Stability of Neuroactive Amino Acids in Cerebrospinal Fluid under Various Conditions of Processing and Storage, Adriano Anesi,1* Mariangela Rondanelli,2 and Gianrico Melzi d’Eril3 1 Laboratorio Analisi, Ospedale Maggiore, Piazza Ospedale, 10, 26900 Lodì, Italy; 2 Dipartimento di Medicina Interna, Università di Pavia, 27900 Pavia, Italy; 3 Laboratorio Analisi, Ospedale di Circolo, Università dell’Insubria, 21100 Varese, Italy; * author for correspondence: fax 371 422138, e-mail anesiadr@tin.it

Free amino acids (AAs) are widely distributed within the central nervous system and play an important role as transmitters of synaptic excitation and/or modulators of synaptic function (1). Because the content of free AAs in cerebrospinal fluid (CSF) has been demonstrated to correlate with brain AA content (2), a reliable measurement of CSF AA concentrations is a potentially useful tool for investigating central nervous system function. Changes in concentrations of AAs with known excitatory function, e.g., aspartate (ASP) and glutamate (GLU), or putative inhibitory function, e.g., glycine (GLY) and taurine (TAU), play an important role in the physiopathology of several neuropsychiatric disorders (3). Varying AA concentrations have been found in the CSF in controls and in neurological disorders. Increased, decreased, and unchanged concentrations of GLU have been observed in the CSF of epileptic patients (4–6). Initial studies provided evidence for increased concentrations of GLU and ASP in the CSF of patients with amyotrophic lateral sclerosis (7–8); however, subsequent studies reported CSF concentrations of ASP and GLU that were more than an order of magnitude lower (9).

These discrepancies have been explained as the effects of different analytical methods or as the result of a lack of systematic evaluation of basic variables (e.g., age, sex, and severity of disease) (10); however, inappropriate CSF sample processing and storage could also explain these conflicting results.

The best method of collection and storage of CSF samples has still not been established (11). We therefore tried several strategies to obtain stable AA concentrations over time.

Samples from five healthy subjects hospitalized for lumbar disk herniation were taken. Each CSF sample was split into several aliquots, which were processed in different ways. An aliquot of the freshly collected sample was deproteinized with ice-cold 100 g/L trichloroacetic acid (TCA; 1:0.2, by volume) and analyzed within 10 min of collection. One group of aliquots was immediately stored untreated at −20 and −80 °C, respectively. A second group was immediately deproteinized with TCA and stored at −20 and −80 °C, respectively. A third group was immediately deproteinized with TCA; after centrifugation (3000g for 10 min at 4 °C) the clear supernatant was immediately adjusted to pH 7.3 with sodium bicarbonate and stored at −20 and −80 °C, respectively.

ASP, GLU, GLY, and TAU were measured by HPLC, using o-phthalaldehyde precolumn derivatization and electrochemical plus fluorometric detection in series (12), in aliquots that had been stored for 1, 2, 3, 7, 14, or 30 days. A Bio-Rad 1350 pump apparatus coupled to a Coullochem 5100A electrochemical detector (ESA Inc.) with a coulometric analytical cell (Model 5011) and a Jasco FP-920 fluorescence detector (Jasco International) was used with a C18 Rosil HL (150 × 4.6 mm i.d.; Bio-Rad Laboratories) reversed-phase column. The phosphate buffer, prepared from 25 mmol/L potassium phosphate and 150 mL/L acetonitrile, was applied as the mobile phase. The flow rate was 1.0 mL/min. The AA content was evaluated after comparison with an external AA calibrator solution.

The data from CSF specimens, as mean values (μmol/L ± SD, are shown in Table 1. Repeated measures ANOVA with the Bonferroni correction for repeated measures was used to compare the results of the stored specimens with the values of the freshly collected samples.

In the untreated CSF samples, ASP was significantly increased (P < 0.001) after 2 days in both storage conditions; GLU increased significantly (P < 0.001) through the second day of storage but decreased progressively from the third day of storage, when stored at either −20 or −80 °C; GLY and TAU showed a significant decrease (P < 0.001) when stored at −20 or −80 °C for >14 days.

The acid-treated CSF specimens had significantly higher ASP values when stored at −20 or −80 °C for >7 days and significantly lower GLY and TAU values when stored at −20 or −80 °C for >14 days than did the fresh aliquots (P < 0.001); the increase of GLU concentrations was progressive and became significant after 1 day of storage at −20 or −80 °C.

Finally, no significant difference was found in AA concentrations of CSF samples stored neutralized at −20 or −80 °C during the 30-day period.

The spurious increase of GLU and ASP concentrations is probably related to the hydrolysis of glutamine, asparagine, and possibly peptides either by enzymes present in the CSF or by incautious use of the TCA used to deproteinize the specimens (11). The pattern of time-related GLU changes in untreated CSF suggests that different types of enzymatic processes could occur in CSF frozen samples, causing the slow formation of new GLU from glutamine or proteins during the first 2 days of storage and the degradation of GLU with the time of storage. This interpretation is supported by previous findings of different enzymes involved in GLU metabolism in the CSF (13).

Thus, the metabolic instability of AAs in CSF, with the possibility of in vitro formation or degradation according to the activation of different enzymes, and the artificial increase of AAs caused by the current methods of CSF acidification to inactivate enzymes could explain the discrepancies in AA concentrations reported in the literature.

From our study, we consider that the native (untreated) CSF can be stored for no longer than a few hours. For a very short storage period, no more than 1 day, we would suggest that the CSF be deproteinized immediately with TCA and that the supernatant be stored either at −20 or at
−80 °C. For a storage period of up to 30 days, we believe that the best procedure of obtaining stable AA concentrations is to deproteinize samples with TCA immediately and neutralize the supernatant of the acidified CSF after centrifugation. After this treatment the sample can be stored at −20 °C. Thus, this method of processing and storing CSF is proposed to avoid artifactual changes of AA concentrations caused by in vitro modifications of the AAs.

Table 1. Effects of temperature and duration of sample storage on the measurement of neuroactive amino acids in CSF treated in different ways.

<table>
<thead>
<tr>
<th>CSF processing</th>
<th>Amino acid</th>
<th>°C</th>
<th>Fresh</th>
<th>2</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated CSF</td>
<td>ASP</td>
<td>−20</td>
<td>0.13 ± 0.03</td>
<td>0.14 ± 0.03</td>
<td>0.16 ± 0.04b</td>
<td>0.21 ± 0.05b</td>
<td>0.22 ± 0.05b</td>
<td>0.22 ± 0.05b</td>
</tr>
<tr>
<td></td>
<td>GLU</td>
<td>−20</td>
<td>0.60 ± 0.21</td>
<td>0.68 ± 0.20b</td>
<td>0.52 ± 0.17b</td>
<td>0.40 ± 0.11b</td>
<td>0.34 ± 0.09b</td>
<td>0.26 ± 0.02b</td>
</tr>
<tr>
<td></td>
<td>GLY</td>
<td>−20</td>
<td>5.60 ± 0.75</td>
<td>5.60 ± 0.67</td>
<td>5.55 ± 0.68</td>
<td>5.46 ± 0.68</td>
<td>5.44 ± 0.68</td>
<td>4.86 ± 0.67b</td>
</tr>
<tr>
<td></td>
<td>TAU</td>
<td>−20</td>
<td>6.54 ± 1.32</td>
<td>6.56 ± 1.30</td>
<td>6.56 ± 1.34</td>
<td>6.60 ± 1.28</td>
<td>6.40 ± 1.34</td>
<td>6.08 ± 1.30b</td>
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<tr>
<td>Acid-treated CSF</td>
<td>ASP</td>
<td>−20</td>
<td>0.13 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.19 ± 0.04b</td>
<td>0.19 ± 0.05b</td>
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<tr>
<td></td>
<td>GLU</td>
<td>−20</td>
<td>0.60 ± 0.21</td>
<td>0.72 ± 0.15b</td>
<td>0.77 ± 0.18b</td>
<td>0.85 ± 0.22b</td>
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<td>−20</td>
<td>5.60 ± 0.75</td>
<td>5.54 ± 0.67</td>
<td>5.62 ± 0.79</td>
<td>5.60 ± 0.76</td>
<td>5.56 ± 0.78</td>
<td>4.96 ± 0.88b</td>
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<tr>
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<td>TAU</td>
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<td>6.54 ± 1.32</td>
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<td>6.56 ± 1.28</td>
<td>6.56 ± 1.30</td>
<td>6.38 ± 1.31</td>
<td>6.02 ± 1.34b</td>
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<td>Neutral CSF</td>
<td>ASP</td>
<td>−20</td>
<td>0.13 ± 0.03</td>
<td>0.11 ± 0.01</td>
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<td>0.11 ± 0.01</td>
<td>0.12 ± 0.02</td>
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<tr>
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<td>GLU</td>
<td>−20</td>
<td>0.60 ± 0.21</td>
<td>0.60 ± 0.21</td>
<td>0.61 ± 0.20</td>
<td>0.62 ± 0.21</td>
<td>0.63 ± 0.19</td>
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<td>6.56 ± 1.28</td>
<td>6.52 ± 1.29</td>
<td>6.68 ± 1.52</td>
<td>6.66 ± 1.28</td>
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</tbody>
</table>

* Results are expressed in μmol/L, mean ± SD.
* Significantly different (P < 0.001) vs freshly analyzed samples (ANOVA).

References

Genotyping Method for Methyleneetetrahydrofolate Reductase (C677T Thermolabile Variant) Using Heteroduplex Technology. Zoe E. Clark,1 Derrick J. Bowen,2 Sharon D. Whalley,1 Michael F. Bellamy,3 Peter W. Collins,2 and Ian F. W. McDowell7* (Departments of 1 Medical Biochemistry, 2 Haematology, and 3 Cardiology, University of Wales College of Medicine, Cardiff CF4 4XN, United Kingdom; *author for correspondence: fax 44-0-1222-766276, e-mail Mcdowell@cf.ac.uk)

Low folate status, with associated increases of plasma homocysteine concentrations, has been implicated as a cause of neural tube defects and premature cardiovascular...