Acta Medica Okayama

Volume 41, *Issue* 4 1987 *Article* 5

AUGUST 1987

Effect of long-term storage on monoamine metabolite levels in human cerebrospinal fluid.

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Abstract

Concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were measured in human cerebrospinal fluid (CSF) following long-term storage at -20 degrees C for intervals of three to 60 months. No significant changes in HVA levels were detected in CSF stored for up to 60 months. On the other hand, 5-HIAA concentrations remained stable for up to 6 months, but decreased significantly in the specimens stored for longer time intervals. The results indicate that 5-HIAA should be determined within 6 months after CSF collection, while HVA determinations may be delayed.

KEYWORDS: stability, homovanillic acid, 5-hydroxyindoleacetic acid, cerebrospinal fluid

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*PMID: 2444074 [PubMed - indexed for MEDLINE]

Acta Med Okayama 41 (4) 179-181 (1987)

— Brief note —

Effect of Long-term Storage on Monoamine Metabolite Levels in Human Cerebrospinal Fluid

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Concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were measured in human cerebrospinal fluid (CSF) following long-term storage at −20°C for intervals of three to 60 months. No significant changes in HVA levels were detected in CSF stored for up to 60 months. On the other hand, 5-HIAA concentrations remained stable for up to 6 months, but decreased significantly in the specimens stored for longer time intervals. The results indicate that 5-HIAA should be determined within 6 months after CSF collection, while HVA determinations may be delayed.

Key words : stability, homovanillic acid, 5-hydroxyindoleacetic acid, cerebrospinal fluid

The measurement of monoamine metabolite levels in human cerebrospinal fluid (CSF) has been widely used to investigate monoamines in neuropsychiatric disorders because of the evidences that these levels reflect the turnover of their parent amines in the brain (1). In clinical studies, however, a variety of factors are known to affect metabolite levels in CSF: age, sex and body height of the subjects, CSF dynamics, rostrocaudal concentration gradients, diurnal rhythms, physical activity, food intake and drugs used. Therefore, consideration of these variables is important to the drawing of accurate conclusions from the CSF findings and also when comparing results from various laboratories. While a number of studies have been carried out with respect to these factors, little is known under what conditions of storage are the monoamine metabolite levels in CSF stable. Since entire patient populations usually can not undergo simultaneous CSF sampling, failure to consider sample stability may result in artifactual variations.

To investigate the stability of monoamine metabolites in CSF, we measured homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), the respective metabolites of dopamine and serotonin, twice within two weeks of CSF collection and after storage for three to 60 months.

CSF obtained from 152 patients with a variety of neuropsychiatric disorders was used in this study. Lumbar punctures were performed between 9-10 AM with the patient in the lateral decubitus position. After collecting CSF in the usual manner, the samples were immediately placed on ice at bedside and then stored at −20°C until analysis. Some samples were thawed and refrozen twice or three times between determinations. All samples utilized were drawn in the course of essential diagnostic pro-

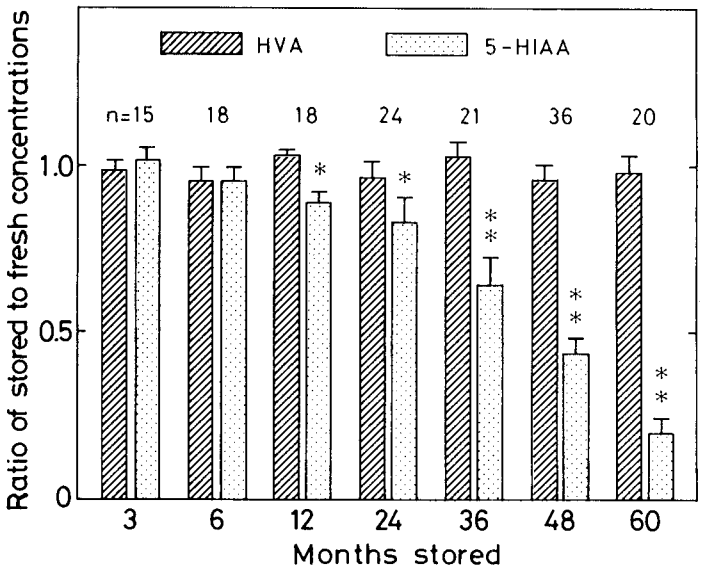

Fig. 1 Mean concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in CSF stored at −20°C for the length of time indicated. Columns and bars represent means ± SEM, respectively, as ratios to control values determined within two weeks of CSF collection. *, $p < 0.05$; **, $p < 0.001$ vs. controls.

cedures.

Concentrations of HVA and 5-HIAA were determined in duplicate by high-performance liquid chromatography (HPLC) employing electrochemical detection as described elsewhere (2). In brief, 0.5 ml of CSF, adjusted to pH 2-3 with two drops of concentrated formic acid, was passed through a column of Sephadex G-10. After washing the column with 3.5 ml of 0.01 N formic acid and 1.0 ml of 0.005 M phosphate buffer (pH 8.5), HVA and 5-HIAA were eluted with 1.5 ml phosphate buffer. The eluate was then dried under a reduced pressure using a centrifugal evaporator. The dried residue was dissolved in 50 μl of the mobile phase, and 10 μl was injected into the HPLC system. Since this study extended over a long period of time, the metabolites were determined in some cases under different conditions of HPLC apparatus and column, methanol concentration and flow rate of the mobile phase. Student's t-test was used for the statistical analysis.

The results from analysis of CSF specimens stored for various intervals up to 60 months are shown in Fig. 1 as ratios to the values determined within two weeks of CSF collection. The ratios of stored sample versus fresh sample HVA concentrations were 0.98 ± 0.05, 0.96 ± 0.04, 1.03 ± 0.03, 0.98 ± 0.04, 1.05 ± 0.06, 0.97 ± 0.04 and 0.99 ± 0.06 for the storage periods of 3, 6, 12, 24, 36, 48 and 60 months, respectively. Thus HVA concentrations remained unchanged in CSF specimens stored at −20°C for at least 60 months. On the other hand, the ratios of 5-HIAA concentrations were 1.03 ± 0.05, 0.96 ± 0.04, 0.90 ± 0.04, 0.85 ± 0.07, 0.66 ± 0.08, 0.44 ± 0.05 and 0.20 ± 0.04 for the same respective storage periods. Although 5-HIAA was found to be stable for intervals up to 6 months following CSF collection, storage over longer periods resulted in a statistically significant decrease in 5-HIAA levels as a function of time.

Banki reported no significant loss in the HVA and 5-HIAA concentrations until 8-10 weeks, if CSF samples were supplemented with 1% cysteine, immediately frozen at −20°C and protected from light (3). Wode-Helgodt *et al.* also demonstrated that storage of samples for 6 months below −20°C did not result in any significant decrease in the levels of HVA, 5-HIAA and 3-methoxy-

4-hydroxyphenylethylene glycol (MHPG), the major metabolite of norepinephrine in the brain (4). The present findings were in good agreement with these data. However, it was found that HVA remains stable for a longer period than previously reported. Although we reported preliminarily that 5-HIAA concentrations did not change for 12 months when stored at −20°C (5), approximately a 10% decrease in 5-HIAA levels was demonstrated during 6 to 12 months of storage in this study. Since Linnoila *et al.* reported that the acidic metabolite levels were unaffected during a period of 36 months when stored at −60°C (6), it might be necessary to freeze CSF at a lower temperature to extend the stability of 5-HIAA.

On the other hand, Langlais *et al.* reported that HVA, 5-HIAA and MHPG remain stable at room temperature for at least 24 h and at −4°C for 72 h following CSF collection (7). They also demonstrated that freezing and thawing do not affect metabolite levels, and that addition of ascorbic acid to samples in order to prevent oxidation of the parent amines and subsequent increase in metabolite levels is not necessary. The present study shows that samples for determinations of the monoamine metabolites do not require the addition of preservatives for their extended storage.

In conclusion, the present study shows that the reliable determination of the HVA level could be carried out after long-term storage of CSF at −20°C, while the 5-HIAA level should be determined within 6 months of CSF collection.

Acknowledgment. We thank Ms. Fumiko Wakasa for her technical assistance.

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Received: March 27, 1987
Accepted: June 10, 1987

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