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Note

Glutamate and glutamine in cerebrospinal fluid and serum from healthy volunteers — analytical aspects

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There are several reports of deviating concentrations of the amino acid glutamate in cerebrospinal fluid (CSF) from patients suffering from schizophrenia or affective disorders [1–3]. However, these findings may be questioned since results in CSF from healthy subjects are inconsistent between different groups. Kim et al. [1], who found differences between healthy subjects and schizophrenics, used an enzymatic method for the determination of glutamate, whereas Perry [4], who used an amino acid analyser, failed to find any differences. The control levels for glutamate in CSF were 25.8 and 1.3 nmol/ml, respectively. The control levels for glutamate in CSF also differ considerably between different laboratories using the same analytical method. Those using an amino acid analyser reported values in the range 1–15 nmol/ml [3,4]. High-performance liquid chromatography (HPLC) with precolumn derivatization gave values between 0.5 and 3 nmol/ml [5–7]. One reason for the spread results may be an in vitro hydrolysis in CSF of glutamine to glutamate. The concentration of glutamine in CSF is ca. 600 nmol/ml. Since hydrolysis of glutamine may be caused by glutaminase, an acid is usually added to the CSF samples to prevent the enzymic activity. However, acidic hydrolysis of glutamine may occur. In order to overcome these problems the samples were deproteinized with ethanol [8] and analyzed by HPLC following derivatization with *o*-phthaldialdehyde (OPT) [9].

The purpose of the present study was to determine the concentrations of glutamate and glutamine in CSF from healthy volunteers at the moment of sampling and to study the in vitro changes of the amino acid concentrations that might occur between sampling and analysis.

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The CSF was collected in fractions in order to investigate if there were concentration gradients of glutamate and glutamine.

EXPERIMENTAL

Reagents and chemicals

Methanol for liquid chromatography was obtained from May and Baker (Dagenham, U.K.). Glutamine, glutamic acid and cysteic acid were from Sigma (St. Louis, MO, U.S.A.). OPT was prepared and handled according to the method used by Lindroth and Mopper [9], except that the concentration of OPT was doubled to ensure an excess of reagent. The phosphate buffer (pH 6.0; 0.067 *M*) was prepared by dissolving potassium dihydrogenphosphate (7.96 g) and disodium hydrogenphosphate heptahydrate (2.2 g) in 1000 ml of water. The borate buffer was prepared from a boric acid solution (0.4 *M*) and was adjusted to pH 9.5 with 1 *M* sodium hydroxide.

Chromatography

HPLC was carried out using Spectra-Physics System 3500 B equipment and with a Valco rotary valve injector with 10-μl loop for syringe injection. The fluorescence detector was an SFM 22 Model from Kontron (Stockholm, Sweden) equipped with a 10-μl flow-through cell. The recorder was a W + W 600 Tarkan. A 150 mm × 4.0 mm I.D. reversed-phase column packed with 5-μm C_{18} Nucleosil (Macherey Nagel, Düren, F.R.G.) was used.

The mobile phase was a mixture of (A) phosphate buffer (pH 6.0) and (B) methanol. The flow-rate was 1.2 ml/min. The detector settings were: excitation, 330 nm; emission 455 nm. The analysis of glutamine was carried out in isocratic mode with a mobile phase of 55% A–45% B. Glutamate was analyzed with a linear gradient from 20% B to 70% B in 20 min.

Subjects

The volunteers were Caucasian subjects with an age range of 22–38 years. Five men and five women volunteered for the study. The health of the subjects was checked by routine physical examination, blood and urine tests. No abnormality was found. No one had a history of severe head injury, abuse, somatic or psychiatric disease. All subjects obtained ratings below 1 for all items on the Comprehensive Psychopathological Rating Scale (CPRS) [10]. No subjects were on medication.

Sampling of CSF and serum

The subjects were admitted to a research ward on the evening before the lumbar punctures were made. They were fasting from 8 p.m. and the lumbar punctures were taken between 8 and 9 a.m. after the subjects had rested in bed for at least 8 h. The samples were taken with the subjects in a sitting position. The punctures were made between L4 and L5 for all subjects but one (L2–L3) and the CSF was collected in twelve fractions. The odd-numbered fractions 1, 3, 5, 7, 9 and 11 (0.5 ml each) were collected in tubes that were kept on ice containing 1.5 ml of 99%

TABLE I

CONCENTRATIONS OF GLUTAMATE AND GLUTAMINE IN SERUM FROM ONE HEALTHY SUBJECT

Time (h)	Glutamate (%)		Glutamine (%)	
	Serum I*	Serum II**	Serum I*	Serum II**
0	100***	100***	100***	100***
0.5	99	–	87	–
1	119	97	88	98
2	210	113	103	100

*The blood was kept at room temperature for 0–2 h before centrifugation.
**The blood was immediately centrifuged and the serum fraction was kept at room temperature. All samples were analyzed in duplicate.
***The concentrations of glutamate and glutamine in serum were 18.2 and 685 nmol/ml, respectively.

ethanol. The tubes were weighed both before and after sampling. Immediately after the samples were withdrawn, they were mixed and centrifuged at 2000 *g* for 10 min. The supernatants were kept at −80 °C pending the analysis of glutamate and glutamine. The even-numbered fractions 2, 4, 6, 8, 10 and 12 (1.5 ml each) were also collected in tubes that were kept on ice. The in vitro changes of glutamate and glutamine in the fluid were studied by taking a 100-μl aliquot from each of the even-numbered fractions. The samples were kept for 1 h at 37 °C before deproteinization.

Blood samples were collected by venipuncture and centrifuged within 30 min at 2000 *g* for 10 min. Two 100-μl serum samples were mixed with two 300-μl portions of 99% ethanol and centrifuged after 10 min. The supernatants were stored at −80 °C pending the analysis of glutamate and glutamine. The in vitro changes of glutamate and glutamine in serum were studied in the blood taken from one of the healthy volunteers: 20 ml of blood were collected by venipuncture and 10 ml were centrifuged within 3 min. Samples of the serum were deproteinized with ethanol at different periods of time as indicated in Table I. The remaining 10 ml of blood were stored at room temperature, and serum was prepared and deproteinized after 0.5, 1 and 2 h.

Sample preparation

Glutamine in CSF and serum. Deproteinized CSF or serum (50 μl) was added to borate buffer (500 μl; pH 9.5) containing the internal standard cysteic acid (6 nmol). Part of the sample (20 μl) was mixed with OPT reagent (50 μl). After 4 min at room temperature, 10 μl of the reaction mixture were injected into the column.

Glutamate in CSF and serum. Cysteic acid (0.8 nmol) in water (25 μl) was added to deproteinized CSF or serum (100 μl). Part of the sample (20 μl) was mixed with the OPT reagent (50 μl) and treated in the same way as the glutamine samples.

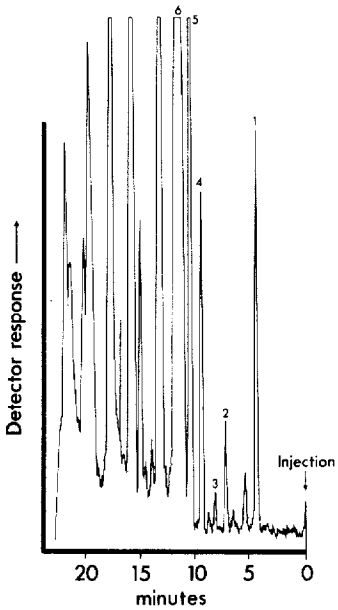

Fig. 1. HPLC of amino acids in CSF as OPT derivatives. Column, 150×4.0 mm I.D. Nucleosil C_{18} (5 μm); gradient, from 20% B to 70% B in 20 min; flow-rate, 1.2 ml/min; injected amounts, 10 μl. Peaks: 1=cysteic acid (internal standard); 2=unknown; 3=glutamate; 4=asparagine; 5=serine; 6=glutamine.

RESULTS

Analysis of glutamate in CSF and serum

Fig. 1 shows a chromatogram of the amino acids in CSF as OPT derivatives. The injected amount corresponded to ca. 1 μl CSF and a glutamate concentration of 0.39 nmol/ml. Cysteic acid, which was used as an internal standard, is not a natural constituent of human CSF or serum. The pH (6.0) of the eluting buffer was of great importance, as a change in pH caused interaction of the glutamate peak with the surrounding peaks. It was not possible to analyse glutamine at the same time as glutamate since its concentration exceeded the linear range of the detector. The detection limit for glutamate in CSF was ca. 0.1 nmol/ml, which gave a detector signal twice the signal-to-noise ratio. The experimental error and the recovery of glutamate from CSF and serum are shown in Table II.

Glutamate and glutamine in CSF and serum from healthy volunteers

The concentrations of glutamate and glutamine in CSF samples were calculated for each subject by taking a mean value of the six fractions (Table III). The range for glutamate was 0.09–0.56 nmol/ml. There was no indication of a gradient for either glutamate or glutamine in CSF. The concentrations tended to be a little higher in the fractions collected first (Fig. 2). The male volunteers had significantly higher levels of glutamine and also tended to have higher levels of glutamate in CSF than the female volunteers (Table III). There was no significant correlation between the glutamate and the glutamine levels in CSF. The analysis

TABLE II

EXPERIMENTAL ERROR AND RECOVERY FOR THE ANALYSIS OF GLUTAMATE (Glu) IN CSF AND SERUM

Sample	*n*	Glu (nmol/ml)	S.D. (%)	Recovery (%)
Frozen pool of CSF	4	1.3	8.1	–
Plus Glu (1.04 nmol/ml)	4	2.2	6.9	87
Individual fresh CSF samples analysed in duplicate at different days	6	0.23	21	–
Fresh pool of serum	1	45	–	–
Plus Glu (31 nmol/ml)	5	78	3.7	104

of glutamate and glutamine in deproteinized CSF was carried out between fourteen days and nine months after sampling. There was no significant correlation between the concentration of the amino acids and the time of storage.

The range for the concentration of glutamate in serum was 12–28 nmol/ml (Table III). There was no significant correlation between glutamate and glutamine in serum. All the serum samples were analysed in the same analysis ca. seven months after sampling. There were significant correlations between the CSF and serum levels of glutamate ($r=0.67$; $p<0.05$) and glutamine ($r=0.84$; $p<0.01$), respectively.

In vitro changes

In CSF samples that had been kept for 1 h at 37°C, the glutamate level was significantly increased ($p<0.01$) and the glutamine level significantly decreased ($p<0.05$) (Fig. 2). The mean increase of the glutamate concentration was 0.18

TABLE III

CONCENTRATIONS OF GLUTAMATE AND GLUTAMINE IN CSF AND SERUM FROM HEALTHY VOLUNTEERS

Results are mean ± S.D. ($n=5$ in each group).

Compound	CSF			Serum		
	Concentration (nmol/ml)		Male versus female	Concentration (nmol/ml)		Male versus female
	Male	Female	*p* value*	Male	Female	*p* value*
Glutamate	0.40 ± 0.13	0.28 ± 0.13	>0.1	20.0 ± 6.0	19.0 ± 6.7	>0.1
Glutamine	618 ± 41	542 ± 46	<0.05	734 ± 88	614 ± 90	<0.1

*Students *t*-test.

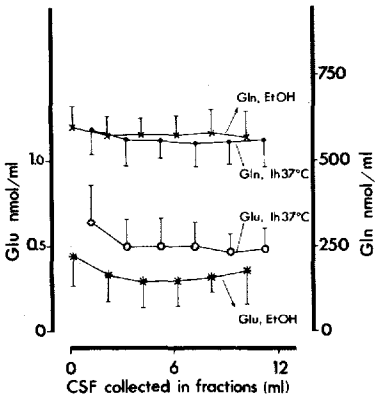

Fig. 2. Concentrations of glutamate (Glu) and glutamine (Gln) in CSF from ten healthy volunteers. The CSF was immediately deproteinized with ethanol or stored for 1 h at 37 °C before deproteinization.

nmol/ml (53%) and the mean decrease of the glutamine concentration was 19 nmol/ml (3.3%). There was a significant negative correlation between the basal glutamate level and the increase of glutamate ($r = -0.68$; $p < 0.05$) and a significant positive correlation between the basal glutamate level and the decrease of glutamine ($r = 0.72$; $p < 0.05$). There were no significant correlations between the changes of glutamate or glutamine and the basal glutamine level.

The glutamine level in serum did not change significantly with time irrespective of whether the samples were centrifuged or stored as whole blood (Table I). The glutamate level started to increase after 1 h and had increased by 100% in serum from blood stored for 2 h at room temperature (Table I).

DISCUSSION

The amount of glutamate in the CSF from healthy volunteers was low in comparison with the published results [1,4,7]. The discrepancy of the results may partly be due to differences in how and when the samples were deproteinized as demonstrated. The difference between the value in the present study (0.34 nmol/ml) and the value in an earlier study (0.53 nmol/ml) [6] in which CSF was deproteinized ca. 30 min after the sampling indicates that it is necessary to precipitate the proteins immediately after the lumbar puncture to obtain reproducible results. Freezing the samples may not be sufficient, as we have noticed an increase of the glutamate level in CSF samples kept at −20 °C. Following immediate deproteinization with ethanol no increase of the glutamate level was observed, even if the samples were stored for nine months at −80 °C.

The glutamate level in CSF increased by 0.18 nmol/ml (53%) when the samples were kept for 1 h at 37 °C. This was in agreement with Ferraro and Hare [11] who noticed a 50% increase of the glutamate level in CSF after 4 h at room temperature. However, it is not likely that the published high glutamate levels (above 1 nmol/ml) in CSF are mainly caused by enzymatic hydrolysis of glutamine after sampling; they are more likely to be due to acidic hydrolysis or other unspecified

differences between the methods. The decrease of glutamine exceeded the formation of glutamate by ca. 19 nmol/ml. It is possible that glutamine was transformed into pyrrolidonecarboxylic acid [12], a compound that we were not able to measure with our method.

The concentrations of glutamate and glutamine in serum were in agreement with the published values [13]. The rapid increase of the glutamate level in serum that occurred when the samples were stored as full blood shows that it is necessary to centrifuge the samples as soon as possible.

The lack of a gradient for glutamate and glutamine in CSF and the findings of significant correlations between their serum and CSF levels suggests that a substantial part of their concentration in CSF is derived from the blood. This is supported by evidence of both active [14] and passive [15] transport of amino acids across the human blood–CSF barrier. The high levels of glutamine may also indicate that extracellular glutamine is not taken up by nervous tissue [15]. In contrast, the existence of effective uptake mechanisms for glutamate may explain its low levels in CSF [16].

The present study demonstrated the possibility of reproducibly analysing low levels of glutamate in CSF without interference from glutamine or changes in concentrations with time.

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