

Short report

Plasma and cerebrospinal fluid γ -aminobutyric acid in neurological disorders

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SUMMARY In 49 patients with various neurological disorders plasma and CSF γ -aminobutyric acid (GABA) concentrations were determined by radioreceptor assay. The CSF GABA concentration of 127 ± 47 pmol/ml (range: 65-275; $n = 52$) was independent of the age, the sex and the intake of various drugs including benzodiazepines, baclofen and antidepressants. Patients with diverse neurological disorders such as multiple sclerosis, ischaemic strokes, intracranial tumour and polyneuropathies had similar CSF GABA levels. The mean plasma GABA concentration was 309 ± 79 pmol/ml (range: 179-498; $n = 44$). The correlation between the GABA concentrations of CSF and plasma was very poor ($r = 0.18$; $n = 44$). Therefore plasma GABA is not a suitable indicator for CSF GABA.

γ -aminobutyric acid (GABA) is considered to be one of the major inhibitory neurotransmitters in the mammalian central nervous system.¹ The GABA system has been implicated in the pathogenesis of several neurological and psychiatric disorders, mainly Huntington's disease²⁻⁴ and a smaller number of patients with Alzheimer's disease,² depression,⁵ schizophrenia,⁶ amyotrophic lateral sclerosis,⁷ and epilepsy.⁸ In Huntington's disease a decreased GABA concentration has been shown in postmortem brain⁹ and in cerebrospinal fluid.^{2,3} Furthermore, the existence of a ventriculo-spinal concentration gradient for GABA¹⁰ and additional experimental data^{11,12} suggest that CSF GABA may reflect brain GABA concentrations. Control values for CSF GABA concentrations frequently stem from a small number of patients with sometimes vaguely defined neurological disorders.¹³⁻¹⁶ In addition, the influence of drugs on CSF GABA has only been evaluated for less commonly used drugs such as isoniazid¹⁷ and haloperidol,¹⁸ while the effect of commonly prescribed drugs such as tranquilizers or antidepressants has not been adequately evaluated. It

has even been suggested that, under special circumstances, changes in blood GABA levels may be an indirect indicator of drug-induced changes in the brain content of GABA.^{12,19} The relevance of human plasma GABA concentrations and the relationship of CSF and plasma GABA levels in patients with neurological disorders is not known. A good correlation between CSF and plasma concentrations would enhance the clinical significance of measuring human plasma GABA concentrations. In the present investigation, we studied GABA concentrations in CSF and plasma of 49 patients with well defined common neurological disorders and a complete history of current drug intake. Some of these results have been reported in preliminary form.²⁰

Methods

CSF and plasma specimens

All patients underwent lumbar puncture as part of their neurological evaluation. A complete drug history was taken and the final clinical diagnosis was recorded. None of the patients was chronically bedridden. Lumbar puncture was performed between 8 and 9 a.m. in standard fashion in the lateral decubitus position. The patient had bed rest for 12 hours and no breakfast was given prior to the lumbar puncture. Bilateral jugular vein compression led to CSF pressure elevation in all patients. Xanthochromic or blood-contaminated CSF was discarded. During lumbar puncture,

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a 2 ml aliquot of CSF was collected into a tube after 15 to 18 ml of CSF had drained from the spinal needle. This sampling procedure was intended to ensure that the same CSF aliquot was obtained from each patient so that the reported ventriculo-spinal gradients in the CSF GABA concentration would not bias the investigation.¹⁰ The CSF sample was put on ice immediately and stored in a freezer (-30°C) within 15 minutes after the lumbar puncture until analysed for GABA. Within 5 minutes after the spinal tap, 5 ml blood were drawn from the antecubital vein, the blood was heparinised, put on ice, and immediately separated in a cooled centrifuge and stored in a -30°C freezer within 15 minutes until analysed for GABA.

Determination of GABA

For the determination of GABA in plasma, 900 μl of ethanol were added to 300 μl of plasma. After mixing, the samples were left for 5 minutes at room temperature to coagulate protein, then centrifuged and the supernatant evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 300 μl of 0.05 M Tris-citrate buffer (pH 7.1 at 4°C) and the samples were centrifuged to remove insoluble residues. The recovery of GABA from plasma during this procedure was 84%. Aliquots of 0.2 ml of the plasma supernatant or 0.3 ml of untreated CSF samples were analysed in duplicate for their GABA content by the radioreceptor assay of Enna *et al*¹⁰ as described in detail previously.¹² This assay is based upon the principle that the amount of [^3H] GABA bound to rat brain synaptic membranes is inversely related to the amount of unlabelled GABA present in the incubation medium. For this assay it has been shown that the only substance in normal CSF or blood which will interfere with the bound [^3H] GABA under the conditions of the assay is GABA itself.^{10, 24} GABA concentrations in CSF and plasma determined with this method are virtually identical to those obtained with other analytical techniques such as gas chromatography-mass spectrometry.^{10, 22} Furthermore, among numerous psychotropic drugs only specific GABA agonists such as muscimol interfered with the radioreceptor assay.^{23, 24}

Results

CSF GABA

The mean GABA concentration of the 52 samples was 127 ± 47 pmol/ml (SD) with a range of 65–275 pmol/ml. The CSF GABA concentration was not influenced by the age, sex, CSF constituents like total protein or cell count and drug intake (table 1). The CSF GABA concentration was similar in a number of different neurological disorders listed in table 2. In three patients CSF could be examined twice. The CSF GABA concentration changed within 14–21 days from 157 to 115 pmol/ml, from 70 to 110 pmol/ml and from 89 to 103 pmol/ml, respectively.

PLASMA GABA

The mean GABA concentration of 44 plasma samples was 309 ± 79 pmol/ml (SD) with a range of 179–498 pmol/ml. The GABA plasma concentration

seemed independent of the age, sex, drug intake and the neurological disorders listed in tables 1 and 2. In three patients two plasma samples were studied. The plasma GABA concentration changed from 227 to 264 pmol/ml, from 304 to 420 pmol/ml and from 277 to 372 pmol/ml, respectively.

CORRELATION OF CSF AND PLASMA GABA

There was no correlation between GABA concentration in CSF and plasma (fig).

Table 1 *The influence of age, sex and CSF constituents and drug intake on the concentration of GABA in cerebrospinal fluid and plasma. Values are shown \pm SD with the number of patients in parenthesis.*

	GABA concentration	
	Cerebrospinal fluid (pmol/ml)	Plasma (pmol/ml)
Age (years)	$r = -0.520$ (49)	$r = -0.278$ (41)
Sex		
female	124 ± 48 (40)	304 ± 83 (32)
male	138 ± 41 (9)	327 ± 62 (9)
CSF protein		
< 45 mg%	129 ± 46 (25)	324 ± 90 (23)
≥ 45 mg%	123 ± 42 (20)	292 ± 65 (17)
CSF cell count		
< 15/3	128 ± 49 (36)	312 ± 87 (31)
$\geq 15/3$	122 ± 34 (11)	324 ± 72 (10)
Drug treatment*		
No drugs	115 ± 29 (9)	296 ± 70 (8)
Antidepressants†	144 ± 49 (8)	299 ± 84 (6)
Antiepileptic drugs‡	117 ± 19 (3)	314 ± 21 (3)
Baclofen (10–20 mg daily)	162 ± 99 (3)	319 ± 50 (3)
Bromazepam	133 ± 47 (20)	299 ± 88 (18)
Diazepam	112 ± 32 (7)	306 ± 60 (6)
Various other drugs§	123 ± 48 (22)	312 ± 81 (17)

* Most patients received more than one drug.

† Amitriptyline, Dibenzapine, Sulpiride, Thioridazine.

‡ Phenytoin, Phenobarbital, Primidone.

§ Digitalis, Multivitamins, Aspirin, Penicillin, Tetracyclines, Antacids, Corticosteroids, Carbidopa, Amantadine, Methylidopa.

Table 2 *The influence of various neurological disorders on the concentration of GABA in cerebrospinal fluid and plasma. Values are shown \pm SD with the number of patients in parenthesis.*

	GABA concentration	
	Cerebrospinal fluid (pmol/ml)	Plasma (pmol/ml)
Epilepsy	128 ± 14 (3)	277 ± 85 (3)
Multiple sclerosis	133 ± 58 (10)	317 ± 92 (9)
Viral meningitis	101 ± 26 (5)	250 ± 73 (5)
Polyneuropathy	121 ± 28 (5)	268 ± 75 (3)
Ischaemic stroke	103 ± 29 (6)	261 ± 74 (4)
Intracranial tumour	148 ± 36 (3)	291.426 (2)
Other neurological disorders*	149 ± 55 (11)	347 ± 75 (10)
No neurological disorders	131 ± 50 (6)	306 ± 97 (5)

*cervical or lumbar disc disease, post-commotional headache, carcinomatosis of the meninges, spastic motor paresis of unknown aetiology, neuralgia of n pudendus, unspecified external brain atrophy, Parkinsonism.

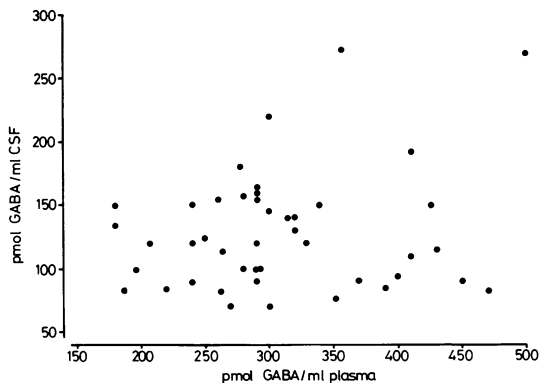


Figure Lack of significant correlation between the GABA concentrations in cerebrospinal fluid and plasma. $r = 0.18$, $n = 44$, \bar{x} CSF = 127 ± 46 pmol/ml (70–275); \bar{x} plasma = 309 ± 79 pmol/ml (179–498).

Discussion

CSF GABA

This study indicates that the CSF GABA concentration is independent of the age and sex of the patients. Furthermore, patients with diverse neurological disorders such as multiple sclerosis, ischaemic stroke, intracranial tumour, alcoholic or diabetic polyneuropathy and patients without neurological disorders had similar CSF GABA concentrations. None of our patients had a disorder reported to be associated with a lower CSF GABA concentration such as schizophrenia,⁶ Huntington's disease,² amyotrophic lateral sclerosis,⁷ spinocerebellar degeneration, or neuro-Behcet's syndrome²⁵ except for three patients with epilepsy.⁸ However, the epileptic patients in this series and other studies,^{10,22,25} did not have lower CSF GABA concentrations than patients with various other neurological disorders. More recent studies have demonstrated a lower CSF GABA when epileptic patients were compared to healthy volunteers and patients without neurological disorders.^{8,26} This suggests that the selection of the control groups is of critical importance. Furthermore, the CSF GABA concentration in patients with epilepsy seems to be influenced by the type of epilepsy⁸ and possibly the antiepileptic drug intake.¹⁰ A number of patients suffered from depression significant enough to be treated with antidepressant drugs. Recently, lower CSF GABA concentrations were reported in severely depressed patients.⁵ In our group of mostly mildly depressed patients we saw no lower CSF GABA concentrations compared to the patients without depression. Earlier suggestions that patients with multiple sclerosis involving the spinal cord have

higher CSF GABA concentrations than patients with cerebral lesions due to multiple sclerosis, could not be confirmed.²⁷ Similarly, we found no correlation between the extent of the functional disability due to the ischaemic brain lesion or the onset of ischaemic brain lesions and the CSF GABA concentration.²⁷

Treatment with various drugs including the GABA analogue baclofen did not clearly influence the CSF GABA concentration in this series. None of our patients received isoniazid¹⁷ or any other drug reported to increase CSF GABA concentrations.

The range of the mean CSF GABA concentrations in our patients was similar to that reported earlier by two independent groups^{22,28} in patients with various neurological disorders including dystonia musculorum deformans and arteriovenous malformations. A number of other reports have shown higher mean values and higher standard deviations of the CSF GABA concentration in similar neurological disorders as well as in healthy volunteers.^{8,14–16,29} The reason for the variability of the results is not obvious, but may be related to differences in sampling, storing and determination techniques^{30,31} as recently reviewed by Wood.³² For instance, repeated deep-freeze and thaw of CSF specimens have been shown to result in irregular and unpredictable elevations in CSF GABA levels which could lead to an incorrect interpretation of data.³³ More studies are needed to establish whether healthy volunteers and patients with neurological disorders do in fact have similar values when diseases known to be associated with lower CSF GABA concentrations are excluded.

PLASMA GABA

In experimental animals changes in blood GABA levels may be an indirect indicator of drug-induced changes in the brain content of GABA.^{12,19} Valproate has been shown to increase plasma GABA concentration in volunteers³⁴ and in patients with alcoholism,³⁵ tardive dyskinesia,³⁵ and schizophrenia.³⁵ Similar relative increases of GABA concentrations have been reported in CSF of schizophrenic patients following valproate medication.³⁶ In our study in patients with various neurological disorders there was no correlation between CSF and plasma GABA concentrations. Therefore, plasma GABA is not a suitable indicator of CSF GABA and probably whole brain GABA concentration in patients with neurological disorders or drug intake not associated with altered CSF GABA levels. Whether human plasma GABA monitoring is of value in disorders or with drug intake which influence CSF GABA concentrations remains to be investigated.

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