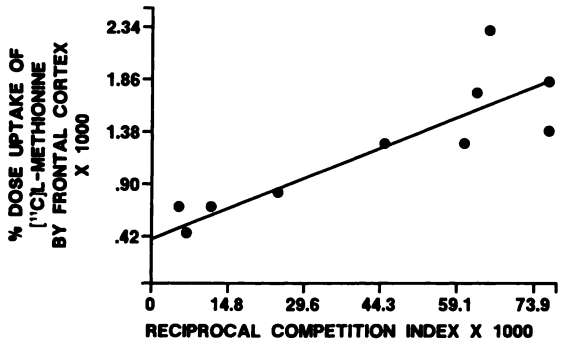


FIGURE 5
Regression analysis of percentage uptake of [^{11}C]L-methionine by normal human frontal cortex (ordinate) versus the plasma level of amino acid competitors, expressed as the reciprocal competition index (abscissa). The competition index is a measure of the effectiveness with which the neutral amino acids will compete at the blood-brain capillary transporter. Uptake of the tracer dose of amino acid (percent dose uptake or K_{in}) is inversely related to:

$(1 + \Sigma\ C_{i/Ki})$ or directly related to $(1 + \Sigma\ C_{i/Ki})$,

where C_i = plasma concentration of each competing amino acid and K^i = Km of each competing amino acid [available for rats from Smith et al. (*12*)].

DISCUSSION

L-MET Transport by Human Brain: Developmental Changes

Our results demonstrate a striking age-dependent decline in brain L-MET uptake in maturing humans, comparable to that previously reported in experimental animals. Furthermore, amino acid uptake in the infant brain, like the adult, appears remarkably sensitive to competition effects.

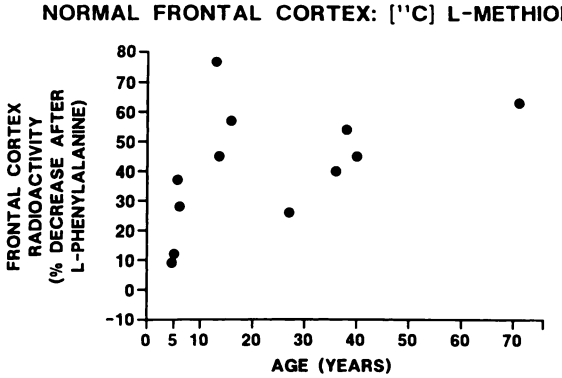


FIGURE 4
Competitive inhibition of the brain transport system for L-MET (ordinate), quantified at different ages (abscissa). The ordinate shows the percentage decrease in L-MET uptake in the volume of frontal cortex defined on the region of interest placed on the reconstructed PET image. This decrease is computed as the difference between the percent dose uptake of the radiotracer in the region of interest observed in the baseline PET study and the percent uptake observed in the same region analyzed in the second PET scan, obtained after pretreatment with non-radioactive L-PHE (100 mg/kg). Age is given as years.

The most likely explanation for the observed reduction in [^{11}C]L-MET uptake with age is a developmental decline in the activity of the blood-brain barrier neutral amino acid transporter. Both the direction and magnitude of the change in L-MET uptake in human brain are similar to that reported for neutral amino acids in experimental animals. Banos et al. (*5*) described a >7-fold reduction in brain L-leucine influx during the first 4 wk of postnatal life in the rat. Sershen and Lajtha (*19*) noted a 2–9-fold reduction in the brain uptake indices of phenylalanine, leucine, methionine, valine, and serine between newborn and adult rats. The developmental changes in brain amino acid uptake are thought to reflect primarily a reduction in the capacity of the blood-brain barrier neutral amino acid carrier and not a change in transport affinity. The rat and rabbit show a 2–3-fold developmental decline in the V_{max} of blood-brain barrier transport of leucine and the non-metabolizable analog, cycloleucine, with no change in transport affinity for either compound (*6,20*). The greater transport capacity for amino acids in maturing animals is thought necessary to provide sufficient substrates to the brain to maintain higher rates of cerebral protein synthesis and amino acid metabolism. In rats, both brain protein synthesis and amino acid influx are high at birth and then decline during the first 4 wk of postnatal life (*2*). A similar developmental decline in brain protein synthesis may occur in humans as well.

Explanations for our findings other than a develop-

mental decline in the transport activity of the blood-brain barrier cannot be totally excluded. For example, an age-dependent decline in brain [^{11}C]L-MET would be expected if plasma amino acid concentrations increased markedly with age (>3.5-fold) from infancy to adulthood or if there was a large developmental increase in the rate of tracer clearance from the circulation. The first would reduce transport by increasing competitive inhibition at the blood-brain barrier, whereas the second would reduce uptake by diminishing the brain exposure to the radioisotope. In an extensive survey, little difference (<15%) was found in plasma-neutral amino acid concentrations between infants, children and adults (*21*). Similarly, for both humans (*22,23*) and animals (Pratt O, *personal communication*, 1990), plasma amino acid clearance has been found to remain fairly stable with age. Consistent with this second point, the plasma time course of [^{11}C]L-MET activity in the one child where blood samples were collected matched well with that observed in adult patients (ages 20–40 yr). Developmental changes in cerebral blood flow would not be expected to influence the pattern of results appreciably as brain [^{11}C]L-MET extraction is quite low (<5%) and, thus, transport is essentially independent of flow (*24*).

Brain protein synthesis has been found to remain quite stable from maturity to old age in rats (*25*). Our study found no evidence for reductions in brain amino acid uptake from maturity to old age in humans. A similar pattern has been reported in rats (*26*).

L-MET Transport by Human Brain: Competition Effects

Competitive inhibition of [^{11}C]L-MET uptake occurred in both developing and adult human brain. The magnitude of inhibition varied from 10% to 80%. Evidence in experimental animals also indicates early development of sensitivity to competitive effects (*27*) and suggests that this sensitivity arises directly from the high affinity (low Km) of the blood-brain barrier neutral amino acid transport carrier. Because of the high affinity, the transport carrier is saturated with amino acids as a group at normal plasma concentrations, and individual amino acids must compete for available transport sites (*12*). The sensitivity of brain amino acid transport to competition effects is unique among body tissues and is thought to have an important role in the selective vulnerability of the brain to amino acid imbalances, such as occurs in phenylketonuria (*28*) and other amino acidurias, and as has also been proposed for diabetes (*29*) and hepatic encephalopathy (*30*).

The variability in the extent of competitive inhibition observed in the current study may be attributable in part to differences in oral L-PHE absorption. Plasma L-PHE concentrations varied among patients after oral administration by ten-fold. In those patients with measured plasma amino acid concentrations, there was a significant negative correlation between the observed percent dose uptake of [^{11}C]L-MET and the summed, weighted concentrations of amino acid competitors (Fig. 5). Such a relation would be expected based on competition kinetics.

Methodologic Issues

To ensure uptake measurements reflect only blood-brain barrier transfer of [^{11}C]L-MET and not incorporation into brain proteins or other aspects of cerebral metabolism, the analysis of uptake was limited to the first 2 min. Previous studies in normal adult humans have established that over such a short interval, L-MET uptake into brain is unidirectional and reflects blood-brain barrier transport (*15*). Meyer et al. (*31*) found that up to 2.5 min after injection, over 95% of tracer in biopsied human brain tissue remained in the form of free [^{11}C]L-MET. Restriction of uptake analysis also helps limit potential artifacts due to peripheral [^{11}C]L-MET metabolism. Hatazawa et al. (*32*) reported that at 5 min after i.v. injection in humans metabolites represented less than 2% of plasma [^{11}C]L-MET activity.

In our study, frontal cortex regions were judged as normal based on CT and/or MR imaging. It is possible that these areas might show biochemical changes similar to those reported for glucose metabolism in sites remote from tumors (*33*). However, the values measured for [^{11}C]L-MET uptake agree within a half order of magnitude with values obtained from our laboratory with a dual-detector probe in normal volunteers (*15*). Considering that the dual-detector probe receives signals not only from the brain but from extracerebral tissues, the agreement is quite good. Furthermore, the mean value for K_{in} in our adult subjects (0.014 ml/cc/min) differs by less than two-fold from estimates obtained in adult rats (*12*).

CONCLUSIONS AND FUNCTIONAL SIGNIFICANCE

During development, substantial changes occur in brain free amino acid concentrations and in rates of brain protein synthesis (*2*). The present study demonstrates two important findings:

1. The rate of amino acid transport into the brain declines with age during the first 20 yr of life in humans.
2. Sensitivity of human blood-brain barrier amino acid transport to competition effects is established at a very early age.

The decline in transport activity during development is consistent with the reported decrease in brain protein synthesis activity over the same period and suggests that barrier amino acid transport is modulated to meet the

needs of brain metabolism. The sensitivity of infant brain amino acid transport to competition effects may enhance the vulnerability of the developing human central nervous system to large amino acid imbalances, such as that occuring in metabolic encephalopathies.

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