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A mathematical approach for assessing the transport of large neutral amino acids across the blood-brain barrier in man

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Changes in the large neutral amino acid (LNAA) transport across the blood-brain barrier (BBB) is thought to contribute to brain dysfunction in a number of clinical conditions, including phenylketonuria, acute liver failure, and sepsis. Here, we present a novel approach for estimating BBB permeability and the LNAA concentrations in brain extracellular fluid, by demonstrating that they can be mathematically derived on the basis of kinetic constants of the BBB available from the literature, if cerebral blood flow and the arterial and jugular venous LNAA concentrations are known. While it is well known that the permeability surface area product of the BBB to a LNAA from blood to brain (PS_1) can be calculated from the arterial LNAA concentrations and kinetic constants of the BBB, we demonstrate that the permeability surface area product from brain to blood (PS_2) can be calculated by deriving the substrate activity of the saturable transporter from the kinetic constants and arterial and jugular venous LNAA concentrations, and that the concentration of the LNAA in brain extracellular fluid can then be determined. This approach is methodically simple, and may be useful for assessing the transcerebral exchange kinetics of LNAAs in future human-experimental and clinical studies.

Key words: branched-chain amino acids, aromatic amino acids, kinetics, permeability, saturable transport parameter zeta

INTRODUCTION

Large neutral amino acids (LNAAs) are critical for maintaining neurotransmitter homeostasis within the brain (Pardridge 1998). The LNAAs in the blood stream enter the brain extracellular compartment through the blood-brain barrier (BBB) and are then further transported into the brain cells. The rate limiting step in the passage of LNAAs from the blood stream to the intracellular compartment is the transport across the BBB, because the total surface area of the cellular membranes within the brain is log orders greater than the total surface area of the BBB (Pardridge 1998).

The LNAA transport system of the BBB encompasses both a saturable and non-saturable component (Knudsen 1994). The saturable component renders LNAA transport across the BBB competitive, so that changes in the plasma concentration of one LNAA will affect the transcerebral exchange kinetics and consequently the intracerebral concentrations of all LNAAs (Shulkin et al. 1995, Smith et al. 1987). Accordingly, changes in the circulating LNAA concentrations, and thus their transport across the BBB, are thought to contribute to brain dysfunction in a wide range of clinical conditions, notably phenylketonuria (Hommes 1989, Knudsen et al. 1995, Novotny et al. 1995, Pietz et al. 1999), acute liver failure (Dejong et al. 2007, Knudsen et al. 1993, Mizock et al. 1990, Strauss et al. 2001), and sepsis (Basler et al. 2002, Berg et al. 2010, Mizock et al. 1990).

A number of human-experimental methods, including the double-indicator technique using either intracarotid and intravenous indicator injections (Knudsen 1994, Knudsen et al. 1994), magnetic resonance spectroscopy (Möller et al. 1997, Novotny et al. 1995, Pietz et al. 1999), and positron emission tomography (Shulkin et al. 1995) have previously been used to investigate BBB permeability to the individual LNAAs, as well as their extracellular concentrations in the brain in various conditions. In the present paper, we provide a novel and methodically simple approach for estimating these parameters in humans, by demonstrating that they can be mathemati-

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cally derived on the basis of kinetic constants of the BBB if the arterial and jugular venous LNAA concentrations and cerebral blood flow (CBF) are known.

Theory and mathematical modelling

Permeability surface area products and apparent K_m

The LNAA transport across the BBB in humans *in vivo* is described by a single-membrane model, in which the BBB is assumed to function as a single membrane, because the intracellular volume in the endothelial cells is considered trivial, and because of high LNAA permeabilities of both the luminal and abluminal endothelial cell membranes (Knudsen et al. 1990, Knudsen 1994, Möller et al. 1997). The transport follows Michaelis-Menten kinetics, and may thus be described by the maximum transport velocity, V_{max}, and the half-saturation constant, K_m (Begley 1998, Gjedde and Christensen 1984). V_{max} is usually quoted in nmol min^{-1} g^{-1}, describing a flux per unit weight of brain tissue, while K_m is quoted in μM. The BBB permeability to a LNAA denoted by X is thus given by:

$$PS_{1,X} = \frac{V_{max,X}}{K_{m,X}^{p,app} + C_{p,X}} + K_{d,X}$$
$$PS_{2,X} = \frac{V_{max,X}}{K_{m,X}^{b,app} + C_{b,X}} + K_{d,X} \quad (1)$$

where PS_1 is the BBB permeability surface area product to X from blood to brain extracellular fluid, while PS_2 is the BBB permeability surface area product to X from brain extracellular fluid to blood. C_p and C_b denote the concentration of X in plasma and in brain extracellular fluid, respectively, while K_m^{app} is the apparent K_m to X and K_d is the non-saturable diffusional constant to X. Both the permeability surface area product and the non-saturable diffusional constant are expressed in ml min^{-1} g^{-1} of brain tissue.

The apparent rather than the absolute K_m-value is used, because the absolute K_m-values are close to the LNAA plasma concentrations *in vivo* (Choi and Pardridge 1986). This causes marked competition between the individual LNAAs for transport across the BBB, so that the apparent K_m becomes higher than the absolute K_m. In Eq. 1, the absolute K_m-value is the half-saturation concentration in the absence of competitors, while the apparent K_m-value is the half saturation concentration under normal physiological conditions where all LNAAs are present in the same fluid (Smith and Stoll 1998). The apparent K_m-values are related to the absolute K_m-values as follows (Mahler and Cordes 1966, Miller et al. 1985):

$$K_{m,X}^{p,app} = K_{m,X} \cdot \left(1 + \sum_{n \in LNAA \setminus \{X\}} \frac{C_{p,n}}{K_{m,n}}\right)$$
$$K_{m,X}^{b,app} = K_{m,X} \cdot \left(1 + \sum_{n \in LNAA \setminus \{X\}} \frac{C_{b,n}}{K_{m,n}}\right) \quad (2)$$

where the summation is done over all LNAAs competing with X. In the sum, the absolute K_m-value equals the so-called inhibition constant as previously described (Pardridge 1977), and it is assumed that the absolute K_m, V_{max}, and K_d are identical for LNAA transport from blood to brain and from brain to blood (Knudsen et al. 1990).

From Eq. 2 alternative expressions of PS_1 and PS_2 can be derived by introducing the substrate activity of the saturable transporter in blood, S_p, and in brain extracellular fluid, S_b (Meier et al. 2002). Let S_p denote the sum of the ratios between the plasma concentration and the absolute K_m-values, and let S_b denote the sum of the ratios between the concentration in brain extracellular fluid and the absolute K_m-values of the individual LNAAs:

$$S_p = \sum_{n \in LNAA} \frac{C_{p,n}}{K_{m,n}}$$
$$S_b = \sum_{n \in LNAA} \frac{C_{b,n}}{K_{m,n}} \quad (3)$$

The substrate activity is a dimensionless parameter describing the capacity of the blood and the brain extracellular fluid to activate the saturable LNAA transporter, which will show half saturation for LNAA influx when $S_p = 1$ and for efflux when $S_b = 1$ (Meier et al. 2002). The formulas $S_p/(S_p+1)$ and $S_b/(S_b+1)$ can be used to calculate the total saturation of the saturable transporter (Smith et al. 1987).

By rewriting Eq. 1, this yields:

$$PS_{1,X} = \frac{V_{max,X}}{K_{m,X}} \cdot \frac{1}{S_p + 1} + K_{d,X}$$
$$PS_{2,X} = \frac{V_{max,X}}{K_{m,X}} \cdot \frac{1}{S_b + 1} + K_{d,X} \quad (4)$$

PS_1 can thus be calculated from V_{max}, the absolute K_m, and K_d, and by calculating S_p from the arterial concentrations and absolute K_m-values of all LNAAs. To calculate PS_2, it is however necessary to know S_b. In the following, we demonstrate that S_b can be determined on the basis of kinetic constants of the BBB, arterial and jugular venous LNAA concentrations, and CBF.

Transcerebral exchange kinetics

After entering the brain extracellular fluid, the LNAAs are either transported back over the BBB or into other cerebral compartments not available for BBB transport, which in the following will be referred to as the brain cells. The time dependent change in the LNAA concentration of the brain extracellular fluid can be calculated by subtracting the flux of LNAA into the brain cells from the difference in BBB fluxes of LNAA (Möller et al. 1997). The fluxes are quoted in nmol min^{-1} g^{-1} of brain tissue:

$$\frac{d}{dt}C_{b,X} \propto \upsilon_{1,X} - \upsilon_{2,X} - \upsilon_{3,X} \qquad (5)$$

Here υ_1 denotes the unidirectional influx from blood to brain extracellular fluid, υ_2 is the unidirectional efflux from brain extracellular fluid to blood, and υ_3 is the flux into the brain cells. It is assumed that the cerebral fluxes are balanced so that steady state concentrations of LNAAs are obtained in the brain extracellular fluid. Hence, the difference between in- and efflux over the BBB, defined as the transcerebral net exchange, equals the flux of LNAA into the brain cells. A positive transcerebral exchange indicates a net influx of LNAA into the brain, while a negative value indicates efflux.

The transcerebral net exchange denoted *J* of the LNAA X can furthermore be calculated according to the Fick principle (Knudsen et al. 1990, 1993):

$$J_X = \upsilon_{1,X} - \upsilon_{2,X} = CBF \cdot V_{c,X} \cdot a\text{-}j\upsilon D_X \qquad (6)$$

where a-jυD is the arterial-to-jugular venous concentration difference of X, and V_c is the distribution volume of LNAA within the flowing blood normalized to the plasma water concentration (Fenstermacher et al. 1981). Since only plasma LNAAs are available for BBB transport, the distribution volume of the LNAAs can be calculated from the erythrocyte volume fraction (Ellison and Pardridge 1990).

The BBB permeability to LNAAs has previously been examined in mouse with *in situ* brain perfusion experiments measuring the cerebral influx of radiolabeled tracers (Begley 1998). The influx of a substance from blood to brain extracellular fluid, defined as the unidirectional transfer constant, K_{in}, and usually measured in the units ml min^{-1} g^{-1} of brain tissue, may be quantified by the clearance of CBF · V_c (Begley 1998, Fenstermacher et al. 1981). This can be done under the assumption that no efflux from brain extracellular fluid is present, so that υ_2=0:

$$K_{in} = \frac{CBF \cdot V_c \cdot a\text{-}j\upsilon D}{C_p} = CBF \cdot V_c \cdot E \qquad (7)$$

Here E is the dimensionless extraction fraction. The unidirectional transfer constant and the permeability surface area product have the same units and are thus two different measures of BBB permeability. K_{in} describes the permeability under physiological conditions where the effect of a low CBF on the extraction fraction can be rate-limiting for BBB transport of LNAAs. PS_1 is an absolute characteristic of the cerebrovascular endothelium and is not affected by CBF.

Here we want to use PS_1 as an approximation of the unidirectional transfer constant K_{in}. For unidirectional transport of a substance over the BBB the Renkin-Crone equation (Crone 1963, Renkin 1959) expresses the relationship between the CBF, PS_1, and E:

$$E = 1 - \exp\left(-\frac{PS_1}{CBF \cdot V_c}\right) \qquad (8)$$

It follows, that CBF · V_c >> PS_1 yields a low extraction fraction, which signifies that CBF is not rate-limiting for the transcerebral exchange, so that K_{in} approaches PS_1 when CBF increases. According to Eq. 7 and Eq. 8, K_{in} approaches PS_1 within 10% of error, when $PS_1/(CBF \cdot V_c)<0.21$ (Fenstermacher et al. 1981).

However under steady state conditions a significant unidirectional efflux of LNAA from brain extracellular fluid to the blood stream is present, and as a consequence the reduction in the capillary LNAA concentration will be less than expected. Hence the Renkin-Crone equation overestimates the magnitude of error between K_{in} and PS_1. In the following we assume that PS_1 can be used as an approximation of K_{in}.

Derivation of S_b

Given that *J*, PS_1, and C_p are known for all LNAAs, the corresponding υ_2-values can be calculated (Knudsen et al. 1990, 1993, Möller et al. 1997) by rewriting Eq. 6:

$$J_X = \upsilon_{1,X} - \upsilon_{2,X} = PS_{1,X} \cdot C_{p,X} - PS_{2,X} \cdot C_{b,X} \quad (9)$$

Now let ρ_1 denote the fraction of unidirectional influx accounted for by saturable transport to the total unidirectional influx of X, and let ρ_2 denote the fraction of unidirectional efflux accounted for by saturable transport to the total unidirectional efflux of X. By using the definition of PS_2 in Eq. 4, ρ_2 can be expressed:

$$\rho_2 = \frac{PS_{2,X} - K_{d,X}}{PS_{2,X}} = \\ = \left(1 + (S_b + 1) \cdot \frac{K_{m,X} \cdot K_{d,X}}{V_{max,X}}\right)^{-1} \quad (10)$$

and similarly for ρ_1 with S_p substituting S_b.

By using ρ_2, K_d can be omitted in the expression of PS_2 in Eq. 4.

$$PS_{2,X} = \frac{1}{\rho_{2,X}} \cdot \frac{V_{max,X}}{K_{m,X}} \cdot \frac{1}{S_b + 1} \quad (11)$$

In order to derive an equation for S_b, the term C_b/K_m can be isolated in the equation for the unidirectional efflux, so that:

$$\frac{C_{b,X}}{K_{m,X}} = \rho_{2,X} \cdot (S_b + 1) \cdot \frac{\upsilon_{2,X}}{V_{max,X}} \quad (12)$$

As previously mentioned, S_b is defined by the sum of C_b/K_m and υ_2 can be determined from Eq. 9 by use of the substrate activity (Eq. 3) and the permeability surface area product (Eq. 4); consequently, summation of Eq. 12 over all LNAAs yields an equation that expresses the relationship between S_b, the kinetic constants of the BBB and υ_2 for all LNAAs. We define ζ as the sum of ratios of $\rho_2 \cdot \upsilon_2/V_{max}$ over all LNAAs:

$$\zeta = \sum_{n \in LNAA} \rho_{2,n} \cdot \frac{\upsilon_{2,n}}{V_{max,n}} = \frac{S_b}{S_b + 1} \quad (13)$$

Thus, ζ equals the total saturation of the transporter. Since ρ_2 is defined in Eq. 10, the only unknown in Eq. 13 is S_b, which can be determined by numerical methods. The total number of complex solutions and solutions with $S_b < -1$ equals the number of LNAAs with non-zero K_d-values. Nevertheless, since the substrate activity is by definition positive (Eq. 3), a physiologically representative value of S_b determined in Eq. 13 should likewise be positive.

The solution can be graphically illustrated if ζ and the total saturation are considered functions of the independent variable S_b. For $S_b \geq 0$ the function $S_b/(S_b+1)$ monotonically increases with an image of [0,1]. The function ζ consists of a sum of terms corresponding to the number of LNAAs. Each term with a non-zero K_d-value is a monotonically decreasing function for $S_b \to \infty$ which converges to 0 as $S_b \to \infty$, while each term with $K_d=0$ is constant. Therefore, ζ monotonically decreases from its positive intersect with the vertical axis at $S_b=0$ to the minimal value obtained when $S_b \to \infty$. A positive solution to Eq. 13 can be found if the sum of constant terms in ζ is below 1, such that an intersect of ζ and $S_b/(S_b+1)$ is found. Thus, a physiologically representative S_b-value can be determined from Eq. 13 by the intersection of ζ and the total saturation.

Together, Eq. 4 and Eq. 13 permit the calculation of PS_2, after which C_b as the only unknown in Eq. 9 can be calculated. Further simplification of Eq. 13 can be achieved under the assumption that the non-saturable component of LNAA transport across the BBB is trivial. Thus, if we assume that $K_d=0$, Eq. 10 is simplified to $\rho_2=1$, and it is consequently possible to obtain an algebraic formula for S_b. We define the saturable transport parameter ζ_s as the sum of ratios of the unidirectional efflux to the maximum transport velocity for the saturable kinetics for all individual LNAAs:

$$\zeta_s = \sum_{n \in LNAA} \frac{\upsilon_{2,n}}{V_{max,n}} = \frac{S_b}{S_b + 1} \Leftrightarrow S_b = \frac{1}{\zeta_s^{-1} - 1} \quad (14)$$

In general the significance of the non-saturable contribution cannot be judged solely from the dimensionless fraction K_dK_m/V_{max}. This estimate may be misleading, since the impact of the competitive effect from a low absolute K_m-value is omitted with the exclusion of S_b and S_p, which in contrast is taken into account by ρ_1 and ρ_2.

In the following section, we demonstrate that the minimal contribution of the non-saturable component, K_d, may be estimated from the cerebral permeability ratio.

Cerebral permeability ratio and estimation of the minimal K_d

A lower limit for K_d, which represents the non-saturable, diffusional component of PS_1 and PS_2 (Eq. 4), as well as an upper limit for the ρ_1 and ρ_2 (Eq. 10) may be estimated by assessing the variation in PS_1- and PS_2-values for all LNAAs. First, the cerebral permeability ratio, R, is defined as the ratio of the permeabilities to a given LNAA in both directions across the BBB (Knudsen et al. 1990). Using Eq. 11, the cerebral permeability ratio of the LNAA X can be expressed as:

$$R_X = \frac{PS_{2,X}}{PS_{1,X}} = \frac{\rho_{1,X}}{\rho_{2,X}} \cdot \frac{S_p + 1}{S_b + 1} \tag{15}$$

When no non-saturable transport is present, this expression can be reduced using $\rho_1 = \rho_2 = 1$ for $K_d = 0$ (Eq. 10). The resulting cerebral permeability ratio depends only on S_p and S_b, so that all amino acids with $K_d = 0$ reach the same value of R designated R_{max} (Knudsen et al. 1990):

$$R_{max} = \frac{S_p + 1}{S_b + 1} \tag{16}$$

Inserting the expression for Eq. 10 into Eq. 15 makes it possible to express R as:

$$R_X = R_{max} \cdot \frac{1 + (S_b + 1) \cdot \frac{K_{d,X} K_{m,X}}{V_{max,X}}}{1 + (Sp + 1) \cdot \frac{K_{d,X} K_{m,X}}{V_{max,X}}} \tag{17}$$

The concentration of LNAAs in the brain extracellular fluid is lower than the concentration of LNAAs in the blood. Hence from the definition of the substrate activity (Eq. 3) we find that $S_p > S_b$, so that $R_{max} > 1$. Since S_p and S_b are constants for a given set of kinetic data, the value of R decreases to 1 as $K_d K_m / V_{max} \to \infty$. Thus given $S_p > S_b$, R will always be in the interval $[1, R_{max}]$ and R_{max} will be the maximum possible permeability ratio.

From the definition of ρ_1 and ρ_2, Eq. 10, we can furthermore derive the following relations:

$$\frac{R_X}{R_{max}} = \frac{\rho_{1,X}}{\rho_{2,X}}$$
$$R_{max} = \frac{\rho_{1,X}^{-1} - 1}{\rho_{2,X}^{-1} - 1} \tag{18}$$

Solving this non-linear system of equations for ρ_1 and ρ_2 yields the expressions:

$$\rho_{1,X} = \frac{R_X - 1}{R_{max} - 1}$$
$$\rho_{2,X} = \frac{(R_X - 1) \cdot R_{max}}{(R_{max} - 1) \cdot R_X} \tag{19}$$

In a set of measured R-values, the obtained maximum R-value, here designated $\max(R_x)$, will always be less than or equal to R_{max}. Thus, $\max(R_x) \leq R_{max}$. ρ_1 and ρ_2 in Eq. 19 are decreasing functions of R_{max}. An upper limit for these may thus be found by insertion of $\max(R_x)$:

$$\rho_{1,X} \leq \frac{R_X - 1}{\max(R_X) - 1}$$
$$\rho_{2,X} \leq \frac{(R_X - 1) \cdot \max(R_X)}{(\max(R_X) - 1) \cdot R_X} \tag{20}$$

From the upper limit of ρ_1 and ρ_2, the non-saturable diffusional constant can be expressed as:

$$K_{d,X} \geq PS_{1,X} \cdot (1 - \rho_{1,X})$$
$$K_{d,X} \geq PS_{2,X} \cdot (1 - \rho_{2,X}) \tag{21}$$

so that a lower limit for K_d can be determined. The most precise limit will be found when $\max(R_x) \approx R_{max}$, but the approach can be used without knowing the

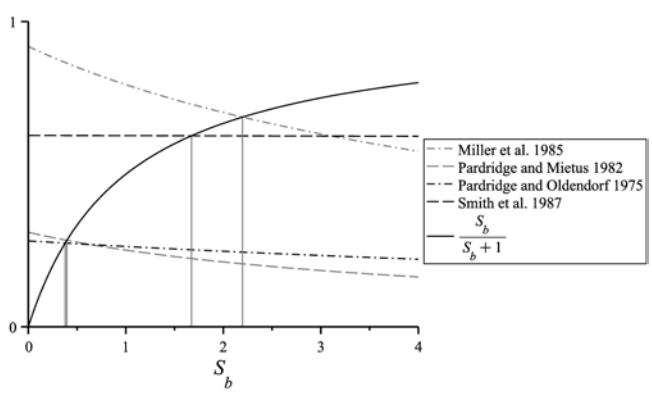

Fig. 1. Graphical determination of the cerebral substrate activity. The cerebral substrate activity (S_b) is determined from the intersect of ζ and the total saturation (Eq. 13). The results are listed in Table III. Data from Hargreaves and Pardridge (1988) are not included in the graph. Horizontal axis: S_b (dimensionless), vertical axis: total saturation and ζ (dimensionless).

Table I

Kinetic constants of the blood-brain barrier

	Hargreaves and Pardridge 1988			Miller et al. 1985			Pardridge and Mietus 1982			Pardridge and Oldendorf 1975			Smith et al. 1987		
	K_m (µM)	V_{max} (nmol min^{-1} g^{-1})	K_d (ml min^{-1} g^{-1})	K_m (µM)	V_{max} (nmol min^{-1} g^{-1})	K_d (ml min^{-1} g^{-1})	K_m (µM)	V_{max} (nmol min^{-1} g^{-1})	K_d (ml min^{-1} g^{-1})	K_m (µM)	V_{max} (nmol min^{-1} g^{-1})	K_d (ml min^{-1} g^{-1})	K_m (µM)	V_{max} (nmol min^{-1} g^{-1})	K_d (ml min^{-1} g^{-1})
Phenylalanine	0.30	6.2	0.66	32	14	0.044	390	62	0.027	110	28	0.014	11	41.4	0.0078
Tryptophan	3.0	15.6	0.57	52	18	0.041	480	55	0.018	160	30	0.002	15	54.6	0.0042
Histidine	5.1	38.9	0.64	164	30	0.033	1340	81	0.043	240	29	0.012	100	61.2	0.003
Methionine	5.1	14.8	0.50	83	18	0.045	1120	90	0.029	180	29	0.019	40	25.2	0.0024
Tyrosine	1.3	11.9	0	86	25	0.039	480	48	0.049	150	31	0.016	64	96.6	0
Valine	8.8	13.3	0.72	168	14	0.037	1470	91	0.017	510	35	0.003	210	49.2	0.0048
Isoleucine	2.7	7.6	0	145	36	0.031	660	57	0.042	280	42	0.005	56	60.0	0
Leucine	3.3	13.4	0.38	87	23	0.045	380	42	0.031	100	22	0.018	29	59.4	0.0036

The kinetic constants have been determined in awake and anaesthetised rats (Miller et al. 1985, Pardridge and Oldendorf 1975, Smith et al. 1987), in anaesthetised newborn rabbits (Pardridge and Mietus 1982), and in human cerebral capillaries obtained post-mortem (Hargreaves and Pardridge 1988).

relative contribution of the saturable and non-saturable components to LNAA transport across the BBB. Furthermore, since $R_x \leq R_{max}$ it follows from the upper part of Eq. 18 that $\rho_1 \leq \rho_2$. Hence, competition for the saturable transport of LNAA from blood to brain is more apparent than from the brain to blood.

Model evaluation of kinetic constants

The mathematical model presented above relies on the kinetic constants K_m, V_{max}, and K_d of the different LNAAs. These are assumed to be identical for the passage of LNAAs from blood to brain and *vice versa*, and have previously been determined in different animals *in vivo* and in human cerebral capillaries obtained post-mortem (Table I). If a human subject, for the sake of example, is assumed to have the arterial and jugular venous concentrations and transcerebral net exchange values for LNAAs presented in Table II, estimates of the associated C_b values can be obtained by the numerical determination of S_b (Eq. 13) illustrated as the positive intersect of ζ and the total saturation (Fig. 1).

For all calculations of BBB parameters, PS_1 is used as an estimate of K_{in}, thus ignoring the decrease in intravascular LNAA concentration across the cerebral capillaries. We assessed the impact of $PS_1/(CBF \cdot V_c)$ on the unidirectional transfer constant K_{in} by solving the Renkin-Crone model (Crone 1963, Renkin 1959), taking into account the efflux of LNAAs due to constant concentrations of amino acids in the brain extracellular fluid. The magnitude of error is given by:

$$\frac{PS_{1,X} - K_{in,X}}{PS_{1,X}} = E_X \cdot \left(\frac{1}{1 - \exp\left(-\frac{PS_{1,X}}{CBF \cdot V_{c,X}}\right)} - \left(\frac{PS_{1,X}}{CBF \cdot V_{c,X}}\right)^{-1} \right) \quad (22)$$

Here K_{in} denotes the clearance of $CBF \cdot V_c$ and the clearance of LNAA due to the unidirectional efflux, which can be expressed $K_{in} \cdot C_p = J + \upsilon_2$. The magnitude of error is proportional to the extraction fraction (Eq. 22), which is $|E| < 0.1$ for all LNAAs (Berg et al. 2010, Strauss et al. 2001). For all LNAAs (Table II) evaluated using different kinetic constants (Table I), we found $PS_1/(CBF \cdot V_c) < 1.18$, so that the magnitude of error is $E \cdot 0.6$ (Eq. 22). Thus, PS_1 can be used to estimate the unidirectional transfer constant of the brain with an error below 6.0%.

Table II

Transcerebral net exchange of large neutral amino acids

	C_p (μM)	C_v (μM)	J (nmol min^{-1} g^{-1})
Phenylalanine	36.4	35.8	0.26
Tryptophan	25.0	25.5	-0.21
Histidine	74.0	71.2	1.16
Methionine	14.0	12.8	0.48
Tyrosine	41.1	36.3	1.98
Valine	223.8	207.8	6.66
Isoleucine	59.4	52.1	3.07
Leucine	113.2	99.8	5.63

Data are average baseline values from a previously published study on healthy volunteers (Berg et al. 2010). The venous concentrations are adjusted for plasma to red blood cell distribution (Knudsen et al. 1994, Hagenfeldt and Arvidsson 1980), and tryptophan concentrations are corrected for albumin binding, assuming a free plasma fraction of 0.725 (Pardridge 1998). C_p: concentration in arterial plasma; C_v: concentration in jugular venous plasma; J: transcerebral net exchange.

Assuming that cerebrospinal fluid (CSF) LNAA concentrations are identical to the extracellular LNAA concentrations in the brain, the calculated concentrations can be compared to the known normal ranges (Table III). When the corresponding ρ-values are calculated, the relative contribution of the saturable and non-saturable component to the total LNAA transport can be derived according to each set of kinetic constants (Table IV).

DISCUSSION

The mathematical approach outlined in the present paper provides a means for estimating the permeability of the BBB (PS_1 and PS_2) to the individual LNAAs, as

Table III

Large neutral amino acid concentrations in brain extracellular fluid

	Hargreaves and Pardridge 1988	Miller et al. 1985	Pardridge and Mietus 1982	Pardridge and Oldendorf 1975	Smith et al. 1987	Normal range
Phenylalanine (μM)	32.9	20.4	19.7	13.1	7.1	4.1–8.9
Tryptophan (μM)	24.6	16.8	16.3	10.5	5.1	0.5–2.1
Histidine (μM)	69.5	36.4	45.2	20.1	10.2	8.6–15.2
Methionine (μM)	12.8	5.3	4.0	2.6	0.8	0.5–3.3
Tyrosine (μM)	-8.2	10.7	13.8	5.0	4.5	3.5–9.3
Valine (μM)	210.5	69.5	89.7	-39.4	-19.1	9.5–20.5
Isoleucine (μM)	-119.2	8.7	19.7	-4.5	3.9	1.9–5.9
Leucine (μM)	95.6	30.5	26.7	15.4	15.1	6.0–14.2
S_p	260.3	5.8	0.8	2.9	12.7	–
S_b	136.8	2.2	0.4	0.4	1.7	–
$S_{b,\,exp}$	22.1–49.8	0.4–0.9	0.1–0.1	0.2–0.4	0.9–2.0	–
R_{max}	1.9	2.1	1.3	2.9	5.1	–

The normal range is based on published cerebrospinal fluid (CSF) concentrations from healthy adult subjects (Lentner 1984). The S_p and S_b parameters are calculated from the values in Table II, as explained in Eq. 3 and Eq. 13. R_{max} is the maximum cerebral permeability ratio (Eq. 16). $S_{b,\,exp}$ is the highest and lowest expected S_b-value, based on the given set of kinetic constants and the normal range of CSF concentrations.

well their concentrations in the brain extracellular fluid (C_b) in man.

According to our single-membrane based model, PS_1 to a given LNAA can be determined from its arterial concentrations, and kinetic constants of the BBB (Eq. 4). By numerically determining the parameter S_b (Eq. 13), PS_2 to a given LNAA may furthermore be calculated (Eq. 4), after which C_b is the only unknown in Eq. 9. The determination of PS_2 and C_b can be simplified even further if the non-saturable component of the transcerebral exchange is considered negligible, that is, if the ρ-values are assumed to be close to 1, since S_b may then be directly derived from the saturable transport parameter ζ_s (Eq. 14).

As with other methods for investigating BBB LNAA permeability parameters, the approach presented here relies on the use of the kinetic constants K_m, V_{max}, and K_d of the different LNAAs. Regardless of which set of kinetic constants is used, the model yields brain extracellular concentrations that are within the same orders of magnitude as the published CSF concentrations (Table III). Some values are, however, negative, which may indeed be a problem in the setting of human studies, where low arterial-to-venous LNAA differences render the transcerebral net exchange estimates particularly susceptible to even small systematic errors in the LNAA and CBF measurements. When assessing the estimated brain extracel-

Table IV

Saturable transport of large neutral amino acids across the blood-brain barrier

	Hargreaves and Pardridge 1988		Miller et al. 1985		Pardridge and Mietus 1982		Pardridge and Oldendorf 1975		Smith et al. 1987	
	ρ_1	ρ_2	ρ_1	ρ_2	ρ_1	ρ_2	ρ_1	ρ_2	ρ_1	ρ_2
Phenylalanine	0.11	0.19	0.60	0.76	0.76	0.81	0.82	0.93	0.97	0.99
Tryptophan	0.03	0.06	0.56	0.73	0.78	0.82	0.96	0.99	0.98	1.00
Histidine	0.04	0.08	0.45	0.63	0.43	0.50	0.72	0.88	0.94	0.99
Methionine	0.02	0.04	0.42	0.60	0.60	0.67	0.68	0.86	0.95	0.99
Tyrosine	1.00	1.00	0.52	0.70	0.53	0.59	0.77	0.90	1.00	1.00
Valine	0.01	0.02	0.25	0.41	0.66	0.72	0.85	0.94	0.78	0.95
Isoleucine	1.00	1.00	0.54	0.71	0.53	0.60	0.88	0.96	1.00	1.00
Leucine	0.04	0.07	0.47	0.65	0.66	0.72	0.76	0.90	0.98	1.00

ρ is the fraction of the total flux of the large neutral amino acid that is saturable, from blood to brain (ρ_1) and from brain to blood (ρ_2), and is calculated by Eq. 10 using the kinetic constants from Table I and values for S_p and S_b from Table III.

lular LNAA concentrations by this approach, it is thus probably more feasible to focus on differences, for example between subjects and conditions, rather than on absolute concentrations. Furthermore, according to the corresponding ρ-values, it is evident that the relative contribution of the saturable and non-saturable component to the total LNAA transport varies markedly depending on which set of kinetic constants is used (Table IV). The highest ρ-values, which indicate that LNAA transport across the BBB is mainly saturable, are obtained when using kinetic constants from the study by Smith and others (1987) (Table IV), which are incidentally the most widely used for modelling BBB function in humans *in vivo* (Knudsen et al. 1990, 1994, 1995, Shulkin et al. 1995). Since the S_b-value derived from the kinetic constants from Smith and others (1987) is within the range of expected S_b values and yields the highest proportion of brain extracellular concentrations that are within the normal range (Table III), the high ρ-values may indeed be representative of conditions in humans *in vivo*. The high ρ-values may thus reflect that LNAA transport across the BBB is predominantly competitive to such an extent, that the contribution of the non-saturable component may be ignored. This implies that it is reasonable to ignore K_d as in previous studies (Knudsen et al. 1990, 1993, 1995, Möller et al. 1997, Pietz et al. 1999), and thus that it is feasible to base the calculations of S_b on the saturable transport parameter ζ_s (Eq. 14). The exact contribution of the non-saturable component to LNAA transport across the BBB *in vivo* may be further evaluated in future studies by measuring the cerebral permeability ratios of LNAAs (Eq. 21).

The mathematical approach for determining BBB LNAA permeability parameters may be useful in studies on healthy human volunteers and on patients, since it is methodically relatively simple, in that it depends only on the collection of paired arterial and jugular venous blood samples, combined with the measurement of CBF. The latter may be achieved by a number of techniques, including the Kety-Schmidt method, magnetic resonance imaging, dynamic computed tomography, and positron emission tomography (Aksoy and Lev 2000, Carroll et al. 2002, Taudorf et al. 2009). The assessment of BBB permeability parameters is particularly relevant in a number

of clinical conditions, where changes in the transcerebral exchange kinetics of LNAAs are thought to contribute to brain dysfunction. Hence, changes in circulating LNAAs are thought to cause intracerebral accumulation of aromatic amino acids in a variety of conditions, such as phenylketonuria (Hommes 1989, Knudsen et al. 1995, Novotny et al. 1995, Pietz et al. 1999), acute liver failure (Dejong et al. 2007, Knudsen et al. 1993, Mizock et al. 1990, Strauss et al. 2001), and sepsis (Basler et al. 2002, Berg et al. 2010, Mizock et al. 1990). In such conditions, the aromatic LNAAs phenylalanine and tyrosine may degrade to phenylethanolamine and octopamine, respectively, which act as so-called 'false' neurotransmitters that inhibit central noradrenergic neurotransmission (Dejong et al. 2007, Fischer and Baldessarini 1971). Treatment with the branched-chain LNAAs leucine, isoleucine, and valine may prevent this through competitive inhibition of the cerebral influx of the aromatic LNAAs (Bower et al. 1986, Holecek 2010, Schindeler et al. 2007), but the exact effects of such interventions on the transcerebral exchange kinetics of LNAAs in the clinical setting have not yet been investigated. Changes in the transcerebral exchange kinetics of LNAAs have furthermore been suggested to contribute to the cerebral pathophysiology in traumatic brain injury (Elkind et al. 2015), spinocerebellar degeneration (Mori et al. 2002), and maple syrup urine disease (Zinnanti et al. 2009), and our mathematical approach is thus relevant in studies focusing on the cerebral pathophysiology of these conditions.

In conclusion, the mathematical approach outlined in the present paper provides a means for assessing the BBB permeability to individual LNAAs in a methodically simple setup. Hence, PS_1, PS_2, and C_b may be estimated in a human subject on the basis of arterial and jugular venous LNAA concentrations, CBF, and kinetic constants of the BBB.

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