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Large neutral amino acids levels in primate cerebrospinal fluid do not confirm competitive transport under baseline conditions

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ABSTRACT

In rodents, transport of large neutral amino acids (LNAAs) across the blood brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier is mediated by high affinity carriers. Net brain LNAA levels are thought to be determined mainly by this competitive transport from plasma. Since the affinity for LNAA transport at the BBB in primates is considerably higher than in rodents, brain influx and by extension LNAA brain levels, should be even more dependent on competitive transport. Given that LNAA levels in CSF and brain interstitial fluid are usually similar, we analyzed serum and CSF of fasted subjects (n=24) undergoing spinal anesthesia and calculated brain influx and transporter occupancy using a conventional model of transport. Despite predicted near-full transporter saturation (99.7%), correlations between CSF levels and brain influx were modest, limited to tyrosine (r=0.60, $p<0.002$) and tryptophan (r=0.50, $p<0.01$) and comparable to correlations between CSF and serum levels. We also analyzed serum and CSF in (n=5) fasted vervet monkeys. Tyrosine and phenylalanine levels in CSF were positively correlated with those in serum, but correlations with calculated brain influx, which takes competition into account, were weaker or absent. We conclude that in primates i) baseline CSF LNAA levels do not confirm competitive transport, ii) brain LNAA levels should not be estimated on the basis of serum indices alone. This has implications for amino acid challenge studies and for neuropsychiatric disorders associated with dysregulated LNAA transport in which quantitative information about brain LNAA levels is needed.

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1. Introduction

The aromatic amino acids tyrosine, phenylalanine and tryptophan have been implicated in a number of neuropsychiatric disorders (Bongiovanni et al., 2013; Johansson et al., 2011; Richardson et al., 1997; Yano et al., 2014). However, brain levels of these and other large neutral amino acids (LNAAs) cannot in general, be measured *in vivo* in man. In species with high affinity blood brain barrier (BBB) transport, brain LNAA levels are largely determined by their competitive influx from plasma (Fernstrom, 2013; Pardridge, 1998). Since the main LNAA transporter at the human BBB has relatively high affinity (Pardridge, 1998) but low expression (Uchida et al., 2011), brain levels of LNAAs in man should be particularly dependent on competitive influx from plasma. Under steady state conditions there is a good correlation between LNAA levels in brain tissue, brain interstitial fluid and cerebrospinal fluid (CSF) (Davson and Segal, 1996; Hamberger et al., 1990; Hamberger and Nystrom, 1984). These considerations have prompted efforts to characterize the relationship between peripheral and CSF LNAA levels in man, with the goal of developing a serum-based index for estimating LNAA brain levels.

A significant but modest correlation (r=0.47) was reported between total serum tryptophan and CSF levels collected under fasting conditions in neurological controls (Young et al., 1975). Significant correlations (r=0.38–0.72) were also noted between fasting serum and CSF levels of tyrosine (Korf et al., 1983; Kruse et al., 1985; McGale et al., 1977) as well as of phenylalanine (Kruse et al., 1985). Pioneering work in the rat demonstrated that the level of a given LNAA in brain tissue was more highly and

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consistently correlated with the serum ratio of that LNAA to the sum of its competitors (LNAA /Σ LNAAs) than with the serum level of the LNAA alone (Fernstrom and Faller, 1978). This approach was extended to human investigations. In the first such study, however, CSF levels correlated more strongly with serum levels of tyrosine (r=0.46–0.92) and phenylalanine (r=0.58) than with the respective serum ratios (Hagenfeldt et al., 1984). In a second report, CSF levels of tyrosine correlated comparably (r=0.67–0.80) with the serum ratio and with serum levels; correlations of CSF tryptophan were gender-dependent (Moller et al., 1996). Thus, the expected transport competition was not evident.

The serum ratio approach, however, is based on the simplifying assumption that all the competing LNAAs have comparable affinity for BBB transport (Fernstrom and Faller, 1978; Moller et al., 1996), whereas both in the rat and in man these affinities vary by some 20-fold (Hargreaves and Pardridge, 1988; Smith et al., 1987). This heterogeneity is taken into account in calculations of brain influx (Pardridge, 1983; Pardridge, 1987) which should therefore be a superior index of brain LNAA levels. Individual LNAA transport affinities can also be used to estimate the net occupancy of the transporter system (Smith et al., 1987). In the rat, for instance, the high calculated transport occupancy (96%) is consistent with competitive LNAA transport at the BBB (Smith et al. 1987).

Until now, the relationship between brain influx and actual CSF levels of LNAAs has been examined only in non-human primates. In juvenile macaques fed graded protein diets, changes in CSF levels qualitatively resembled changes in estimated brain influx for tyrosine but less so for phenylalanine (Grimes et al., 2009). Possible quantitative relationships between CSF levels and brain influx of LNAAs were not, however, examined and transport site occupancy was not determined (Grimes et al. 2009).

For these reasons, we now report on the relationship between LNAA levels in plasma, CSF and calculated brain influx in fasting subjects undergoing spinal anesthesia. Our primary hypothesis was that the CSF level of a given LNAA would correlate more strongly with its calculated brain influx than with either its serum ratio (Fernstrom and Faller, 1978) or its serum level. Our secondary hypothesis was that transport sites in man would also show a degree of saturation at least as high as that in the rat. For comparison, we analyzed a set of CSF and plasma samples from non-human primates. In both samples we calculated brain influx using published kinetic parameters (affinity (K_m), maximal transport velocity (V_m), non-saturable constant (K_D)) derived from blood capillaries in man (Hargreaves and Pardridge, 1988).

2. Results

2.1. Patient sample

The patient sample (n=24, Caucasian=19, African American=4, Asian American=1) was all male (mean age 66.7 ± 2.1 yr.). Serum levels of LNAAs exceeded those in CSF by 5–15-fold. The apparent affinity ($K_{m(app)}$) exceeded K_m by 100–300-fold. Over half of the transport sites were occupied by phenylalanine, with remaining occupancy tyrosine > leucine > valine, isoleucine > histidine (5%) > tryptophan > methionine (1%) (Table 1). Overall, the transport system was calculated to be 99.7% saturated.

Significant correlations between CSF levels and serum-derived indices were evident only for tyrosine and tryptophan. CSF tyrosine levels showed a moderate and comparable correlation (r=0.57–0.61) with tyrosine serum levels, serum ratio or influx (Table 2, Fig. 1). CSF levels of tryptophan were moderately correlated (r=0.5) with serum tryptophan or tryptophan influx but not with the serum ratio (Table 2, Fig. 2) (not significantly affected by exclusion of one outlying value (influx =1.39 pmol/min*mg_{cp}). For each LNAA, the correlation between its serum level and corresponding estimated influx was significant. There were also strong (r=0.78–0.93) correlations between the serum ratio and calculated influx for each LNAA with the exception of phenylalanine and tryptophan. Within the CSF, there were significant correlations between all the LNAAs (Table 3). There were no significant correlations between subject age and CSF levels of any of the LNAAs (data not shown).

2.2. Non-human primate sample

The group consisted of 3 adult males and 2 females. Serum levels of LNAAs exceeded those in CSF 7–34-fold. Significant correlations between CSF and serum levels were evident for tyrosine ($r=0.97$, $p<0.008$) and phenylalanine ($r=0.98$, $p<0.003$). CSF levels of phenylalanine were also correlated with phenylalanine influx ($r=0.89$, $p<0.05$). There was an inverse correlation ($r=-0.88$, $p<0.05$) between CSF tryptophan and serum tryptophan.

3. Discussion

We found that despite very high estimated transporter occupancy (99.7%) (Table 1), correlations between CSF levels and various serum indices of LNAAs in man were modest, limited to tyrosine and tryptophan, and were not improved by taking into account transport competition (Table 2). In vervet monkeys, limited correlations were found between serum indices and CSF levels of LNAAs (Table 3). These conclusions must be understood in light of experimental limitations and assumptions.

Our human sample was older and exclusively male, reflecting the population that undergoes spinal anesthesia in our facility. Human aging is associated with ultrastructural changes of brain capillaries (Stewart et al., 1987), decreases in the density of certain BBB transporters (van Assema et al., 2012) and a general increase in BBB permeability (Farrall and Wardlaw, 2009). While normal aging is not associated with changes in LNAA uptake as measured

Table 1
Large neutral amino acid levels and kinetic parameters in patient sample (n =24).

	Serum (μM)	Km*(μM)	$K_{m\ (app)}$ (μM)	$K_{m\ (app)}$/ K_m	fractional occupancy	Influx pmol/min mg_{cp}	CSF μM
TYR	56.52 ± 2.81	1.3	359 ± 15	276	13%	1.63 ± 0.06	11.10 ± 0.95
VAL	200.56 ± 8.40	8.8	2610 ± 104	297	7%	0.96 ± 0.03	23.39 ± 2.16
ISO	60.62 ± 2.75	2.7	802 ± 31	297	7%	0.53 ± 0.01	7.26 ± 0.50
LEU	102.11 ± 5.18	3.3	952 ± 37	288	9%	1.29 ± 0.03	15.09 ± 0.92
PHE	56.72 ± 2.41	0.3	39 ± 2	130	55%	3.66 ± 0.04	12.22 ± 0.83
TRP	29.09 ± 3.35	3	929 ± 36	310	3%	0.47 ± 0.05	1.99 ± 0.17

Means + SEM, Km(app)= Km apparent, cp = capillary protein. Estimated fractional occupancy histidine 5%, methionine 1% (see Methods).
(ISO – isoleucine, LEU – leucine, PHE – phenylalanine, TRP – tryptophan, TYR – tyrosine, VAL – valine)

* Km from (Hargreaves and Pardridge 1988).