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Abstract

Levels of gamma-aminobutyric acid (GABA) in cerebrospinal fluid (CSF) were measured by radioreceptor assay (RRA) in 25 normal controls and in 121 patients with various central nervous system disorders. CSF-GABA levels could be measured down to 5 pmoles/ml reliably by this assay. In normal controls, the mean (+/- SEM) GABA level in CSF was 127 +/- 5.2 pmoles/ml. There was no correlation between age, sex and the CSF-GABA level in normal controls. The lowest CSF-GABA level, which was 60 +/- 6.0 pmoles/ml, was observed in alcoholic patients suffering from cerebellar ataxia. The CSF-GABA levels were quite low in patients with Alzheimer’s disease, late cortical cerebellar atrophy, neuro-Behcet’s syndrome, olivopontocerebellar atrophy, Huntington’s chorea, Parkinson’s disease and cerebral hemorrhage. On the other hand, the CSF-GABA levels of meningitis patients were significantly increased. These findings suggest that measuring the CSF-GABA level is quite beneficial in the diagnosis and pathophysiological determinations of some diseases.

KEYWORDS: cerebrospinal fluid (CSF), gamma-aminobutyric acid (GABA), radioreceptor assay (RRA), neurological and psychiatric diseases

*PMID: 6224397 [PubMed - indexed for MEDLINE]
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GAMMA-AMINOBUTYRIC ACID (GABA) IN CEREBROSPINAL FLUID

Hiroo Kuroda

Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan (Director: Prof. Z. Ota)

Received November 18, 1982

Abstract. Levels of gamma-aminobutyric acid (GABA) in cerebrospinal fluid (CSF) were measured by radioreceptor assay (RRA) in 25 normal controls and in 121 patients with various central nervous system disorders. CSF-GABA levels could be measured down to 5 pmoles/ml reliably by this assay. In normal controls, the mean (± SEM) GABA level in CSF was 127 ± 5.2 pmoles/ml. There was no correlation between age, sex and the CSF-GABA level in normal controls. The lowest CSF-GABA level, which was 60 ± 6.0 pmoles/ml, was observed in alcoholic patients suffering from cerebellar ataxia. The CSF-GABA levels were quite low in patients with Alzheimer's disease, late cortical cerebellar atrophy, neuro-Behçet's syndrome, olivopontocerebellar atrophy, Huntington's chorea, Parkinson's disease and cerebral hemorrhage. On the other hand, the CSF-GABA levels of meningitis patients were significantly increased. These findings suggest that measuring the CSF-GABA level is quite beneficial in the diagnosis and pathophysiological determinations of some diseases.

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Gamma-aminobutyric acid (GABA), a putative inhibitory neurotransmitter, is one of the most widely distributed substances in the brain, and 30 % of all brain synapses are believed to be GABAergic (1-6). Recent biochemical studies suggest that disorders of GABA metabolism may exist in certain neurological and psychiatric diseases (7-10). Cerebrospinal fluid (CSF) is believed to be formed in the choroid plexus and is in contact with both the brain and spinal cord. Total CSF-GABA concentrations are related primarily to brain GABA levels and are minimally affected by the changes in the peripheral GABA concentration (11, 12). Therefore, CSF-GABA may reflect disorders of the central nervous system. In the present study, GABA concentrations in CSF were measured by radioreceptor assay (RRA) to gain further insight into GABAergic disorders in various neurological and psychiatric diseases.

MATERIALS AND METHODS

Controls. The control group consisted of normal volunteers and hospitalized patients without neurological and psychiatric diseases, and not receiving any drug which would have
had an influence on this study. There were 15 men and 10 women, and the mean age was 34 ± 3.2 year (mean ± SEM).

Patients. There were 121 patients investigated who suffered from various neurological or psychiatric diseases. The diagnosis and numbers of patients are shown in Fig. 5.

Procedure. All individuals were kept in bed, and oral intake was avoided for 15 h prior to taking a CSF sample. Those patients on medication stopped for at least 14 days preceding lumbar puncture with the exception of those with epilepsy. Lumbar puncture was performed at 9 a.m. in the standard fashion with the patients in the lateral decubitus position. Ten ml of CSF was withdrawn, and the final 5 ml was immediately frozen in an acetone-dry ice tube and kept at -70°C until assay.

Assay. The receptor preparation and standard GABA radioreceptor assay (GABA-RRA) was performed principally as previously reported (13). For the GABA-RRA, 0.5 ml of receptor preparation was placed into a glass tube containing 0.2 ml of CSF, 0.2 ml of Tris-HCl buffer (50 mM, pH 7.6) and 0.1 ml of [3H]-GABA (Amersham, specific activity 57 Ci/mmol, final concentration 6.4 nM). The samples were incubated in ice for 30 min and the reaction was terminated by filtration through glass fiber filters (Whatman GF/C) under reduced pressure. Finally, each filter was washed twice with 5 ml of cold Tris-HCl buffer and then placed into a scintillation vial with 10 ml of scintillation fluid for counting. All samples were analyzed in duplicate.

Amino acid effect on [3H]-GABA binding. There are various amino acids in CSF which might influence [3H]-GABA binding. Therefore, the cross-reactivity of the main amino acids (glycine, L-glutamic acid, taurine) in CSF was checked. Furthermore, artificial CSF (14) was also examined by the GABA-RRA.

Relationship between CSF fraction and each CSF-GABA level. Since the existence of a rostrocaudal concentration gradient of CSF-GABA has been reported (15, 16), the GABA concentration in each fraction (0-5, 5-10, 10-15, 15-20 ml) of CSF was examined.

RESULTS

With this radioreceptor assay, as little as 5 pmoles/ml of GABA could be measured reliably (Fig. 1). As shown in Fig. 1, the dilution curve of CSF was parallel to the standard GABA-RRA curve. Artificial CSF (14) had no effect on GABA binding. Judging from Fig. 2, the 50% of inhibitory concentration ($IC_{50}$) of glycine was over 100 $\mu$M, L-glutamic acid also over 100 $\mu$M, taurine about 26 $\mu$M and GABA 5.1 nM. The cross-reactivity of these amino acids except GABA in the GABA-RRA was, therefore, less than 0.02%. These data showed that the CSF-GABA concentration measured by the GABA-RRA was the GABA concentration itself and the cross-reactivity of other amino acids in the GABA-RRA was negligible.

The existence of a rostrocaudal concentration gradient of CSF-GABA was verified as shown in Fig. 3. The mean rate of increase of the CSF-GABA concentration was 27% of the GABA level in the first 0-5 ml of CSF for each following 10 ml. In this study, the 5th-10th ml of CSF was used in the assay. There was no correlation ($\gamma = 0.40$) between age and the CSF-GABA level in normal controls (Fig. 4).
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Fig. 1. The standard GABA-RRA curve and the CSF dilution curve. ▲▲, CSF of Parkinson's disease patients; ■■, CSF of meningitis patients; ○○, artificial CSF.

Fig. 2. Effects of amino acids on [3H]-GABA binding. ○○, glycine; ▲▲, L-glutamic acid; ■■, taurine.

CSF-GABA levels of various patients are summarized in Fig. 5. In normal controls, the mean (± SEM) GABA level in CSF was 127 ± 5.2 pmoles/ml. The mean CSF-GABA level in males was 122 ± 5.4 pmoles/ml (N = 15), and that in females was 134 ± 9.9 pmoles/ml (N = 10), which were not significantly different. Among the patients tested, the lowest CSF-GABA level, which was 60 ± 6.0 pmoles/ml, was observed in alcoholic patients suffering from cerebellar ataxia. The
next lowest GABA level in CSF, 63 pmoles/ml, was detected in patients with Alzheimer's disease. In patients with late cortical cerebellar atrophy (LCCA), neuro-Bechter's syndrome, olivopontocerebellar atrophy (OPCA), Huntington's chorea, Parkinson's disease and cerebral hemorrhage, the CSF-GABA levels were significantly low. On the other hand, an increased CSF-GABA level was found in patients with meningitis. In all other diseases tested, CSF-GABA levels did
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Fig. 5. CSF-GABA levels in normal controls and in patients with various neurological and psychiatric diseases. Each bar represents mean ± SEM. Asterisk indicates statistical significance compared with normal controls by Student's t test.

(): number of cases; Abbreviations: OPCA = olivopontocerebellar atrophy, LCCA = late cortical cerebellar atrophy, FSP = familiar spastic paraplegia, ALS = amyotrophic lateral sclerosis.

not differ from normal controls (Fig. 5).

DISCUSSION

Tissue levels of GABA are measured by various techniques such as ion exchange chromatography, paper chromatography-fluorometry, an enzymatic-fluo-
rometric technique and a dansylation technique (17). Recent advancements in analytical technique have resulted in methods for quantifying GABA to a sensitivity approximately 1,000 fold greater than that of conventional amino acid analysis. In the present study, the CSF-GABA level could be measured down to 5 pmoles/ml exactly by radioreceptor assay (Fig. 1, Fig. 2).

There are some GABAergic system related neurological and psychiatric diseases. The purpose of the present investigation was to clarify the degree of the relation to the GABAergic system by measuring CSF-GABA levels of patients with these diseases. As shown in Fig. 5, the CSF-GABA level in normal controls was lower than that which some other groups have reported (18-20). This discrepancy may be due to the rostrocaudal concentration gradient of CSF-GABA (Fig. 3), since the 5-10ml CSF fraction was used in this study, but other investigators (18, 19) measured GABA levels in the 0-15ml CSF fraction.

The lowest CSF-GABA level was seen in alcoholic patients suffering from cerebellar ataxia whose cerebella observed on a CT scan were atrophic. Though the GABA concentration in the cerebellum is low, because of its size, the total GABA content is large (21). Therefore, the low CSF-GABA level was attributed to the degeneration of the cerebellum due to alcoholic intoxication.

The CSF-GABA levels were significantly low in patients with LCCA and OPCA, which levels were 65 ± 7.8 pmoles/ml (± SEM) and 71 ± 12.0 pmoles/ml, respectively. LCCA and OPCA are diseases of spinocerebellar degeneration (SCD), characterized mainly by cerebellar degeneration. Therefore, the reduced level of CSF-GABA was probably due mostly to cerebellar degeneration as in the cases of alcoholism with cerebellar ataxia. Familial spastic paraplegia (FSP) is also one subtype of SCD, but which is usually limited to degeneration of the corticospinal tract in the spinal cord where the GABA content is quite low. The CSF-GABA in FSP patients was not different from controls. The CSF-GABA level thus appears to be closely related to the severity of cerebellar degeneration, and OPCA and LCCA should be differentiatable from other types of SCD by measuring the CSF-GABA level (22).

In patients with presenile dementia (Alzheimer’s disease and Pick’s disease), the level of CSF-GABA was quite low. Recently, similar results have been reported (18, 19). Presenile dementia is characterized by extensive degenerative changes in the brain and clinically progressive dementia and dysphasia in the presenium. The atrophy in Alzheimer’s disease is more diffuse and often affects the entire frontal and temporal lobes. In the cerebral cortex, the GABA concentration is low, and about 40% of the GABA in the substantia nigra and globus pallidus. The total GABA content is, however, high because of the total weight of the cerebral cortex. The total GABA content in the cerebral cortex thus becomes lower as these diseases progress.

The GABA content of CSF from patients with Parkinson’s disease was about 57% of normal controls. Low CSF-GABA levels in patients with Parkinson’s
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Disease compared with controls has been reported by others (19, 23). Teychenne et al. also reported low CSF-GABA in parkinsonian patients who responded poorly to levodopa or suffered from the "on-off" phenomenon (24). Glutamic acid decarboxylase (GAD) catalyses the decarboxylation of glutamic acid to GABA and regulates the steady-state concentration of GABA. The highest concentrations of GAD and GABA are in the substantia nigra and globus pallidus (25). The activity of GAD is decreased in certain areas of the brain in patients with untreated Parkinson's disease and is almost normal in brains of patients treated with levodopa, whereas brain levels of GABA are unchanged in both groups of Parkinson's disease patients (26, 27). The CSF-GABA level in patients suffering from Parkinson's disease in the present study was very low compared with that in Enna et al.'s report, which analyzed levodopa-treated patients (18). The decrease of CSF-GABA in patients with Parkinson's disease may be due to the change of GABA turnover because patients in this study were not treated with levodopa for at least 14 days. In severe parkinsonism in which there is no response to therapy, it is suspected that there are fewer functionally intact nigrostriatal dopaminergic neurons and postsynaptic dopamine receptors (28). Therefore, the low CSF-GABA levels in poor responders and patients with the "on-off" phenomenon may represent a compensatory decrease in GABAergic activity (24).

In neuro-Behçet's syndrome, the main pathological changes are a mild inflammatory reaction in the meninges and in the perivascular space of the cerebrum, basal ganglia, brain stem and cerebellum and degenerative changes in the ganglion cells. A quite low CSF-GABA level was observed in this study. The reason for the low CSF-GABA level may be due to changes of GABA metabolism, decrease of GABA content in the brain or alterations of the meninges. It may be possible to differentiate neuro-Behçet's syndrome from other types of Behçet's syndrome. Moreover, measuring CSF-GABA might become a useful method to diagnose neuro-Behçet's syndrome at an early stage.

Perry et al. (29) detected a deficiency of GABA in brain tissue removed after death from a patient with Huntington's disease, especially in the substantia nigra, globus pallidus, putamen and caudate. They suggested that the pathogenesis of Huntington's disease might be related to the decreased GABA levels. A decreased activity of GAD was detected in the basal ganglion of brains of huntingtonian patients (30, 31). Since then, many studies about CSF-GABA that might reflect the abnormalities of brains of patients with Huntington's disease have been reported (18, 19, 23, 32, 33). In the present study, patients with Huntington's disease also had low levels of CSF-GABA, namely, 72 ± 16.2 pmoles/ml. It is not known yet whether the low GABA level was a genetic defect in Huntington's disease or the consequence of this disease. Manyam et al. (8) reported the CSF-GABA of individuals who had a parent with diagnosed Huntington's disease. Some of them showed low CSF-GABA levels, and Manyam et al. suggested that these individuals might develop Huntington's disease. If their suggestion bears
out, the etiology of Huntington’s disease will be better understood, and assay of the CSF-GABA level may be a useful method to diagnose Huntington’s disease at an early stage. Recently, isoniazid (isonicotinic acid hydrazid, INH) was reported to increase brain and CSF-GABA content, evidently through inhibition of GABA aminotransferase (GABA-T). A substantial clinical improvement with INH therapy, however, was not be observed because GABA level did not increase in the basal ganglia nor did the imbalance in concentrations of neurotransmitters change (34, 35).

In patients with cerebral hemorrhage without penetration to the ventricle, CSF-GABA was at a low level. This might be due to the destruction of parts which contained high concentrations of GABA. It was observed that cerebral anoxia increased brain tissue concentrations of GABA (36). High levels of CSF-GABA were detected in patients with a cerebral infarction of less than 2 week’s duration, vertebrobasilar insufficiency and transient cerebral ischemia (37, 38). In this study, the CSF-GABA level of cerebral infarction patients was not different from that of the controls. The difference in the results might be due to the severity of the cerebral infarction and the timing of the lumbar puncture.

A significant increase of CSF-GABA, 2.5 times that of normal controls, was observed in patients with meningitis. The meningitis was not caused by bacteria but by virus. Buryakova et al. (39) observed increased CSF-GABA levels in children with acute bacterial meningitis but not those with serous meningitis and normal children. They suggested that the detection of CSF-GABA might be used as a test to differentiate between serous and bacterial meningitis. Heiblim et al. (40) reported the increase of amino acids in bacterial meningitis. They suspected that the reason for increased amino acid concentration in the meningitis group might be due to alterations in brain metabolism, changes in the kinetics of CSF formation or alterations in the removal of amino acids by an active transport mechanism. In the present study, the cause of the increased CSF-GABA may also be due to what Heiblim et al. (40) have suggested. It is unclear, however, which has the most important role in increasing CSF-GABA levels in meningitis patients, and which type of meningitis has the highest level of CSF-GABA. If many more patients with various types of meningitis are studied, the detection of CSF-GABA may become a useful method to diagnose and differentiate various types of meningitis.

In other neurological and psychiatric patients tested, there were no differences in CSF-GABA levels from normal controls, although abnormalities of CSF-GABA levels in various psychiatric diseases have recently been reported (41-43).

These findings indicate that the CSF-GABA level might reflect brain disorders due to alteration of GABA metabolism, destruction of brain tissue and changes of permeability in the meninges among other causes. In such cases, measuring CSF-GABA may be an important method in the diagnosis and pathophysiological determination of these diseases, and furthermore, to obtain new information on
these and other diseases.

Acknowledgement. The author wishes to thank Prof. Zensuke Ota for his kind guidance and critical review of this manuscript, and Dr. Norio Ogawa for his helpful advice and encouragement during this study. Some of the CSF samples were kindly provided by Dr. Mitsutoshi Yamamoto, Department of Neuropsychiatry, Okayama University Medical School.

REFERENCES

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