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ARTICLE

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What's Known on This Subject

Reference values for cerebrospinal fluid/plasma ratios are difficult to establish, because cerebrospinal fluid can not be obtained in healthy children. Furthermore, the influence of age, gender, and antiepileptic therapy on these ratios has not been described previously.

What This Study Adds

The effects of age, gender, and antiepileptic therapy on amino acid cerebrospinal fluid/plasma ratios in children were investigated. Cerebrospinal fluid/plasma ratios—in particular for essential neutral amino acids and for serine, asparagine, and glutamine—may be influenced by age, gender, and antiepileptic therapy

ABSTRACT

OBJECTIVE. The purpose of this work was to investigate the influence of age, gender, and antiepileptic therapy on amino acid cerebrospinal fluid/plasma ratios in children.

PATIENTS AND METHODS. Concentrations of 17 amino acids measured by ion-exchange chromatography with ninhydrin detection in plasma and cerebrospinal fluid from 68 patients with neurologic diseases were used to calculate their cerebrospinal fluid/plasma ratios (70 measurements; 28 female patients [29 punctures] and 40 male patients [41 punctures]). Age dependence and the effects of gender and antiepileptic medication on amino acid cerebrospinal fluid/plasma ratios were investigated by linear multiple regression analysis, and nonstandardized predicted mean values for 2 age groups were calculated (cutoff: 3 years old).

RESULTS. The cerebrospinal fluid/plasma ratios ranged between 0.02 for glycine and 0.93 for glutamine. Age had a significant influence on cerebrospinal fluid/plasma ratios for valine, isoleucine, leucine, and tyrosine, with higher ratios in younger children. Gender had a significant influence only on the glutamine cerebrospinal fluid/plasma ratio (female patients had lower ratios). Cerebrospinal fluid/plasma ratios of glutamine and tyrosine were significantly elevated by valproate therapy and those of serine, asparagine, glutamine, valine, methionine, and phenylalanine by phenobarbital therapy. No significant influence of age, gender, and antiepileptic drugs was detectable on cerebrospinal fluid/plasma ratios of threonine, proline, glycine, alanine, histidine, ornithine, lysine, and arginine.

CONCLUSIONS. Cerebrospinal fluid/plasma ratios, especially for essential neutral amino acids and for serine, asparagine, and glutamine were influenced to different degrees by age, gender, and antiepileptic therapy.

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Key Words
amino acids, blood-brain barrier, CSF/plasma ratios, child

Abbreviations
BBB— blood-brain barrier
CSF— cerebrospinal fluid

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THE BLOOD-BRAIN BARRIER (BBB) is a unique dynamic regulatory interface separating bloodstream from brain extracellular fluid and, thus, maintaining the autonomy of the brain's extracellular space from the remaining extracellular space of the body. The BBB is composed of 2 membranes in series, the luminal and abluminal membranes of the endothelium of cerebral capillary vessels.[1] Transporters for neutral, anionic, and cationic amino acids have been identified, which show an overlap of their transporter specificities.[2] A detailed examination of amino acid transporters at the BBB in humans in vivo is currently not available, because only blood and cerebrospinal fluid (CSF), but not membranes, can be investigated. In addition, CSF is mainly produced by the choroid plexus and is not identical to the extracellular fluid surrounding the brain tissue. This may be the reason why so far no inborn error of metabolism has been described because of an amino acid transport defect across the BBB, as has been shown for the glucose transporter GLUT1, for example.[3,4] Furthermore, substrate overlap seen in amino acid transporters protects the brain from amino acid deficiencies. A possible method for the investigation of amino acid transporters at the BBB may be the calculation of amino acid CSF/plasma ratios. However, establishing reference values for CSF/plasma ratios in children is difficult, because CSF

cannot be obtained in healthy children. Furthermore, the influence of age, gender, and antiepileptic therapy on CSF/plasma ratios has not been described previously. The aim of this study was, therefore, to investigate the influence of age, gender, and antiepileptic treatment on CSF/plasma ratios in children.

MATERIALS AND METHODS

Analytic Methods

Within a few minutes after sample collection, lithium heparin blood was centrifuged for 10 minutes at 1930 × *g*. CSF and plasma samples were stored at −20°C. Before analysis lithium heparin plasma and CSF samples were deproteinized by mixing 500 μL of the sample with 50 μL Li-S buffer (Beckman Coulter, Fullerton, CA) containing *S*-2-aminoethyl-L-cysteine internal standard (2.5 μmol/mL) and 50 μL of 50% sulfosalicylic acid. The mixture was then centrifuged for 5 minutes at 10930 × *g*. The supernatant (300 μL) was mixed with 300 μL of Li-S buffer and 30 μL, 1 N NaOH, and 50 μL used for amino acid analysis. The external standard contained among other amino acids 100 μmol/L each of threonine, serine, asparagine, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, histidine, lysine, and arginine (Beckman Coulter). Analysis of amino acid concentrations was performed by using ion-exchange chromatography with ninhydrin detection on a Jeol system (AminoTac, Model JLC-500/V, Tokyo, Japan). A 120-mm × 4-mm analytical column with the cation exchanger resin was used. Elution was conducted using 5 lithium citrate buffer solutions (pH 2.83 to 3.65) successively. The duration of analysis was 85 minutes.

Clinical Information

During a 3-year period (May 2003 to April 2006), 117 simultaneous venous and lumbar punctures were performed in 110 patients with seizures and/or mental retardation or other neurologic symptoms for determination of routine parameters (cell count, glucose, protein, and lactate), as well as amino acid and neurotransmitter concentrations. The inclusion criteria for this retrospective study were the simultaneous performance of venous and lumbar puncture. Lumbar and venous punctures were conducted under general anesthesia for magnetic resonance tomography (with or without magnetic resonance spectroscopy) or computed tomography or in acute indications without anesthesia. In none of the patients was an epileptic status reported within the day before puncture was performed. General anesthesia was done routinely as total intravenous anesthesia with opioids plus propofol or as balanced anesthesia combined with inhalative agents.

For calculation of reference values, patients with known inborn errors of metabolism, elevated CSF-protein, and nonoptimal preanalytical conditions (blood contamination of CSF, missing CSF protein determination, and missing simultaneous venous puncture) were excluded. This retrospective analysis was performed with the approval of the ethics committee of the Medical University of Innsbruck.

Collection and Storage of Samples

Because of the rostrocaudal gradient,[5] the collection of the CSF samples was conducted in a standardized manner: depending on the age of the patients 3, 6, or more fractions of 1 mL and, in infants of <1 year, of 0.50 to 0.75 mL each were collected. The first fraction was used for cytological and chemical investigations (glucose, protein, and immunoglobulins), the second fraction for the determination of neurotransmitter concentration was immediately frozen in liquid nitrogen, and both the third fraction for the determination of amino acids and the fourth fraction for lactate were analyzed immediately or frozen (−20°C) until analysis. Venous puncture for the determination of protein, immunoglobulins, lactate, and plasma amino acids was performed within ±2 hours.

Statistical Analysis

Statistical analysis was performed by using Excel 2003 (Microsoft, Redmond, WA) and SPSS 12.0 (SPSS Inc, Chicago, IL). Age dependence, as well as the effect of gender and medication on amino acid CSF/plasma ratios, was investigated by linear multiple regression analysis. A P value of <.05 was considered statistically significant. To take the influences of age, gender, and antiepileptic therapy into account, nonstandardized predicted mean values for CSF/plasma ratios were calculated in addition to crude means ± SDs for 2 age groups. Variables were distributed according to the Gaussian function.

RESULTS

Assay Performance

Intra-assay and interassay variations of amino acids were tested by measuring 10 samples of external standards of 100 μM concentration. The intra-assay and interassay coefficients of variability are shown in Table 1. The quality of amino acid measurements was assured by participating in the European Research Network of Inherited Disorders of Metabolism quantitative amino acid scheme and proficiency testing program.[6]

Patients

Criteria for accepting samples for the calculation of reference values were fulfilled in 70 of 110 punctures (68 patients; age: median: 4.1 years, mean: 5.1 years, range: 3 days to 17.2 years; 28 female patients [29 punctures] and 40 male patients [41 punctures]). Thirty punctures were done for the investigation of seizures, epilepsy, and electroencephalography changes plus mental retardation; 19 for mental retardation alone; 15 for seizures, epilepsy, and electroencephalography changes alone; and the remainder (6 punctures) because of other reasons (eg, to exclude infectious, autoimmune, or movement disorder). In 36 punctures, patients received antiepileptic therapy (valproate: $n = 12$; valproate in combination with other antiepileptic drugs: $n = 8$; phenobarbital: $n = 12$; phenobarbital plus other antiepileptic drugs: $n = 2$; others: $n = 2$).

Routine Laboratory Variables in Plasma and CSF

Table 2 summarizes the mean, SD, and range of the routine parameters measured (glucose, lactate, and pro-

TABLE 1 **Intra-Assay and Interassay Coefficients of Variability for Amino Acid Concentrations in External Standard**

Variable	Concentration, μmol/L	Intra-Assay Variability			Interassay Variability		
		Mean, μmol/L	SD, μmol/L	CV, %	Mean, μmol/L	SD, μmol/L	CV, %
Threonine	100	100.5	0.5	0.5	101.4	1.8	1.8
Serine	100	100.4	0.6	0.6	101.2	1.8	1.8
Asparagine	100	100.5	0.7	0.7	98.8	0.9	0.9
Glutamine	100	89.8	0.4	0.4	91.5	2.4	2.6
Proline	100	107.5	2.5	2.3	104.9	2.1	2.0
Glycine	100	100.0	0.4	0.4	101.4	1.7	1.7
Alanine	100	100.3	0.3	0.3	101.3	1.5	1.4
Valine	100	99.8	0.4	0.4	100.9	1.6	1.6
Methionine	100	100.0	0.3	0.3	101.5	1.7	1.6
Isoleucine	100	99.9	0.3	0.3	101.6	1.9	1.8
Leucine	100	101.3	0.9	0.9	102.6	1.9	1.9
Tyrosine	100	100.8	0.8	0.8	102.5	1.8	1.8
Phenylalanine	100	102.9	0.6	0.6	102.5	2.4	2.4
Ornithine	100	101.5	0.6	0.5	102.5	1.6	1.5
Histidine	100	96.8	0.9	0.9	98.9	2.1	2.1
Lysine	100	100.3	0.7	0.7	103.9	2.1	2.0
Arginine	100	99.7	0.6	0.6	102.1	1.3	1.3

For each amino acid, 10 samples containing 100 μM of the respective analyte were measured.
CV indicates coefficient of variation.

tein). Because normal CSF-protein concentration was an inclusion criterion, CSF-protein concentrations were available for all 70 of the punctures. Mean CSF-protein concentration was 237 mg/L (SD: ±80; median: 219 mg/L; range: 100–467 mg/L). Mean CSF-glucose concentration was 2.9 mmol/L (SD: ±0.4; median: 2.9 mmol/L; range: 2.0–4.0 mmol/L) and the mean plasma-glucose concentration was 4.7 mmol/L (SD: ±0.9; median: 4.6 mmol/L; range: 2.6–8.5 mmol/L) resulting in a mean CSF/plasma ratio of 0.63 (SD: ±0.13; median: 0.63; range: 0.36–1.28). Mean CSF-lactate concentration was 1.4 mmol/L (SD: ±0.2; median: 1.4 mmol/L; range: 1.0–1.9 mmol/L) and the mean plasma-lactate concentration was 1.3 mmol/L (SD: ±0.4; median: 1.3 mmol/L; range: 0.7–2.5 mmol/L), resulting in a mean CSF/plasma ratio of 1.12 (SD: ±0.30; median: 1.06; range: 0.48–1.94).

Age and Gender Dependence for Amino Acid CSF/Plasma Ratios

To investigate the statistical significance of age dependence, multiple linear regression analysis was performed (variables: age, gender, therapy with valproic acid, and phenobarbital; Table 3). No significant influence ($P > .05$) of age, gender, and antiepileptic drugs was detectable on proline, glycine, alanine, histidine, threonine, ornithine, lysine, and arginine (Table 3). A significant influence of gender was only detectable for glutamine (female, standardized coefficient: −0.18; $P < .05$). Age dependence was significant ($P < .05$) for the essential neutral amino acids valine, leucine, isoleucine, and tyrosine, with higher CSF/plasma ratios in younger children (Fig 1). Amino acid CSF/plasma ratios were usually higher in the younger children, with the exception of glutamine, proline, histidine, and methionine (P value not significant; Table 3). Because of age dependence, reference values for 2 age groups were calculated: values for patients <3 years are shown in Table 4 and for patients >3 years are shown in Table 5. The comparison of our amino acid CSF/plasma ratios with those from the literature showed that our values were in the range of previously published ratios or estimated values with the exception of arginine, which was higher in our patients (Table 6). However, in contrast to most reference values from the literature, our samples were obtained under standardized preanalytical conditions as paired samples.

Because there is an overlap of transport specificities

TABLE 2 **Summary of CSF and Plasma Routine Parameters in 70 Lumbar and Venous Punctures (Protein and Glucose Concentrations Were Determined in 2 Different Laboratories)**

Variable	*n*	Mean	SD	Median	Minimum	Maximum
CSF-glucose, mmol/L	69	2.9	0.4	2.9	2.0	4.0
Plasma-glucose, mmol/L	65	4.7	0.9	4.6	2.6	8.5
Glucose CSF/plasma ratio	65	0.63	0.13	0.63	0.36	1.28
CSF-lactate, mmol/L	63	1.4	0.2	1.4	1.0	1.9
Plasma-lactate, mmol/L	62	1.3	0.4	1.3	0.7	2.5
Lactate CSF/plasma ratio	58	1.12	0.30	1.06	0.48	1.94
CSF-protein, mg/L	70	237	80	219	100	467

TABLE 3 **Results of Multiple Linear Regression for Age and Therapy With Valproic Acid and Phenobarbital (Standardized Coefficient)**

Group	Variable	Age	Phenobarbital	Valproic Acid
1	Serine	−0.20	0.26[a]	−0.06
	Asparagine	−0.12	0.26[a]	0.16
	Glutamine	0.19	0.20[a]	0.68[a]
	Proline	0.12	0.13	−0.32
	Glycine	−0.16	−0.10	−0.04
	Alanine	−0.14	0.11	−0.08
	Histidine	0.16	0.16	−0.04
2	Threonine	−0.13	0.23	0.13
	Valine	−0.34[a]	0.28[a]	−0.16
	Methionine	0.02	0.28[a]	0.12
	Isoleucine	−0.39[a]	0.19	−0.22
	Leucine	−0.41[a]	0.20	−0.05
	Tyrosine	−0.30[a]	0.09	0.25
	Phenylalanine	−0.18	0.27[a]	0.30[a]
3	Ornithine	−0.26	0.15	0.13
	Lysine	−0.27	−0.02	−0.04
	Arginine	−0.16	<0.01	0.10

[a] Data are significant at $P < .05$.

and capacities in the different amino acid transporters at the BBB,[2] classification of amino acids in the figures and tables in general follows the chemical properties (neutral or cationic) and the need for external supply in mammalians (essential or nonessential) as follows: (1) nonessential neutral amino acids: serine, asparagine, glutamine, proline, glycine, and histidine (transported by several transporters)[2]; (2) essential neutral amino acids: threonine, valine, methionine, leucine, isoleucine, phenylalanine and tyrosine (mainly transported by the L1 system [leucine preferring])[2]; and (3) cationic amino acids: ornithine, lysine, and arginine (mainly transported by the y^+[= CAT1] system).[2]

Influence of Antiepileptic Drugs on Amino Acid CSF/Plasma Ratios

Thirty-six punctures were obtained from patients who received antiepileptic drugs. To investigate the influence of phenobarbital and valproic acid on amino acid CSF/plasma ratios, multiple linear regression analysis was performed (variables: age, gender, therapy with valproic acid, and phenobarbital). CSF/plasma ratios of serine, asparagine, glutamine, valine, methionine, and phenylalanine were significantly increased by phenobarbital therapy, whereas valproate therapy increased glutamine and tyrosine ratios ($P < .05$; Table 3). The influence of other antiepileptic drugs was not calculated, because only a few patients received these drugs.

DISCUSSION

In our retrospective analysis of standardized sampling for the determination of amino acid CSF and plasma concentrations in children, CSF/plasma ratios were calculated. Amino acid concentrations in CSF have been reported for children using various analytic methods.[7–11] For amino acid CSF concentrations, age dependence was shown for several amino acids with often higher levels in the younger children. However, there are no reference values available for CSF/plasma ratios in children with the exception of a few amino acids such as glycine, where a CSF/plasma ratio of >0.08 is considered diagnostic for nonketotic hyperglycinemia.[12] Because Applegarth et al[9] and Gerrits et al[13] have reported amino acid concentrations in plasma and CSF, we were able to calculate the CSF/plasma ratios from the mean values of CSF and plasma concentrations (nonpaired samples). In contrast, reference values for amino acid CSF/plasma ratios or plasma/CSF ratios in adults are available.[14–17] In addition to age dependence of CSF and plasma concentrations published previously, we found the CSF/plasma ratios for valine, isoleucine, leucine, and tyrosine to be significantly higher in children of <3 years.

In 36 punctures, patients received medication with antiepileptic drugs mostly with valproic acid or phenobarbital. Ko et al[18] showed that plasma glycine and ammonia concentrations were influenced by therapy with valproic acid. In contrast, in our patients, CSF/plasma ratios of glutamine and phenylalanine but not glycine were significantly elevated by valproate therapy as reported by Applegarth and

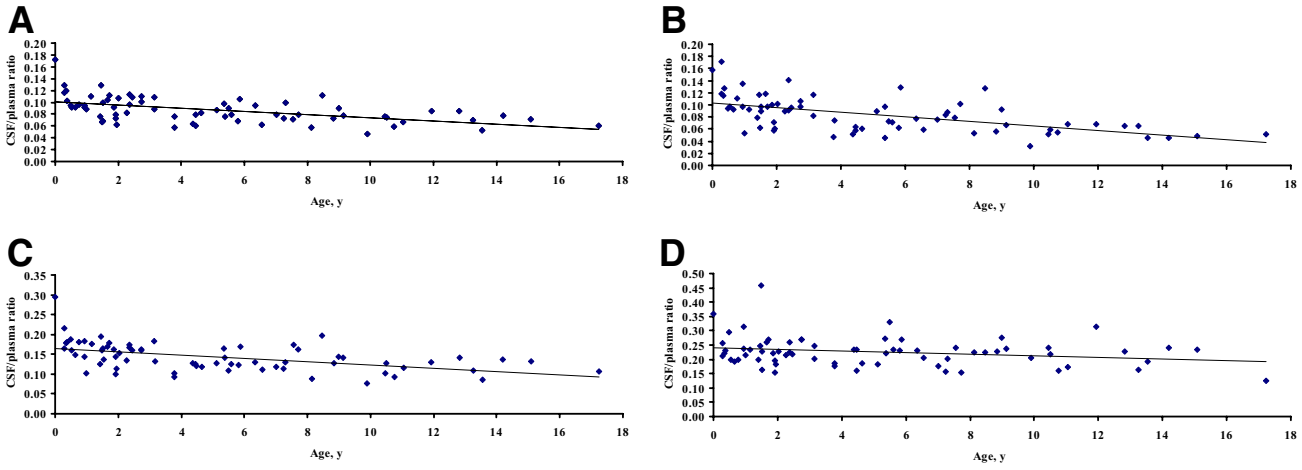

FIGURE 1
Age dependence of CSF/plasma ratios of valine (A), isoleucine (B), leucine (C), and tyrosine (D).

TABLE 4 **Summary of CSF/Plasma Ratios and CSF and Plasma Concentrations in 31 Patients <3 Years of Age Grouped in Nonessential Neutral Amino Acids, Essential Neutral Amino Acids, and Cationic Amino Acids**

Group	Variable	*n*	CSF/Plasma Ratios, Mean ± SD (Crude Values)	CSF/Plasma Ratios, Mean ± SD (Unstandard Predicted Values)	CSF Concentrations, Mean ± SD, μmol/L	Plasma Concentrations, Mean ± SD, μmol/L
1	Serine	31	0.328 ± 0.093	0.270 ± 0.030	39.5 ± 10.3	127 ± 36
	Asparagine	31	0.134 ± 0.037	0.129 ± 0.012	5.4 ± 2.1	41 ± 12
	Glutamine	31	0.757 ± 0.154	0.775 ± 0.079	469.2 ± 118.2	627 ± 123
	Proline	14	0.033 ± 0.020	0.036 ± 0.004	4.6 ± 1.6	148 ± 59
	Glycine	31	0.027 ± 0.010	0.027 ± 0.001	6.5 ± 2.3	258 ± 82
	Alanine	31	0.101 ± 0.036	0.098 ± 0.007	27.3 ± 7.0	299 ± 117
	Histidine	31	0.200 ± 0.090	0.202 ± 0.023	13.8 ± 6.6	69 ± 11
2	Threonine	31	0.268 ± 0.067	0.273 ± 0.018	26.5 ± 13.4	101 ± 48
	Valine	31	0.099 ± 0.022	0.098 ± 0.008	16.0 ± 2.9	167 ± 37
	Methionine	31	0.150 ± 0.049	0.152 ± 0.016	2.6 ± 0.8	18 ± 5
	Isoleucine	31	0.101 ± 0.027	0.100 ± 0.008	4.4 ± 1.0	45 ± 13
	Leucine	31	0.164 ± 0.035	0.160 ± 0.009	14.4 ± 3.4	90 ± 21
	Tyrosine	31	0.239 ± 0.059	0.236 ± 0.010	12.6 ± 5.1	53 ± 18
	Phenylalanine	31	0.222 ± 0.055	0.217 ± 0.018	9.7 ± 2.7	45 ± 11
3	Ornithine	31	0.095 ± 0.032	0.093 ± 0.007	4.8 ± 2.0	54 ± 21
	Lysine	31	0.247 ± 0.051	0.241 ± 0.005	29.7 ± 8.6	122 ± 34
	Arginine	31	0.411 ± 0.130	0.408 ± 0.015	18.6 ± 4.3	50 ± 21

CSF/plasma ratios are crude mean values and unstandardized predicted mean values (adjusted for age, gender, and antiepileptic treatment with valproic acid and phenobarbital); CSF and plasma concentrations are measured mean values.

Toone.[12] Vossler et al[19] showed elevated CSF and plasma glutamine levels in valproate-related hyperammonemic encephalopathy. They concluded that glutamine levels may be useful diagnostic adjuncts to the serum ammonia level in the diagnosis of valproate-related hyperammonemic encephalopathy. Although our patients had no clinical signs for hyperammonemic encephalopathy, CSF/plasma ratios for glutamine were significantly elevated when valproic acid was used as antiepileptic drug. This may be caused by increased glutamine synthesis for detoxification of ammonia in the brain and CSF. Ko et al[18] showed further that plasma tyrosine concentrations in convulsive children were significantly elevated when phenobarbital was used as an antiepileptic drug. In our patients, phenobarbital

TABLE 5 **Summary of CSF/Plasma Ratios and CSF and Plasma Concentrations in 39 Patients >3 Years of Age Grouped in Nonessential Neutral Amino Acids, Essential Neutral Amino Acids, and Cationic Amino Acids**

Group	Variable	*n*	CSF/Plasma Ratios, Mean ± SD (Crude Values)	CSF/Plasma Ratios, Mean ± SD (Unstandard Predicted Values)	CSF Concentrations, Mean ± SD, μmol/L	Plasma Concentrations, Mean ± SD, μmol/L
1	Serine	39	0.272 ± 0.071	0.273 ± 0.021	34.4 ± 5.0	132 ± 29
	Asparagine	39	0.114 ± 0.037	0.118 ± 0.008	4.1 ± 1.2	38 ± 10
	Glutamine	39	0.928 ± 0.177	0.914 ± 0.155	498.3 ± 98.4	543 ± 83
	Proline	18	0.035 ± 0.014	0.031 ± 0.006	4.7 ± 1.6	146 ± 54
	Glycine	39	0.024 ± 0.011	0.025 ± 0.002	6.2 ± 1.5	279 ± 85
	Alanine	39	0.082 ± 0.022	0.084 ± 0.008	23.2 ± 4.4	300 ± 100
	Histidine	39	0.207 ± 0.090	0.205 ± 0.015	14.5 ± 6.7	72 ± 12
2	Threonine	39	0.259 ± 0.048	0.255 ± 0.014	25.1 ± 7.1	99 ± 30
	Valine	39	0.077 ± 0.015	0.078 ± 0.008	14.6 ± 2.9	192 ± 32
	Methionine	39	0.146 ± 0.035	0.145 ± 0.008	2.8 ± 0.7	19 ± 4
	Isoleucine	39	0.070 ± 0.022	0.071 ± 0.013	3.7 ± 1.1	55 ± 16
	Leucine	39	0.127 ± 0.026	0.130 ± 0.013	13.7 ± 2.7	111 ± 28
	Tyrosine	39	0.216 ± 0.043	0.219 ± 0.021	10.5 ± 2.8	48 ± 7
	Phenylalanine	39	0.200 ± 0.040	0.203 ± 0.020	8.8 ± 1.6	45 ± 8
3	Ornithine	39	0.078 ± 0.024	0.080 ± 0.010	3.4 ± 0.8	46 ± 13
	Lysine	39	0.209 ± 0.062	0.213 ± 0.016	26.7 ± 5.9	134 ± 26
	Arginine	39	0.378 ± 0.187	0.380 ± 0.033	18.5 ± 3.8	54 ± 16

CSF/plasma ratios are crude mean values and unstandardized predicted mean values (adjusted for age, gender, and antiepileptic treatment with valproic acid and phenobarbital); CSF and plasma concentrations are measured mean values.

TABLE 6 **Summary of CSF/Plasma Ratios Shown in Tables 4 and 5 and Published in or Calculated From Literature**

Group	Variable	Mean[a]	Mean[a]	Mean[b]	Mean[c]	Mean[d]	Mean[e]	Mean[f]	Mean[g]
1	Serine	0.328	0.272	0.304	0.203	0.250	0.153	0.23	0.227
	Asparagine	0.134	0.114	0.126	0.178	0.147	—	0.12	0.238
	Glutamine	0.757	0.928	—	—	1.000	—	0.86	0.828
	Proline	0.033	0.035	—	—	—	—	—	0.023
	Glycine	0.027	0.024	0.027	0.028	0.029	0.029	0.02	0.039
	Alanine	0.101	0.082	0.099	0.077	0.085	0.082	0.08	0.098
	Histidine	0.200	0.207	0.072	0.174	0.141	0.100	0.16	—
2	Threonine	0.268	0.259	0.254	0.333	0.217	0.238	0.25	0.179
	Valine	0.099	0.077	0.072	0.096	0.079	0.078	0.07	0.093
	Methionine	0.150	0.146	0.144	0.142	0.135	0.069	0.10	0.205
	Isoleucine	0.101	0.070	0.097	0.098	0.081	0.090	0.09	0.107
	Leucine	0.164	0.127	0.118	0.123	0.112	0.093	0.10	0.096
	Tyrosine	0.239	0.216	0.193	0.203	0.154	0.109	0.14	0.173
	Phenylalanine	0.222	0.200	0.252	0.257	0.172	0.108	0.17	0.155
3	Ornithine	0.095	0.078	0.083	0.080	0.102	—	0.06	0.082
	Lysine	0.247	0.209	0.128	0.126	0.147	0.122	0.12	0.188
	Arginine	0.411	0.378	0.250	0.270	0.233	0.213	0.31	0.214

— indicates no data.

[a] Data are measured means.

[b] Data are from Applegarth et al[9] and include plasma ($n = 27$), CSF ($n = 21$), ages 3 months to 6 years (plasma), ages 3 months to 10 years (CSF), no paired samples, and CSF/plasma ratios calculated as quotient of mean values.

[c] Data are from Gerrits et al[13] and include plasma ($n = 26$), CSF ($n = 12$), age 2 to 59 months (plasma), age 3 days to 12 months (CSF), no paired samples, and CSF/plasma ratios calculated as quotients of the 50th percentile.

[d] Data are from Perry et al[14] and include plasma ($n = 77$), CSF ($n = 47$), age 14 to 56 years (plasma), age 14 to 74 years (CSF), no paired samples, and CSF/plasma ratios calculated from plasma/CSF ratios.

[e] Data are from Plum[16] and include paired CSF and plasma samples ($n = 15$) and CSF/plasma ratios calculated from plasma/CSF ratios.

[f] Data are from McGale et al[17] and include paired CSF and plasma samples ($n = 37$) and age 10 to 69 years.

[g] Data are from Kruse et al[15] and include paired CSF and plasma samples ($n = 72$) and age 16 to 85 years.

therapy had a significant influence on the CSF/plasma ratio for serine, asparagine, glutamine, valine, methionine, and phenylalanine, with higher values for those patients treated with phenobarbital. The reason for this finding was not clear until now, because these amino acids are not transported by the same carrier across the BBB and back. In contrast, CSF/plasma ratio for tyrosine was not influenced by phenobarbital therapy.

CSF/plasma ratios are only an estimation of the sum of all of the transport mechanisms (influx and efflux) at the BBB, the blood-CSF barrier, and between extracellular fluid and CSF, but the only possibility available in vivo. Possibly, CSF/plasma ratios can help differentiating between amino acid accumulation produced in CNS or in the periphery. In phenylketonuria (McKusick's Mendelian Inheritance in Man catalogue no. 261600), the best known amino acid disorder, phenylalanine hydroxylase deficiency results in the accumulation of phenylalanine and its ketones in the brain with cerebral damage. Antoshechkin et al[20] reported on phenylalanine and tyrosine concentrations in CSF and plasma in 8 phenylketonuria patients (average age: 2.5 years). The mean phenylalanine CSF/plasma ratio (0.277) was not elevated compared with our reference values (0.222), but the tyrosine CSF/plasma ratio was higher (0.347 vs 0.239). Similar results were found by Ratzmann et al,[21] who investigated 6 phenylketonuria patients. The underlying mechanism for these findings is unknown.[21] In 2 of our patients (not included as references) with hyperammonemic coma because of arginase or ornithine transcarbamylase deficiency, CSF/plasma ratios for glutamine were massively elevated (7.407 and 3.580 vs 0.757; Table 4), indicating synthesis and/or trapping of glutamine in the brain (S. Scholl-Bürgi, unpublished results, 2007). For both patients, CSF/plasma ratios of all of the essential neutral amino acids (group 2) were elevated, whereas CSF/plasma ratios for cationic amino acids, especially arginine, were within the reference range or sightly decreased (group 3, S. Scholl-Bürgi, unpublished results, 2007). 3-Phosphoglycerate dehydrogenase deficiency, an inborn disorder of serine biosynthesis, leads to low CSF and plasma serine concentrations and is associated with congenital microcephaly, intractable seizures, and mental retardation.[22] de Koning et al[23] reported CSF and plasma serine concentrations in 4 patients with 3-phosphoglycerate dehydrogenase deficiency; the CSF/plasma ratios were 0.285 (case 5, age: 10 months), 0.207 (case 1, age: 1 year), 0.125 (case 3, age: 5 years), and 0.122 (case 4, age: 7 years). Especially in the 2 older patients, these ratios were decreased compared with our patients, indicating an even more pronounced serine deficiency of the brain compared with plasma.[23] These examples lead to the conclusion that, in metabolic diseases, which affect CNS and peripheral organs, amino acid CSF/plasma ratios will change because of the degree of metabolic disturbance. In contrast, in diseases with changes only detectable in the liver, CSF/plasma ratios will remain unchanged, indicating that the ratio of influx to efflux of the amino acid occurs in the same proportion. However, the primary metabolic changes may lead to secondary changes in the transport of other amino acids like tyrosine in phenylketonuria

and glutamine in arginase deficiency. We speculate further that, through changes seen in CSF/plasma ratios, new primary inborn errors of amino acid transport over the BBB will be detected. Therefore, it is important to have reference values as shown in this study.

CONCLUSIONS

This is the first time that the effect of age, gender, and antiepileptic therapy on amino acid CSF/plasma ratios in children has been investigated with the result that CSF/plasma ratios in particular for essential neutral amino acids and for serine, asparagine, and glutamine may be influenced by age, gender, and antiepileptic therapy.

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