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BRAIN RESEARCH

Research Report

# Relationships between large neutral amino acid levels in plasma, cerebrospinal fluid, brain microdialysate and brain tissue in the rat

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ABSTRACT

Experimental limitations may preclude direct measurement of large neutral amino acid (LNAA) levels in brain tissue. Some data suggest that serum or cerebrospinal fluid (CSF) may provide an index of LNAA brain levels. We examined this in a series of experiments in rats, administering tyrosine, phenylalanine or valine IP 60 min prior to harvesting of blood, CSF or brain tissue or during *in vivo* microdialysis of the brain. Serum indices of the administered LNAA generally showed a significant ($r>0.8$) correlation with brain tissue levels but the linear relationships varied significantly across brain regions, the LNAA and its dose. Increases in levels of an administered LNAA were consistently greater in CSF than in brain tissue. In contrast, changes in LNAA levels in brain tissue and *in vivo* microdialysate were generally comparable. We confirm that changes in serum and CSF LNAA levels can support limited, qualitative inferences about changes in brain tissue LNAA levels; quantitative inferences should not be drawn without prior validation under relevant experimental conditions.

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## 1. Introduction

Large neutral amino acids (LNAAs) such as tyrosine (TYR), phenylalanine (PHE) and tryptophan (TRP) have been implicated in various neuropsychiatric disorders (Bjerkenstedt et al., 2006; Jans et al., 2007; Richardson et al., 1997). Research and treatment paradigms that target brain LNAA levels in man (Munafo et al., 2007; Richardson et al., 2003; Ruhe et al., 2007), however, face a common limitation. Human brain tissue or extracellular fluid cannot in general, be sampled *in vivo*. It would be important to know, therefore, how well serum or cerebrospinal fluid (CSF) LNAA levels would support quantitative inferences about brain LNAA levels.

Fernstrom and Wurtman were the first to report (Fernstrom and Wurtman, 1972) that brain tissue TRP levels in the rat showed a highly significant linear correlation with the ratio serum TRP/Σ (competing LNAAs). This relationship for TRP was confirmed (Fernstrom et al., 1973, 1975) and extended to other LNAAs (TYR, PHE, valine (VAL), leucine, and isoleucine) (Fernstrom et al., 1976; Fernstrom and Faller, 1978). In those

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*Abbreviations:* BBB, blood brain barrier; CSF, cerebrospinal fluid; ECF, extracellular fluid; LNAA, large neutral amino acid; MPFC, medial prefrontal cortex; PHE, phenylalanine; TRP, tryptophan; TYR, tyrosine; VAL, valine; VEH, vehicle

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early studies, however, fasted rats were typically presented with an acute meal that affected most LNAAs simultaneously (Fernstrom et al., 1976; Fernstrom and Faller, 1978). In contrast, the goal of recent clinical research studies, has been to elevate or lower levels of a single LNAA (Hood et al., 2005; Mahoney et al., 2007; Ruhe et al., 2007). The relationship between serum and brain indices under such conditions has not been characterized.

While CSF is considered to approximate the composition of brain ECF (Davson et al., 1987), relatively few studies have directly compared LNAA levels in brain and CSF. Two groups reported modest but significant correlations between brain tissue and CSF levels of tryptophan (TRP) in response to acute dietary manipulations (Modigh, 1975; Young et al., 1976). Another group, however, found that acute IP administration of TRP induced a much larger and earlier elevation of TRP levels in CSF than in brain extracellular fluid (Hutson et al., 1985). Other LNAAs have not been similarly examined.

To address these issues, we undertook a series of studies in the rat, comparing LNAA levels across different compartments following IP administration of selected doses of TYR, PHE or VAL. Given the role of PHE in neuropsychiatry (Richardson et al., 2003; Weglage et al., 2001), we were surprised that a PubMed search did not locate full dose–response data for acute effects of PHE administration on PHE or TYR levels in the rat brain; there was only one limited study examining such effects in the retina (Fernstrom et al., 1989). For that reason, we examined a wider range of PHE doses.

In the first series of experiments, we harvested trunk blood and brain tissue. In the second series, trunk blood and CSF were collected. In the third series, microdialysate was collected *in vivo* from medial prefrontal cortex (MPFC). Our primary hypothesis was that under conditions of rapidly changing LNAA levels, there would be a high and significant correlation between the serum ratio (LNAA/ΣLNAA) and brain levels of a given LNAA. Our secondary hypothesis was that the percentage change in the level of a given LNAA would be comparable in CSF, brain tissue and microdialysate. An additional aim was to generate a dose–response curve for TYR and PHE levels after IP administration of PHE.

## 2. Results

### 2.1. *Comparing serum and brain tissue levels*

In Experiment 1, trunk blood and brain tissue were harvested 60 min after IP vehicle, ($n$=11), TYR 100 mg/kg ($n$=8), or VAL 200 mg/kg ($n$=8). Only levels of TYR, PHE, TRP and VAL are reported (Table 1), although leucine and isoleucine levels were measured as well.

**Table 1 – Tyrosine (TYR), phenylalanine (PHE), tryptophan (TRP), valine (VAL) levels in serum and brain tissue harvested 60 min after IP administration of vehicle (control), TYR 100 mg/kg or VAL 200 mg/kg. Serum ratio=LNAA/ΣLNAA. Correlations (Spearman) were determined between brain tissue levels and either absolute serum levels or the serum ratio. Tissue levels are expressed per mg protein. Only significant correlations are shown. Significantly different from control: a, $p<0.05$; b, $p<0.01$; c, $p<0.0001$. Medial prefrontal cortex (MPFC) and hypothalamus (HYP).**

Experiment 1 — serum and brain tissue

| Dose (mg/kg) | LNAA | % of control | | | | |
|---|---|---|---|---|---|---|
| | | Serum | Serum ratio | MPFC | Striatum | HYP |
| TYR 100 | TYR | 289±97 c | 300±35 c | 250±23 c | 239±20 c | 219±40 c |
| | PHE | 95±13 | 75±3 c | 102±6 | 94±3 | 93±14 |
| | TRP | 99±33 | 76±9 b | 81±5 b | 81±6 a | 82±17 |
| | VAL | 93±13 | 67±3 | 94±5 | 93±4 | 113±19 |
| VAL 200 | TYR | 97±22 | 40±3 c | 86±5 | 74±3 a | 85±20 |
| | PHE | 99±10 | 41±2 c | 90±4 | 78±4 c | 87±19 |
| | TRP | 84±14 | 34±2 c | 74±5 c | 67±4 c | 76±11 a |
| | VAL | 619±78 c | 561±27 c | 559±24 c | 489±18 c | 549±126 c |

Correlations

| Groups compared | LNAA | MPFC vs. | | Striatum vs. | | HYP vs. | |
|---|---|---|---|---|---|---|---|
| | | Serum | Serum Ratio | Serum | Serum Ratio | Serum | Serum Ratio |
| Vehicle, TYR 100, VAL 200 mg/kg | TYR | 0.88 c | 0.95 c | 0.86 c | 0.96 c | 0.84 c | 0.88 c |
| | PHE | | | | 0.64 c | 0.53 b | |
| | TRP | 0.49 b | 0.61 c | 0.37 a | 0.65 c | | 0.35 a |
| | VAL | 0.98 c | 0.99 c | 0.98 c | 0.99 c | 0.91 c | 0.93 c |

Control levels

| | Serum (nmol/ml) | Serum ratio | MPFC (nmol/mg) | Striatum (nmol/mg) | HYP (nmol/mg) |
|---|---|---|---|---|---|
| TYR | 98.88±14.12 | 0.180±0.026 | 0.851±0.152 | 0.939±0.199 | 1.131±0.282 |
| PHE | 65.91±9.47 | 0.113±0.017 | 0.706±0.093 | 1.296±0.173 | 1.030±0.232 |
| TRP | 84.73±11.45 | 0.151±0.024 | 0.268±0.031 | 0.377±0.062 | 0.293±0.072 |
| VAL | 157.37±39.16 | 0.331±0.148 | 1.151±0.116 | 1.491±0.177 | 1.332±0.273 |