Aging Effect on Neutral Amino Acid Transport at the Blood-Brain Barrier Measured with L-[2-^{18}F]-Fluorophenylalanine and PET

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Neutral amino acids (NAAs) are transported from the blood to the brain using the same carrier system in a competitive fashion. The purpose of this study is to establish a method for evaluating neutral amino acid transport at the blood-brain barrier (BBB) in humans and to examine the aging effect of amino acid transport. **Methods:** A dynamic PET study with L-(2-^{18}F)-fluorophenylalanine (^{18}F-Phe) was performed in 14 normal volunteers (age 21–71 yr; mean ± s.d., age range 48.0 ± 17.1 yr). By using a two-compartment model analysis and a weighted integration technique, the influx rate constant K_1, the efflux rate constant k_2 and distribution volume V_d of ^{18}F-Phe were estimated in various brain structures. **Results:** The value of K_1 was inversely correlated with plasma NAA concentration ($r = -0.69$, $p < 0.01$). The cerebellum showed the highest value of K_1, while the white matter showed the lowest. There was no significant change in K_1 during aging. The value of k_2 was significantly increased with age. **Conclusion:** No decline of K_1 during aging indicated that NAA transport from the blood to the brain is a limiting process of age in amino acid incorporation. Fluorine-18-Phe PET imaging is a feasible method to study NAA transport at the BBB in vivo in humans and can be applied to pathological conditions of the brain.

Key Words: fluorine-18-fluorophenylalanine; positron emission tomography; brain; amino acid transport; aging effect

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Neutral amino acids are transported from the blood to the brain using the same carrier system in a competitive fashion (*1,2*). There have been some reports describing the changes in amino acid transport at the blood-brain barrier (BBB) in humans during aging. O'Tuama et al. (*3,4*) used [^{11}C]-L-methionine and reported that amino acid transport decreased with advancing age. Koeppe et al. (*5*) used a synthetic amino acid of [^{11}C]-aminocyclohexanecarboxylate (ACHC) as a marker and demonstrated no significant decline in neutral amino acid transport with age.

The aim of this study was to establish a method to evaluate neutral amino acid transport at the BBB in humans using L-(2-^{18}F)-fluorophenylalanine (^{18}F-Phe) and PET. We previously reported (*6–8*) that this ligand is transported by the same carrier system as phenylalanine and other neutral amino acids in rat brain. The HPLC analysis demonstrated that only a negligibly small amount of the metabolized ligand was found in the blood. First, we examined a two-compartment model to see if it is suitable to describe the ^{18}F-Phe kinetics in human brain. Second, the effect of age on amino acid transport at the BBB was examined in normal volunteers.

MATERIAL AND METHODS

Subjects

A PET study was performed on 14 normal volunteers, including 13 men and 1 woman (aged 48.0 ± 17.1 yr; mean ± s.d., age range 21–71 yr) (Table 1). All subjects had brain by MRI (T1- and T2-weighted images) and blood laboratory tests. They had no neurological deficits. The subjects did not have to fast before the study. Informed consent was obtained from all subjects. The project was approved by the PET Research Program Committee of the Institution.

Fluorine-18-Phe PET Study

Fluorine-18-Phe PET imaging was performed on all subjects (Table 1). Following a 1-min intravenous infusion of 222 MBq ^{18}F-Phe, a dynamic PET scan was obtained. The scan schedule was as follows: ten 1-min frames, five 2-min frames and five 4-min frames. Thirty samples of arterial blood were taken periodically. Total radioactivity of plasma for each sample was estimated as an input function. Total radioactivity of whole blood for each sample was measured for correction of cerebral blood volume (CBV) in six subjects (Table 1). The PET scanner (*9*) provided seven axial images. Inplane resolution was 8 mm FWHM, and axial resolution was 10 mm FWHM. Image slices were set parallel to the anterior commissure-posterior commissure (AC-PC) line determined on MRI using the MRI midsagittal section and lateral skull radiograph taken with the headholder in a PET scanner system

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TABLE 1
Subject Profiles

Subject no.	Age/Sex	$C^{15}O$ study	C' (nmole/ml)*
1	61/M	+	227.4
2	34/M	+	214.8
3	70/M	+	203.3
4	60/M	+	202.5
5	61/M	+	208.1
6	26/M	+	184.2
7	39/M	–	180.9
8	21/M	–	197.4
9	23/M	–	268.7
10	42/M	–	253.8
11	57/M	–	287.9
12	71/M	–	238.4
13	52/F	–	183.8
14	55/M	–	266.0

*Weighted sum of plasma concentration of NAAs (normal range of fasting state: 180.7–247.2 nmole/ml).

(*10*). The pulse sequence for the MRI camera was: spin-echo technique, TR = 500 msec, TE = 25 msec (T1-weighted image). Attenuation correction was performed using the data from the transmission scan which was obtained just before the PET study using a ^{68}Ge line source. The specific activity of ^{18}F-Phe was around 7.4 GBq/mmole. The average amount of the administered ligand was 11.4 mg/370 MBq. Radiochemical purity determined by HPLC was more than 99%.

Carbon PET Study

Just before ^{18}F-Phe PET imaging, a $C^{15}O$ PET study (*11,12*) was performed on six subjects (Table 1, Subjects 1–6) for correction of intravascular radioactivity by estimating CBV. After 1 min of continuous inhalation of $C^{15}O$ gas, a 2-min static PET scan and two arterial blood samplings were obtained. To register PET images between the ^{18}F-Phe and $C^{15}O$ studies, a head fixation system composed of forming materials was used.

Data Analysis

Fluorine-18-Phe is transported in almost the same rate constant as natural phenylalanine from the blood to the brain (*8,13*) and is metabolized slowly in the brain (*7,8,14*) but is not metabolized in the blood (*8*). Therefore, a two-compartment model was used to analyze ^{18}F-Phe kinetics in the brain. Based on this model, the following equation is expressed:

$$\frac{dC_b(t)}{dt} = K_1 \cdot C_a(t) - k_2 \cdot C_b(t), \qquad \text{Eq. 1}$$

where $C_b(t)$ is the concentration of radioactivity in the brain; $C_a(t)$ is the arterial input function; K_1 is the influx rate constant (ml/g/min); k_2 is the efflux rate constant (1/min); and V_d is the distribution volume ($=K_1/k_2$) (ml/g).

Solving Equation 1 provides:

$$C_b(t) = K_1 \cdot C_a(t) \otimes e^{-k_2 \cdot t}, \qquad \text{Eq. 2}$$

where $\otimes$ denotes the convolution integral.

For calculating K_1 and k_2 in Equation 2, the weighted integration technique was applied (*15–20*), and images of K_1, k_2 and V_d values were calculated. In this study, the weighted integration technique with the table look-up procedure reported by Alpert et al. was used (*17*).

A three-compartment model, which includes an additional rate constant k_3 for ^{18}F-Phe metabolism in the brain, was used. By a nonlinear least-squares fitting technique, rate constants K_1, k_2 and k_3 were determined and compared with the results of the two-compartment model analysis in six subjects (Table 1, Subjects 1–6).

To correct ^{18}F-Phe radioactivity for intravascular ^{18}F-Phe, the CBV image was used (*21,22*). In six subjects (Table 1, Subjects 1–6), K_1, k_2 and V_d values were estimated with and without correction.

Data analysis was performed on a UNIX work station (TITAN-750, Kubota Computer Corp., Yamanashi, Japan). On ^{18}F-Phe PET images, regions of interest (ROIs) were placed in the brainstem, cerebellum, thalamus, basal ganglia, frontal cortex, temporal cortex, parietal cortex, occipital cortex, centrum semiovale and whole brain, corresponding to MRI images which gave the same slices as PET images. ROIs other than whole brain were ellipzed with a 16-mm, short-axis and 32- or 64-mm long-axis.

Simulation Study for Effect of CBV

To estimate errors caused by noncorrected CBV, a simulation study was performed. First, the brain radioactivity curve including the radioactivity in capillary vessels, $C_b(t)$, was generated for a K_1 range of 0.0200–0.0600 ml/g/min according to Equation 2 in which the V_d value was 0.590 ml/g and the CBV values were 4%, 5% or 6%. A typical arterial input function was used for $C_a(t)$. Second, for each calculated $C_b(t)$, K_1 and V_d values were estimated by two-compartment model analysis.

Measurement of Neutral Amino Acid Concentration in Plasma

In all subjects, natural neutral amino acid (NAA) concentration in plasma was measured by a JLC-300V amino acid autoanalyzer system (Nihon Densi Corp., Tokyo, Japan). These amino acids are phenylalanine, tryptophan, leucine, tyrosine, histidine, methionine, isoleucine, valine and threonine which are transported through the same carrier-termed L-system (*1*). The affinity of the carrier system for these the nine NAAs is different. When the affinity ratio of phenylalanine to the nine NAAs is defined as $K_{m(i)}/K_m$, where K_m is the half-saturation constant for phenylalanine and $K_{m(i)}$ is the half-saturation constant for each NAA, the concentration C_i of each NAA divided by $K_{m(i)}/K_m$ is the concentration corresponding to phenylalanine for the NAA carrier system. The sum of $C_i/(K_{m(i)}/K_m)$ for the nine NAAs, a weighted sum of the NAAs (C'), is the phenylalanine corresponding concentration of the nine NAAs for the carrier system (*23*) (Table 1). The $K_{m(i)}$ on rat brain was used as follows: phenylalanine 0.011; tryptophan 0.015; leucine 0.029; tyrosine 0.064; histidine 0.100; methionine 0.040; isoleucine 0.056; valine 0.21; and threonine 0.22 (μmole/ml) (*24*).

RESULTS

Two-compartment Model Analysis Validation

K_1 values obtained by two-compartment model analysis using the nonlinear least squares fitting technique with no CBV correction were consistent with those obtained by three-compartment model analysis with no CBV correction ($Y = 1.01X + 0.005$, $r = 0.89$, $p < 0.001$) (Fig. 1).

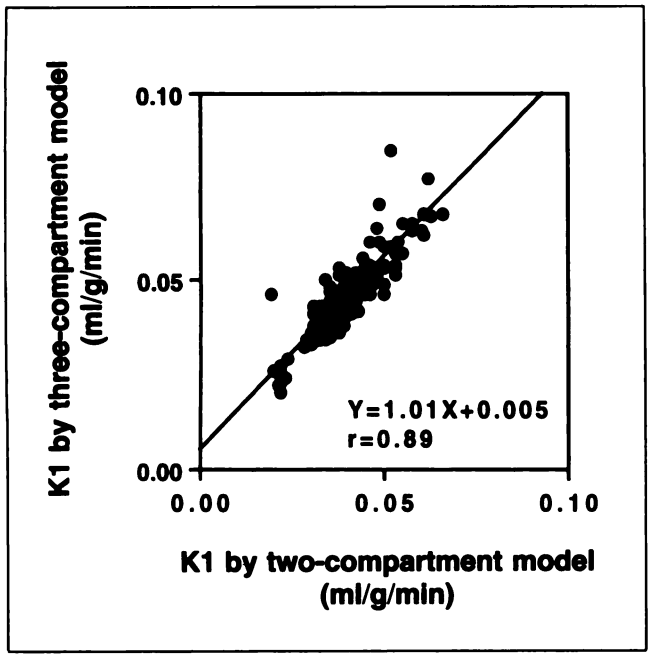

FIGURE 1. Correlation between K_1 values obtained by two-compartment model analysis and three-compartment model analysis using a nonlinear least squares fitting technique with no CBV correction.

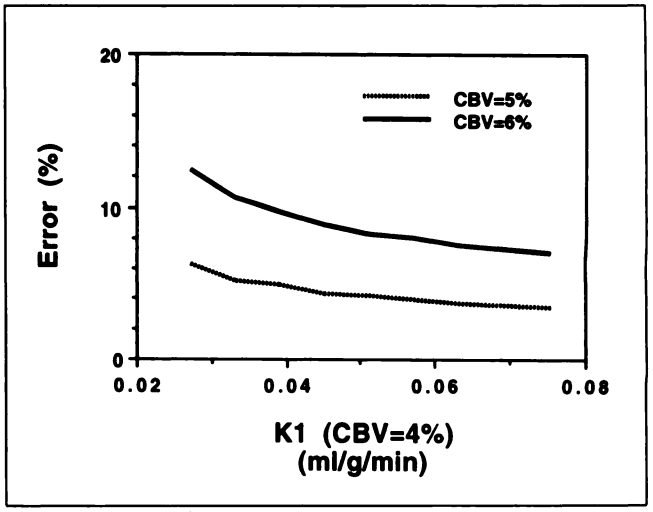

FIGURE 3. The simulation of error for K_1 caused by CBV non-correction.

K_1 Value Correction and Correlation

Figure 2A shows the correlation between K_1 values with CBV correction and those with no CBV correction by a weighted integration technique based on the two-compartment model. The mean K_1 value with no CBV correction was 20.0% higher than that with CBV correction. Good correlation and linearity were observed between K_1 values with CBV correction and those with no CBV correction ($Y = 0.914X + 0.011$, $r = 0.92$, $p < 0.001$). V_d values with no CBV correction were consistent with those with CBV correction ($Y = 0.970X + 0.019$, $r = 0.97$, $p < 0.001$) (Fig. 2B). In addition, it was confirmed that K_1 and V_d values obtained by the weighted integration technique were consistent with those obtained by the nonlinear least squares fitting technique based on two-compartment model, respectively.

The simulation study (Fig. 3) indicates that errors for K_1 values for 6% of CBV compared to 4% of CBV were less than a 10.0% overestimation when the K_1 value for a 4% CBV was larger than 0.0370 ml/g/min. On the other hand, errors for V_d values were always less than 2.0%.

K_1 values of the cerebral cortex region, including frontal, temporal, parietal and occipital cortex obtained by the weighted integration technique based on a two-compartment model were inversely correlated with the weighted sum of plasma concentration of nine NAAs ($Y = -0.000122X + 0.069$, $r = -0.69$, $p < 0.01$) (Fig. 4).

Regionality of the Rate Constant of Fluorine-18-Phe in the Brain

Table 2 shows the ratio of K_1, k_2 and V_d values for each region to those for the whole brain (K_1 value for whole brain: 0.042 ± 0.006 ml/g/min; k_2: 0.080 ± 0.008 1/min; V_d: 0.542 ± 0.105 ml/g (mean ± s.d.)). K_1 values of cerebellum, thalamus and occipital cortex were higher than other regional data (occipital cortex versus brainstem: $p < 0.01$, other pairs: $p < 0.005$). K_1 values of centrum semiovale were lower than other regional data ($p < 0.005$). V_d values of the brainstem were lower than other regional data ($p <$

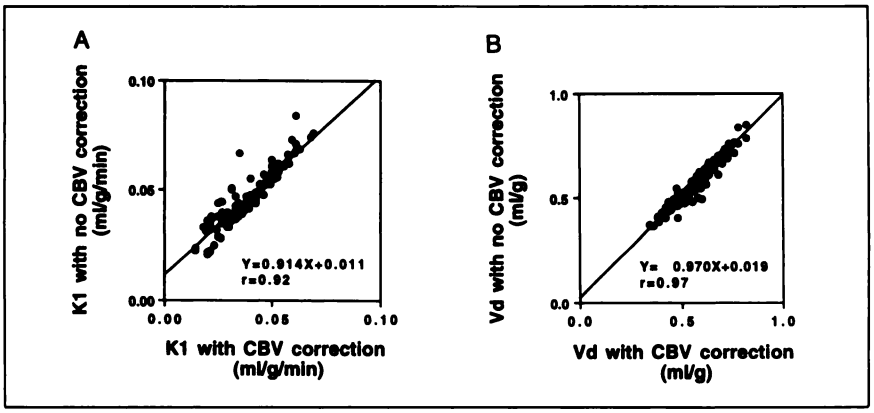

FIGURE 2. (A) Correlation between K_1 values with CBV correction and those with no CBV correction by a weighted integration technique based on the two-compartment model. Mean K_1 value with no CBV correction was 20.0% higher than that with CBV correction. (B) Correlation between V_d values with CBV correction and those with no CBV correction by weighted integration technique based on the two-compartment model. V_d values with no CBV correction were consistent with those with CBV correction.

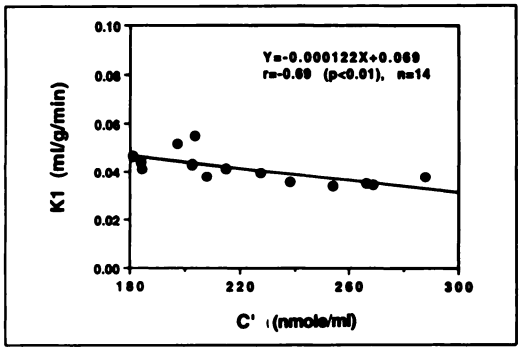

FIGURE 4. Comparison of K_1 values of the cerebral cortex region with C' (weighted sum of plasma concentration of nine NAAs). K_1 values were inversely correlated with C'.

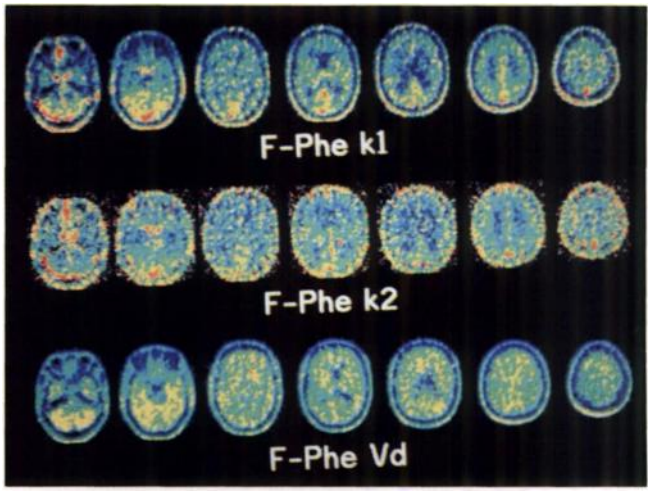

FIGURE 5. K_1, k_2 and V_d images of Subject 3 obtained with the weighted integration technique. K_1 values of the cerebellum, thalamus and occipital cortex were higher than those of other regions. K_1 values of the centrum semiovale were lower than those of other regions.

0.005) and V_d values of centrum semiovale were lower than other regional data excluding the brainstem and occipital cortex ($p < 0.005$). k_2 values of the brainstem, cerebellum and occipital cortex were higher than other regional data ($p < 0.005$), and k_2 values of the thalamus were higher than the basal ganglia, frontal cortex, temporal cortex and centrum semiovale data ($p < 0.005$). k_2 values of the basal ganglia were lower than other regional data excluding centrum semiovale and temporal cortex ($p < 0.005$), and k_2 values of the centrum semiovale were lower than other regional data ($p < 0.005$, paired t-test).

Figure 5 shows K_1, k_2 and V_d images of a normal volunteer (Subject 3 in Table 1).

Aging Effect of Fluorine-18-Phe Rate Constant on the Brain

Figure 6 shows a comparison of K_1, k_2 and V_d values of the cerebral cortex region, including the frontal, temporal, parietal and occipital cortex, obtained by a weighted integration technique based on a two-compartment model with age, for 10 subjects whose weighted sum of NAA plasma concentrations was within the normal range of a fasting state (Table 1). No significant correlation was observed between K_1 values and age (mean K_1 ± s.d.: 0.0436 ± 0.0059 ml/g/min) (Fig. 6A). A significant positive correlation, however, was observed between k_2 values and age ($Y = 0.000198X + 0.067$, $r = 0.55$, $p < 0.05$) (mean k_2 ± s.d.: 0.0764 ± 0.0065 1/min) (Fig. 6B). A significant negative correlation was observed between V_d values and age ($Y = -0.0030X + 0.74$, $r = -0.59$, $p < 0.05$) (mean V_d ± s.d.: 0.590 ± 0.093 ml/g) (Fig. 6C).

TABLE 2
Regional K_1, k_2 and V_d Values of Fluorine-18-Phe for Whole Brain Ratios

Region	K_1 (mean ± s.d.)	k_2 (mean ± s.d.)	V_d (mean ± s.d.)
Brainstem	1.007 ± 0.098	1.297 ± 0.136	0.787 ± 0.050
Cerebellum	1.263 ± 0.089	1.119 ± 0.102	1.113 ± 0.081
Thalamus	1.182 ± 0.086	1.057 ± 0.074	1.100 ± 0.053
Basal ganglia	0.976 ± 0.104	0.877 ± 0.070	1.110 ± 0.080
Frontal cortex	0.960 ± 0.046	0.963 ± 0.050	0.996 ± 0.053
Temporal cortex	0.950 ± 0.039	0.894 ± 0.030	1.061 ± 0.027
Parietal cortex	1.000 ± 0.062	1.022 ± 0.034	0.969 ± 0.044
Occipital cortex	1.139 ± 0.101	1.177 ± 0.078	0.960 ± 0.075
Centrum semiovale	0.578 ± 0.070	0.688 ± 0.063	0.887 ± 0.074

K_1, k_2 and V_d values of ^{18}F-Phe for whole brain are 0.042 ± 0.006 ml/g/min, 0.080 ± 0.008 1/min and 0.542 ± 0.105 ml/g (mean ± s.d.), respectively.

DISCUSSION

Amino acid transport across the BBB has been studied in humans by using PET and natural amino acid labeled with ^{11}C (*3,4*). The metabolic products of labeled natural amino acids in plasma and brain, however, reduced the accuracy of determined kinetic rate constants (*25*). Koeppe et al. used [^{11}C]ACHC to measure the transport rate of NAAs at the BBB specifically (*5*). They used a two-compartment model because ACHC is a synthesized amino acid and is not metabolized in the brain. No metabolic products appeared in the blood. These features simplified the application of the kinetic model (*26*). For ^{18}F-Phe, in rat brain, Murakami et al. demonstrated that fractions other than ^{18}F-Phe were negligible (*7*).

We previously reported that k_3 in humans in comparison to corresponding to ^{18}F-Phe incorporation to amino acid is negligible (*27*). In this study, the three-compartment model analysis also yielded small k_3 values. A two-compartment model in which k_3 was assumed to be 0 yielded the K_1 values obtained by the three-compartment model (Fig. 1). These results indicate that the amount of ^{18}F-Phe irreversibly trapped in the brain is not significant during a 40-min scan procedure. The two-compartment model could be used in the ^{18}F-Phe PET study.

When first-pass extraction fraction of a tracer is less than 100%, the tracer uptake rate constant is overestimated due

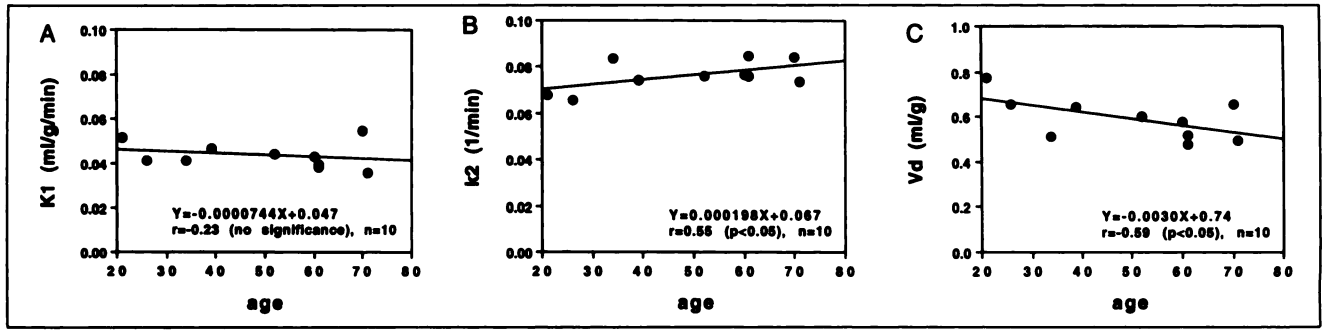

FIGURE 6. Comparison of K_1, k_2 and V_d values of the cerebral cortex region with age in 10 subjects within fasting normal range of plasma concentration of NAAs. (A) No significant correlation was observed between K_1 values and age. (B) A significant positive correlation was observed between k_2 values and age. (C) A significant negative correlation was observed between V_d values and age.

to radioactivity in the intravascular component (*21,22*). In this study, the mean K_1 value of ^{18}F-Phe with no CBV correction was 20.0% higher than that with CBV correction. Good correlation and linearity were observed between K_1 values with CBV correction and those with no CBV correction (Fig. 2A). Leenders et al. reported on CBV reduction with aging (Y = 7.3 − 0.046X; X: age, Y: CBV) (*28*), however, the simulation study indicates that the differences in K_1 values between 4% and 6% of CBV were less than 10.0% (Fig. 3). V_d values of ^{18}F-Phe with no CBV correction were consistent to those with CBV correction (Fig. 2B), and the simulation study indicates that the differences in V_d values between 4% and 6% of CBV were always less than 2.0%. Therefore, we analyzed ^{18}F-Phe rate constant values without CBV correction in this study.

There were regional differences in K_1, k_2 and V_d of ^{18}F-Phe within the brain's structure (Table 2, Fig. 5). Similar regionality was also found in the ACHC study (*5*). In both studies, the K_1 was highest in the cerebellum and second highest in the thalamus. The cerebral white matter showed the lowest K_1values. These regional parameters of NAA transport at the BBB is similar to CBF and metabolic regionality (*29*).

As for the effect of age on NAA transport, the four subjects with NAA plasma concentrations above the normal fasting state range were excluded because rate constant values were affected by NAA plasma concentrations (Fig. 4). Mean K_1, k_2 and V_d values in the cerebral cortex region of the other ten subjects were plotted against age. In this analysis, we did not find any age-dependent changes in K_1, that is, an indicator of amino acid transport from the blood to the brain (Fig. 6A). This result was consistent with the ACHC result (*5*). It is noteworthy that transport from the brain to the blood as indicated by k_2 was increased during aging (Fig. 6B). k_2 might be affected by the intracellular NAA brain concentration since K_1 is affected by plasma NAA concentration. Therefore, increasing k_2 during aging would indicate decreased competition between ^{18}F-Phe and natural NAAs due to decreased intracellular NAA concentration in the brain, which would be indicated by decreasing V_d during aging (Fig. 6C). On the other hand, decreased V_d during aging also might be affected by brain atrophy, which would cause a decrease in the fraction of brain tissue mass per given ROI.

Bustany et al. reported that protein synthesis in human brain decreased during aging, which must be related to atrophic brain changes (*30*). On the other hand, the protein synthesis in rat brain was reported to decline during aging (*31*). The transport of neutral amino acids at the BBB, however, may not be the limiting process of decreased amino acid incorporation in the aging brain because no decline of K_1 value was found in normal volunteers.

The present study demonstrated that ^{18}F-Phe PET imaging is feasible to study NAA transport at the BBB in vivo in humans. The method can be applied to analysis of functional change in amino acid transport under various pathological conditions such as ischemic brain damage (*32*) and degenerative diseases.

CONCLUSION

A two-compartment model analysis was valid for measuring amino acid transport at the BBB using ^{18}F-Phe dynamic PET datasets and input functions. There was no indication of age-dependent changes in NAA transport from the blood to the brain in our subjects. Our results indicate that NAA transport from the blood to the brain might not be the limiting process of decreasing amino acid incorporation during aging.

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