Gamma Aminobutyric Acid (GABA) and Glutamate Levels in the CSF of Epileptic Children

Ahmed T. Mahmoud [1] and Ahmed M. Abdel-Tawab [2]

From the departments of Pediatrics,[1] Faculty of Medicine, Menofiya University and department of Pharmacology and Therapeutics,[2] Faculty of Medicine, Ain Shams University, Egypt.

Abstract:

Thirty-one children divided into 3 groups were studied. Group I included 13 neurologically free children, aged 3-9 years and not receiving any medications. Group II consisted of 7 recently diagnosed non medicated epileptic children, aged 3.5-8 years. Eleven medicated epileptic children; aged 4-9 years represented group III. All children were examined thoroughly. EEG and brain CT scan were done for epileptic children. Gamma Aminobutyric acid (GABA) and Glutamate levels in CSF were assessed for all children by HPLC. The mean CSF levels of GABA were lowest in non-medicated epileptic children. Control of the seizures by anti-epileptic drugs (AEDs) was associated by a concomitant re-increase in the CSF GABA levels. The mean glutamate CSF levels did not differ significantly among the three studied groups. Nevertheless, it was noticed that the GABA / Glutamate ratio was higher in medicated than in non-medicated epileptic children. It could be concluded that CSF GABA levels as well as the GABA / Glutamate ratio can reliably monitor the epileptic control by pharmacotherapy.

Introduction:

The release of neurotransmitters via regulated exocytosis is the primary mode of communication in nervous system.(1) For the majority of human epilepsy syndromes, the molecular and cellular basis for the epileptic activity could be attributed to defects in inhibitory amino acid and enhanced or abnormal excitatory amino acid neurotransmitters.(2) Gamma Aminobutyric acid (GABA is the major inhibitory neurotransmitter in the CNS(3) and glutamate is the principal fast excitatory neurotransmitter in the brain.(2)

Currently, designing new antiepileptic drugs (AEDs) is planned to target the mechanisms underlying either the defective inhibitory activity or the enhanced excitatory activity.(3)

The aim of the present work is to study the CSF levels and clinical applications of GABA and, glutamate as well as their ratio in epileptic children.

Subjects and Methods

Thirty-one children were recruited in the present study after having obtained consent from their guardians. The recruited children were grouped as follows:

Group I (control group): Thirteen children (6 boys and 7 girls) having fever of unknown origin, after clinical exclusion of neurological abnormalities and provided that their CSF findings rule out CNS infections. Their ages ranged between 3 and 9 years (mean 5.3 years).

Group II: Seven, recently diagnosed epileptic children (4 boys and 3 girls) aged 3.5-8 years (mean 4.5 years) not receiving AEDs.

Group III : Eleven epileptic children (6 boys and 5 girls) aged 4-9 years (mean 5.1 years) receiving AEDs for more than one year (mono and polytherapy).

All epileptic children included in this study were subjected to a detailed history and thorough clinical examination with special emphasis on seizures, AEDs regimens, epilepsy control and neurological findings. EEG study using (ATES Medico, Italy) was done. At the same time, brain CT scan using Picker CT-Q2000 USA, was done to exclude neurological diseases.

CSF sample collection:

Lumbar puncture was done for all children. One ml of CSF was collected for GABA and glutamate analysis after 2 ml CSF had been drained from the spinal needle, so that the reported ventriculospinal gradient in the CSF neuro-transmitters concentrations would not bias the investigation.(4)

Sample preparation & derivatization(5):

Samples were stored in -70°C after been lyophilized. The average storage was 18 months. When ready for analysis, samples were extracted in 1 ml 80% ethanol, vortexed, then 100 μl were aspirated, and dried in a centrivap concentrator (Labconco) at 35°C. After being dried, derivatization of the samples was performed as follows: 20μl of solution I (ethanol: water: triethylamine; 2:2:1) was added, vortexed and then evaporated in the centrivap con-

centrator at 35°C for 10 minutes. Thirty μl of solution II (ethanol: water: triethylamine: phenylisothiocyanate; 7:1:1:1) were added, left to react at room temperature for 20 minutes, then evaporated at 35°C for 10 minutes in the centrivap concentrator. The dried, derivatized samples were reconstituted in 100 μl of the eluted mobile phase (mixture of solvent A and solvent B).

HPLC apparatus and conditions[6] :

Hewlett Packard (HP) system consists of quaternary pump series 1050, Rheodyne injector with 20 μl loop, on-line degasser and spectrophotometer detector series 1050. The system was provided with built-in data acquisition software. Lichrosorb RP-18 column (200 x 4.6 mm), particle size 5 μm (Hewlett Packard) was used.

Isocratic separation was performed using a mobile phase consisting of 80% solvent A "0.1 M acetate buffer" and 20% solvent B "acetonitrile:water" (60:40, v:v). Solvent A was prepared by adding 8.205 g sodium acetate, 0.5 ml triethylamine, 0.7 ml acetic acid and 5 ml acetonitrile, completed to 1 L with water and adjusted to pH 5.8. Flow rate was maintained at 0.61 ml/min, and detection was at wave length 254 nm.

Results:

Table I shows the clinical data of all studied children as regards family history of epilepsy, age, sex, neurological examination, pharmacotherapy, control of epilepsy, EEG and brain CT findings.

From table II, it could be depicted that the mean GABA concentrations in the CSF of the control (non-epileptic) group was 167.41 ± 79.89 pmol/ml. Contrary wise, the mean GABA concentration was 77.01 ± 28.41 pmol/ml in the group of epileptic non-medicated children (group II). However, this level was found to be as high as 337.5 ± 93.46 pmol/ml in epileptic children on long-term AEDs (group III). The GABA levels in CSF were significantly different among the three groups (p = 0.034) using the Kruskal-Wallis statistical analysis.

The mean glutamate level in the CSF of the control (group I) was 527.98 ± 109.81 pmol/ml, this level was found to be numerically lower in group II: 300 ± 71.93 pmol/ml" and 393.91 ± 69.32 pmol/ml in group III. However, The difference between the glutamate levels in the CSF of the three groups was not significant (p = 0.683).

The mean GABA/Glutamate ratio was 0.22 ± 0.17 in group I. A much higher ratio was found in group II: 0.54 ± 0.15. However, the highest ratio was that of group III: 1.09 ± 0.17. The differences among the three groups were statistically significant (p = 0.007). This significance was calculated after omission of two outlier figures using the appropriate Wilk-Shapiro/Rankits and Box & Whiskers plots. However, even before this omission, the statistical significance of this difference was still substantial (p = 0.062). These statistical differences among the three groups were still the same when studied by the parametric ANOVA test. P values were 0.026, 0.699 and 0.003 for GABA, glutamate levels and their ratio respectively.

Table III shows a sort of subgroup analysis from which it could be seen that the uncontrolled epileptic children had GABA level 96.88 pmol/ml and a GABA/Glutamate ratio of 0.625. The controlled epileptics by mono and polytherapy showed higher GABA levels:142.65 and 656.04 pmol/ml respectively with high GABA/Glutamate ratio: 1.254 and 1.129 respectively. The partially controlled epileptics showed an intermediate GABA level "122.9 pmol/ml"; however the GABA/Glutamate ratio was low: 0.352".

Discussion:

The three studied groups were well matched for age and sex. Bias was minimized throughout the diagnosis "clinical, EEG and CT", CSF sampling "timing and ventriculospinal gradient", storage (-70°C and lyophilization) and HPLC analysis (blinded).

The mean GABA level in CSF was 167.4 pmol/ml in neurologically free non-epileptic children. This is in agreement with the results of Loscher and Siemes (1985).[8] In the present work, the mean GABA level in recently diagnosed epileptic children, not receiving AEDs, was 77.01 pmol/ml. This also, agrees with several earlier findings.[8,9] On the other hand, the decrease in the GABA levels in the CSF of non-medicated epileptic children was not in harmony with few other studies.[10,11]

The present study showed that anticonvulsant pharmacotherapy was associated with an increase in CSF GABA levels in epileptic children, its mean value became 337.5 pmol/ml. This finding was also reported by others.[8] However, Ito et al., (1984)[12] found a significantly lower GABA concentrations in epileptic children both treated and untreated.

In our work, treated epileptic children were subgrouped according to whether their seizures were controlled by the pharmacotherapy, or not. The GABA level in the CSF of the uncontrolled children was 96.88 pmol/ml, i.e. not markedly different from the mean of the group of non-medicated epileptic children (77.01± 28.41 pmol/ml). This is strikingly in agreement with earlier findings.[8]

Table I: Clinical data among studied children

Variable	Group I *(control)*	Group II *(No AEDs)*	Group III *(AEDs)*
Family history			
Positive	0	2	1
Negative	13	5	10
Age (years)			
Mean	5.3	4.5	5.1
SD	2.9	2.7	3.2
Sex			
Males	6	4	6
Females	7	3	5
Neurological examination	Normal	Normal	Normal
Anticonvulsants			
None	13	7	0
Monotherapy	0	0	6
Polytherapy	0	0	5
Clinical control			
Controlled		0	7
Partially controlled		0	3
Uncontrolled		7	1
EEG	-	Positive	Positive
Brain CT scan	-	Normal	Normal

Table II: Comparison between the GABA, Glutamate levels and their ratio in the CSF of control (group I), epileptic non-medicated (group II) and epileptic medicated (group III) children.

Variable	Group I *(control)*	Group II *(No AEDs)*	Group III *(AEDs)*
GABA			
Number	9	7	10
Mean (pmol/ml)	167.41±79.89	77.01±28.41	337.50±93.46
P (Kruskal-Wallis)	0.034*		
P (ANOVA)	0.026*		
Glutamate			
Number	10	7	11
Mean (pmol/ml)	527.98±109.81	300 ± 71.93	393.91±69.32
P (Kruskal-Wallis)	0.683		
P (ANOVA)	0.699		
Ratio (GABA/Glutamate)			
Number	8	7	9
Mean	0.22 ± 0.17	0.54 ± 0.15	1.09 ± 0.17
P (Kruskal-Wallis)	0.007*		
P (ANOVA)	0.003*		

** Significant*

GABA/Glutamate ratio after omission of outliers by 2 tests[7] : Wilk-Shapiro/Rankit plot; Box and Whisker plot.

N.B. 4 samples from GI and 1 sample from GIII were missed during measurement of GABA levels; 3 samples from GI were missed during measurement of glutamate levels.

Table III : GABA and Glutamate levels in CSF of medicated epileptic children of Group III

Subgroup	Number	GABA (pmol/ml)	Glutamate (mmol/ml)	GABA / Glutamate ratio
Group III $_{cm}$	2	142.65	113.75	1.254
Group III $_{cp}$	5	656.04	580.88	1.129
Group III $_{pc}$	3	122.90	348.77	0.352
Group III $_{uc}$	1	96.88	154.88	0.625

cm = controlled by monotherapy "Carbamazepine" , cp = controlled by polytherapy, pc = partially controlled by monotherapy "Carbamazepine" , uc = uncontrolled by monotherapy "phenobarbital".

The mean GABA level in CSF was significantly higher in those partially controlled (122.9 pmol/ml) and still higher in epileptic children controlled by monotherapy (142.65 pmol/ml). The highest mean GABA level was found in those controlled by polytherapy (656.04 pmol/ml).

The mean Glutamate level in CSF did not show statistically significant differences among epileptic children, whether medicated and non-medicated, and whether their seizures were controlled or not. This suggests that CSF glutamate level, by the currently used methods, may not directly reflect the actual neurochemical mechanisms. However, Croucher et al., (1997)[13] reported a role of glutamate in both epileptogenesis and spread of epileptic neuronal hyperactivity in the brain. This should emphasize on the need for a method to correlate CSF glutamate levels with the neuronal levels. There may be a need for a method of inactivating CSF enzymes to provide stable glutamate levels under different storage conditions.[14] On the contrary, a correlation has been demonstrated between changes in brain GABA concentration and its concentration in the CSF.[15,16] Experimental and clinical studies[17] have strongly suggested that cerebrospinal fluid GABA reflects alterations in brain GABA metabolism. No such findings were reported as regard glutamate.

In our work, when GABA /Glutamate ratio was calculated, the statistically significant differences among the three studied groups became more elaborate. Mean ratios after omission of outliers were 0.22, 0.54 and 1.09 in group I, II and III respectively. The ratio in the uncontrolled epileptic child was 0.625 which is not much different form that of the non-medicated epileptics. The ratio was clearly higher in the children whose seizures were controlled: 1.254 and 1.129 respectively for those on monotherapy and polytherapy. The partially controlled epileptics had a ratio close to that of healthy children, *viz.* 0.352 versus 0.22.

It can be concluded, from the present work, that GABA level in CSF and GABA/Glutamate ratio are satisfactory indicators for whether the epileptic child was controlled by the pharmacotherapy or not. Some earlier studies[18-21] recommended monitoring pharmacotherapy by assaying GABA levels in CSF. Different AEDs can be individually studied through recruiting larger sample size of epileptic children subdivided according to the anticonvulsant used.

References:

1. Bajjalieh SM, Scheller RH. The biochemistry of neurotransmitter secretion. J Biol Chem 1995; 270(5): 1971-4.
2. Meldrum BS. Excitatory amino acid transmitters in epilepsy. Epilepsia 1991; 32(suppl 2): S1-S3.
3. Gram L. Epilepsy care : image of the future. Epilepsia 1995; 36(suppl 6): S22-S24.
4. Enna SJ, Wood JH, Snyder SH. GABA in human cerebrospinal fluid : radioreceptor assay. J Neurochem 1977; 28: 1121-4.
5. Guanawan S, Walton NY, Treiman DM. High performance liquid chromatographic determination of selected amino acids in rat brain by precolumn derivatization with phenyl isothiocyanate. J Chromatogr 1990; 503: 177-87.
6. Rossetti V, Lombard A. Determination of glutamate decarboxylase by high performance liquid chromatography. J Chromatogr 1996; 681: 63-7.
7. Altman DG. Practical Statistics for Medical Research. London, UK : Champman & Hall, 1991.
8. Loscher W, Siemes H. Cerebrospinal fluid GABA levels in children with different types of epilepsy : effect of anticonvulsant treatment. Epilepsia 1985; 26(4): 314-9.
9. El-Sebaie B. GABA in cerebrospinal fluid of children with idiopathic epilepsy. MS Thesis, Cairo University, 1983.
10. Perry TL, Hansen S. Amino acid abnormalities in epileptogenic foci. Neurology (NY) 1981; 31: 872-6.
11. Schmidt D, Loscher W. Monitoring of GABA in human cerebrospinal fluid : downward revision of previous control values. Ther Drug Monit 1984; 6: 227-31.
12. Ito M, Mikawa H, Taniguchi T. Cerebrospinal fluid GABA levels in children with infantile spasms. Neurology (NY) 1984; 34: 235-8.
13. Croucher MJ, Ruffle KL, Bradford HF. The effect of focal N-methyl-D aspartate pretreatment on the parameters of amygdaloid electrical kindling. Eur J Pharmacol 1997; 319(2-3): 207-13.
14. Ferrarese C, Pecora N, Frigo M, Appollonio I, Frattola L. Assessment of reliability and biological significance of glutamate levels in cerebrospinal fluid. Ann Neurol 1993; 33(3): 316-9.
15. Bohlen P, et al. Automated assay of GABA in human cerebrospinal fluid. Clin Chem 1978; 24: 256-60.
16. Loscher W. GABA in plasma and CSF of different species : effects of Y-acetylenic GABA, Y-vinyl

GABA and sodium valproate. J Neurochem 1979; 32: 1587-91.
17. Hare TA, Manyam NVB, Glaeser BS. Evaluation of cerebrospinal fluid GABA content in neurological and psychiatric disorders. In: Wood JH, ed. Neurobiology of CSF. New York : Plenum, 1980; 171-87.
18. Manyam BV, Tremblay RD. Free and conjugated GABA in human CSF : effect of degenerative neurologic diseases and isoniazid. Brain Res 1982; 307: 217-23.
19. Ben-Menachem E. Pharmacokinetic effects of vigabatrin on cerebrospinal fluid amino acids in human. Epilepsia 1989; 30(suppl 3): S12-4.
20. Jacken J. Disorders of neurotransmitters. In: Fernades J, Saudubray J, Tada K, eds. Inborn Metabolic Diseases. Berlin : Springer, 1990; 637-48.
21. Giroux JD, et al. GABA concentrations in the CSF of newborn infants determined by high performance liquid chromatography. Acta Paed 1996; 85: 595-7.