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Cerebrospinal fluid GABA and seizure control with vigabatrin

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1 To evaluate the relationship between the clinical response and enhancement of GABAergic neurotransmission, for 6 months we administered vigabatrin (γ-vinyl-GABA, GVG) to 75 patients with complex partial epilepsy. Total GABA (TGABA), free GABA (FGABA), homocarnosine (HC), and GVG concentrations were measured in CSF of these patients before and during GVG treatment.

2 Over 50% reduction in seizures was found in 55% of the patients. Dose-reduction resulted in a relapse, i.e. the return of seizures.

3 At baseline TGABA, FGABA, and HC did not differ in responders and non-responders. After GVG treatment, the TGABA and HC levels were lower in non-responders ($P < 0.001$), but the GVG and FGABA levels did not differ. The GVG dose reduction resulted in a concomitant decrease in TGABA, FGABA, HC and GVG ($P < 0.001$).

4 According to our results GVG is an effective anticonvulsant drug in complex partial seizures. In nonresponders the poor anticonvulsant response may be related to the lower elevation of the CSF markers of GABAergic neuronal activity in this group compared with the responders.

Keywords vigabatrin GABA epilepsy

Introduction

In one-third of patients with complex partial seizures (CPS) the seizures are not properly controlled by classical anticonvulsant drugs. In addition, severe cognitive and other side-effects accompany the drug treatment (Kutt & Solomon, 1980). These problems could possibly be overcome by developing molecules that affect the pathophysiological mechanisms of epilepsy specifically.

Neuroanatomical, neurochemical, and neuropharmacological studies have recently demonstrated that the balance between inhibitory and excitatory neurotransmission is important for the initiation of focal seizures (Gale, 1986; Turski *et al.*, 1986). Studies of brain samples taken during epilepsy surgery suggest that in about 50–70% of the patients with CPS GABAergic transmission is impaired (Lloyd *et al.*, 1986). GABA seems to be the major inhibitory neurotransmitter in the different nigral efferents, which modulate the spread of the motor component of seizures in rats (Gale, 1986; McNamara *et al.*, 1983). On the other hand, some data also suggest that the primary defect in epilepsy may be a genetically determined overavailability of the excitatory amino acid transmitter, glutamate (Van Gelder *et al.*, 1980; Janjua *et al.*, 1982). This amino acid seems to mediate the excitatory drive from the cortex via pyramidal cells to the hippocampus, thalamus, striatum, substantia nigra, and to many other subcortical targets (Fonnum *et al.*, 1981).

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Table 1 Clinical data of the patients in CSF study

	Total	*(n)*	*Male*	*(n)*	*Female*	*(n)*
Age	31.0 ± 9.7	(67)	32.5 ± 10.3	(31)	29.7 ± 9.1	(36)
Duration of epilepsy (years)	19.5 ± 9.1	(67)	19.8 ± 9.0	(31)	19.4 ± 9.3	(36)
Mean number of seizures per month	29.7 ± 74.1	(67)	31.2 ± 90.8	(31)	28.5 ± 58.1	(36)
Number of other anti-convulsant drugs used						
CBZ	7		4		3	
CBZ + CLN	21		9		12	
CBZ + VPA	15		3		12	
CBZ + DPH	5		3		2	
Other	19		12		7	

Abbreviations: number of patients (n), CBZ (carbamazepine), CLN (clonazepam), VPA (sodium valproate), DPH (phenytoin), Other (different combinations of carbamazepine, clonazepam, phenytoin, valproate, phenobarbitone, and ethosuximide). Values are expressed as mean ± s.d.

The clinical applications of this balance theory in the treatment of epilepsy have previously concentrated on strengthening inhibitory transmission in the epileptic brain. This is achieved by elevating GABA levels in the brain (sodium valproate), by using GABA agonists (progabide), or by enhancing GABA transmission with benzodiazepines. The more recent strategy is to elevate GABA levels in the brain by an irreversible GABA-transaminase (GABA-T, EC 2.6.1.19) inhibitor, vigabatrin (γ-vinyl-GABA, GVG), which has been shown to be an effective anticonvulsant drug in both animal and human seizure disorders (see review: Hammond & Wilder, 1985).

The purpose of this study was to determine whether there are differences in the CSF levels of total GABA (TGABA), free GABA (FGABA), or homocarnosine (HC) which are the markers of GABAergic neurotransmission) in the CSF samples of patients whose seizures decline during GVG treatment and in those patients who do not respond to GVG. These results may give further information about the significance of GABAergic inhibition in the pathophysiology of seizures and especially in seizure control.

Methods

Patients

Seventy-five patients with seizures of complex partial onset were included in this study (34 males, 41 females; age 15–55 years, mean 31). The mean age at the onset of seizures was 10 years. The duration of epilepsy was 20 years, and the frequency of monthly seizures varied from 2 to 556. Detailed information about the patients from whom CSF samples were taken is shown in Table 1.

The study design is shown in Figure 1. The first CSF sample was taken at the end of the 2-month baseline period (Phase I). Thereafter the GVG-treatment was started and continued for 3 months in an open trial (Phase II). Each patient received 3 g GVG day^{-1} in two doses. The second CSF sample was taken at the end of this period.

For the next 3 months patients who had 50% or more reduction in seizures or marked improvement in global performance were randomized into two groups: patients who received 1.5g GVG day^{-1} and patients who received 3g GVG day^{-1}. The third CSF sample was taken at the end of this double-blind period (Phase III).

The CSF study was performed with the permission of the ethics committee of Kuopio University Central Hospital and with the oral consent of the patients. The patients had been immobilized overnight and lumbar CSF samples were taken between 08.00 and 10.00 h. CSF samples were frozen immediately and stored at −80° C.

Chemicals

The standards of GABA and homocarnosine were purchased from Sigma (St. Louis, MO). Pure vigabatrin was obtained from Centre de Recherche Merrell International (Strasbourg,

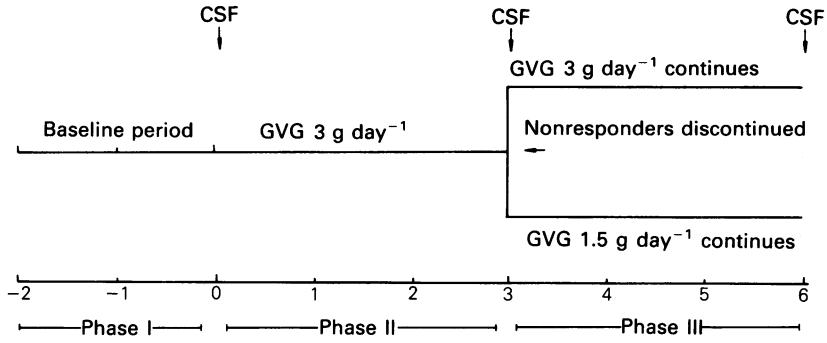

Figure 1 Study design

France). Other chemicals (analytical or h.p.l.c. grade) were from E. Merck (Darmstadt, G.F.R.). Water with a resistance greater than 15 MOhms cm (Millipore, Milli Q-system) was used to prepare the h.p.l.c. solvents.

Apparatus

The gradient h.p.l.c. system contained two Waters 501 h.p.l.c. pumps, Waters 680 automated gradient controller, Waters column heater module, Ultra Techsphere ODS (150 × 4.6 mm, particle size 5 μm) column, and a Merck-Hitachi F-1000 fluorescence spextrophotometer using an excitation wavelength of 340 mn and emission wavelength of 450 nm. Injections were made by a Rheodyne 7125 injector with a 20 μl loop. The chromatographic data was processed by a Varian 4270 integrator.

GVG, TGABA, FGABA and HC analysis

GVG, TGABA, FGABA, and HC concentrations in CSF were assayed by h.p.l.c. as their *o*-phthaldialdehyde (OPA)/ethanethiol derivatives, by a modification of the method of Pfeifer & Hill (1983).

Briefly, 100 μl of internal standard (Norleucine; 50 nmol ml^{-1} methanol) was added to 200 μl of CSF or amino acid standard followed by 100 μl of saturated Borax buffer, pH 9.5, 100 μl of ethanethiol (20 μl ml^{-1} methanol) and 200 μl OPA (10 mg ml^{-1} methanol). After a 2 min reaction, 10 μl was injected into the h.p.l.c. column. Solvent A was 50 mM sodium phosphate-acetate buffer, pH 7.5: methanol:tetrahydrofurane (96:2:2, v/v/v). Solvent B was methanol:water (65:35, v/v). The gradient program was: time 0 min, 0% B; time 25 min, 100% B followed by a 5 min isocratic eluation with 100% B; The column was run to starting condition in 5 min. The flow rate of 1.5 ml min^{-1} was used at 35° C. Total GABA assay: 200 μl of CSF or GABA standard (5 nmol ml^{-1}) was added to 100 μl 20% sulphosalicylic acid (SSA), containing 100 nmol Norleu ml^{-1} SSA. After 30 min incubation on ice and centrifugation, the supernatant (120 μl) was hydrolyzed at 100° C for 18 h. The hydrolysate was neutralized with 1 M NaOH. The amino acids were derivatized and assayed by the procedure described above, except that the internal standard was added before hydrolysis and the gradient program was reduced to 15 min.

Statistics

Statistical significance of the results was tested by Student's *t*-test and Wilcoxon's test as indicated in the texts of figures.

Results

Clinical response

The seizure frequencies at different phases are shown in Figure 2. After 3 months of GVG administration the median frequency of seizures was 65% lower than the baseline level ($P < 0.01$). In 41 patients (55%) the decrease in seizure frequency was more than 50%. Twenty-one of these patients received 3 g GVG day^{-1} during Phase III and their seizure frequency remained at the same level as in Phase II. On the other hand, the seizure frequency in the group with 1.5 g GVG day^{-1} during Phase III was 235% higher than in Phase II ($P < 0.01$).

FGABA, TGABA, HC and GVG in CSF

After 3 months administration of 3 g of drug day^{-1} the TGABA, FGABA, and HC levels were elevated 151%, 103%, and 194% ($P <$

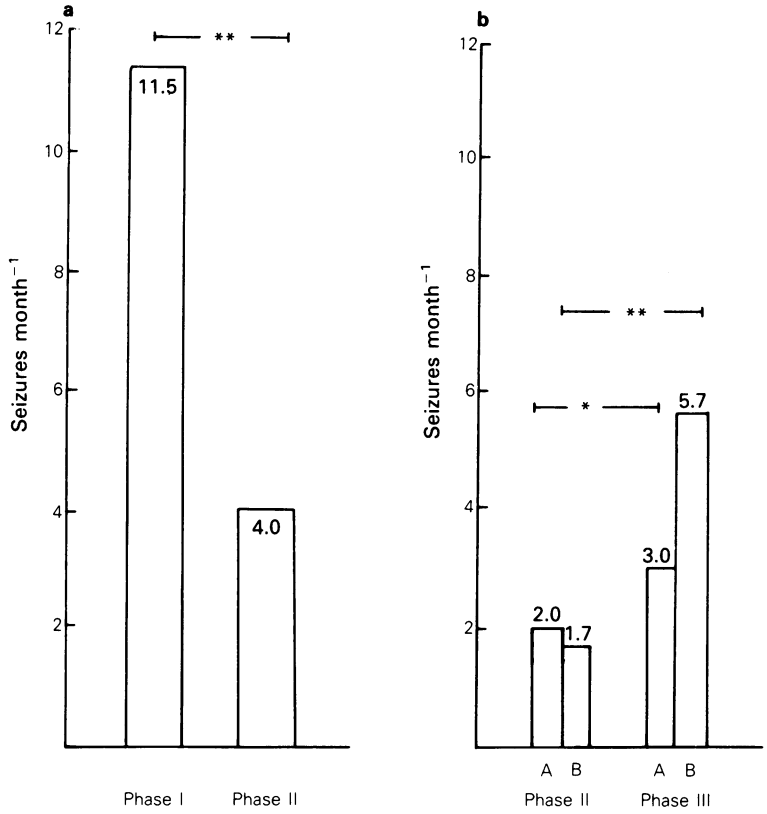

Figure 2 The median frequency of seizures of patients at different phases (I, II, III; see Figure 1) during GVG-treatment. (a) Whole patient group at baseline (Phase I) and after 3 months GVG treatment (3 g day^{-1}) (Phase II). (b) Patients with over 50% reduction in the number of seizures; these patients received either 3 g (A) or 1.5 g (B) GVG per day during Phase III. $^{**}P < 0.01$, $^{*}P < 0.05$ (Wilcoxon's test).

0.001), respectively (Figure 3a). Patients receiving vigabatrin 3 g day^{-1} had similar concentrations of GVG in both Phases II and III (Figure 3b). The halving of the drug dose to 1.5g day^{-1} resulted in a 43% decrease of GVG levels in the CSF ($P < 0.001$). In the same figure are also presented the TGABA, FGABA and HC concentrations in patient groups receiving different doses of GVG. At the end of Phase III the TGABA (24%), FGABA (25%), and HC (25%) concentrations were lower ($P < 0.001$) in patients receiving 1.5 g day^{-1} compared with the levels in the same patients in Phase II. In patients receiving 3 g day^{-1} during both of these phases the values for these amino acids at the end of Phase III did not differ significantly from the values at the end of Phase II. FGABA and GVG levels in CSF were in correlation at both phases (phase II: $r = 0.341$, $P < 0.02$; phase III: $r = 0.438$, $P < 0.001$), whereas the TGABA and HC concentrations in CSF were not correlated with CSF levels of GVG (Pearson's correlation test).

The levels of TGABA, FGABA, and HC in groups of patients with different responses to GVG are shown in Figure 4. At baseline the TGABA, FGABA, and HC did not differ between responders and nonresponders. Instead, after 3 months administration of GVG, responders had higher levels of TGABA (42%, $P < 0.001$) and HC (75%, $P < 0.001$) than nonresponders did. The FGABA, and especially GVG, levels did not differ between these groups.

Discussion

In the present study more than a 50% reduction in seizure number was found in 55% of the patients with complex partial epilepsy. Our re-

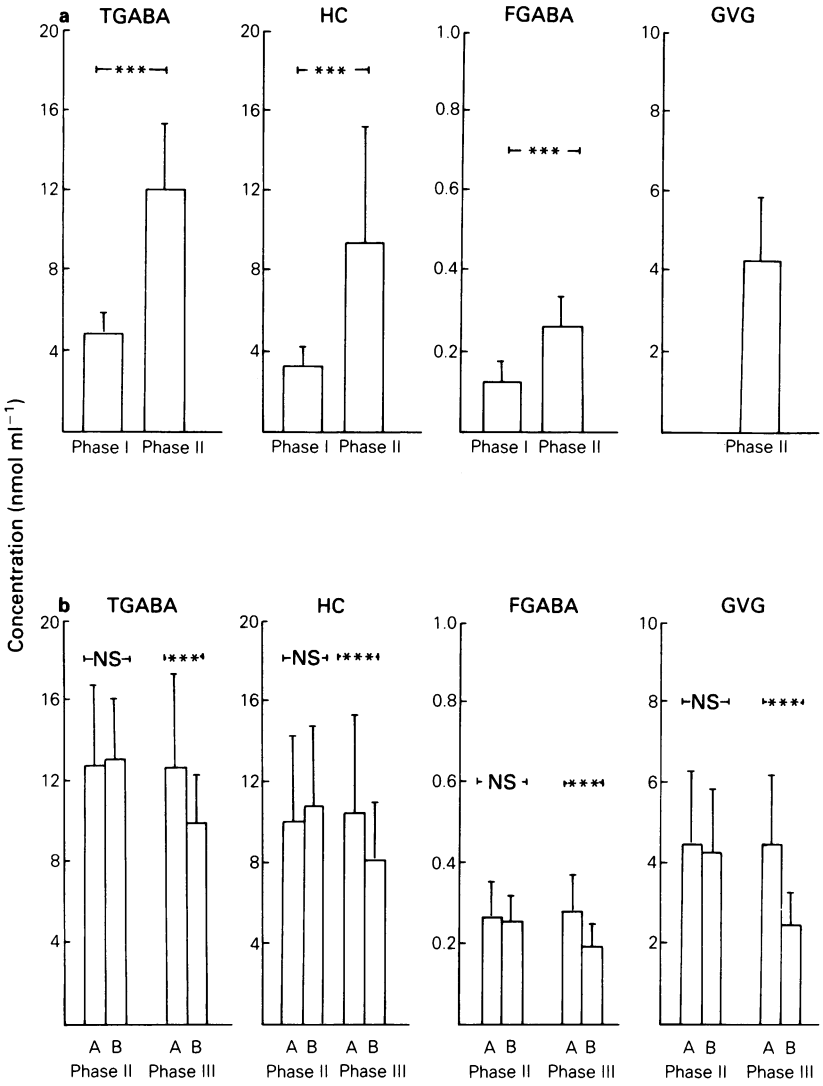

Figure 3 TGABA, HC, FGABA, and GVG concentrations in CSF of patients at different phases (I, II, III; see Figure 1) and with different doses of GVG. (a) Whole patient group at baseline (Phase I) and after 3 months GVG treatment (3 g day^{-1}) (Phase II). (b) Responders, who received either 3 g (A) or 1.5 g (B) GVG day^{-1} at Phase III. ***P = 0.001, NS = nonsignificant (Student's t-test).

sults further extend the beneficial clinical results presented in short-term and long-term studies of GVG (Sivenius *et al.*, 1987). Furthermore, in our study the TGABA, FGABA, and HC levels increased two to three fold during GVG administration, which agrees with the results of Schechter *et al.* (1984) and Ben-Menachem *et al.* (1986). Interestingly, the TGABA, FGABA, and HC elevations differed in magnitude in responders and nonresponders. In addition, a relationship was found between the clinical response and the GVG dose.

Reduction of the GVG dose in Phase III from 3 g to 1.5 g GVG day^{-1} significantly increased the number of seizures. Previously it has been speculated that, owing to the irreversibility of GABA-T inhibition by GVG, even a small drug dose could be sufficient to eliminate the metabolic activity of GABA-T. Our findings do not support this proposal. Possibly, the number of enzyme molecules inhibited by 1.5 g GVG day^{-1} is not enough to block GABA metabolism to the extent that presynaptic GABA levels would be elevated enough to block the seizures. We

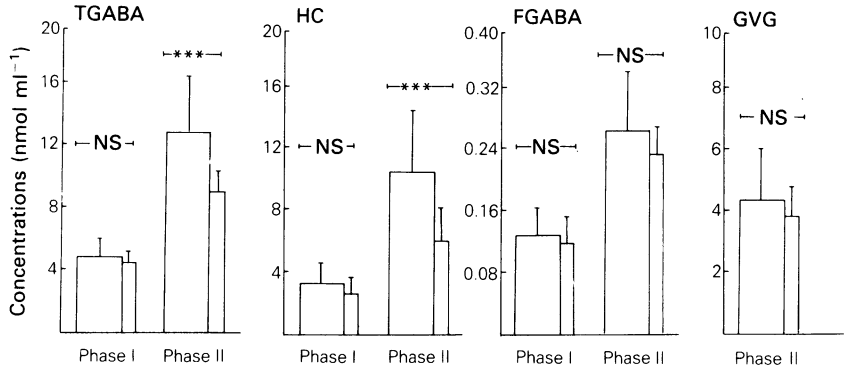

Figure 4 TGABA, HC, FGABA, and GVG concentrations in CSF of responders (⌐¬) and nonresponders (⊓) at baseline (Phase I) and after 3 months treatment with 3 g GVG day^{-1} (Phase II). *** $P < 0.001$, NS = nonsignificant (Student's t-test).

administered the drug twice a day, which suggests that new GABA-T synthesis does not exceed the number of enzyme molecules eliminated by GVG during the intervals between drug administration.

The histopathological findings from the brains of drug-resistant epileptics show that in partial epilepsy gliosis increases, especially in the limbic structures (Engel *et al.*, 1982). GABA-T is localized in neurons but also in glial cells (McGeer & McGeer, 1981). In a recent study Gale (1986) suggested that during GVG administration the glial GABA-T is inhibited first, leading to the elevation of glial GABA. In our study the lower clinical response at a 1.5 g dose of GVG than with 3 g could be associated with the higher proportional capture of GVG to glial cells, which results in the elevation of GABA in the nontransmitter pool. This would reduce the amount of GABA-T inhibition in presynaptic terminals and further reduce the GABAergic inhibitory neurotransmission in brain. During treatment with a higher dose of drug, however, the presynaptic GABA-T is also suggested to be inhibited more.

We measured GVG levels in the CSF of the patients and found that the GVG concentrations were about twice as high at a dose of 3 g GVG as at a dose of 1.5 g. This suggests that GVG goes through the blood-brain barrier in a more-or-less dose-dependent manner. The CSF levels of GABA correlate with the concentrations of GABA in the brain (Grove *et al.*, 1983). In our study we found no differences in the CSF levels of GVG in the responders and nonresponders receiving the same drug dose in Phase II. Instead, after 3 months of GVG treatment with 3 g day^{-1} the HC and TGABA levels of the nonresponders were lower than those of the responders. We suggest that the access of GVG to the brain does not explain the poor clinical response or the lower GABA levels in the CSF of nonresponders. Instead, the lower GABA levels in the CSF of these patients after GVG treatment could be related to the degree of destruction of their GABAergic neurons. In surgical samples from patients with partial epilepsy a defect in GABA neurons and/or neurotransmission is found in about 50–70% of these patients (Lloyd *et al.*, 1986). The number of GABA-synthesizing neurons in nonresponders might be destroyed even more than in responders, thus resulting in lower GABA levels even in the presence of GVG-induced metabolic inhibition.

Other factors may be connected with the poor response in part of the patients. First, there may be defects in the epileptic brain such as transmission mediated by glutamate and aspartate (Turski *et al.*, 1986; Van Gelder *et al.*, 1972). This suggests that the artificial elevation of GABAergic inhibition alone may not be able to block the seizure activity. Second, animal studies suggest that the total level of GABA in the brain, which is correlated to the GABA concentration in CSF, is poorly correlated with the seizure threshold (Gale, 1986). Recent studies propose that there are many areas in the brain, especially in basal ganglia structures, that regulate progression of a seizure by a GABAergic mechanism (Gale, 1986, McNamara *et al.*, 1983). This implies that we should evaluate the local concentrations of GABA in the brains of responders and nonresponders, a procedure that is not possible in CSF studies. This suggestion is supported by our findings that in individual cases the similar elevation of GABA in CSF could be connected with either good or poor clinical

response. Third, the GABA-receptors of non-responders could be more sensitive than those of responders to down-regulation after a GVG-induced increase in the GABA concentration of the brain. In animal studies no down-regulation has been observed (Hammond & Wilder, 1985).

In the present study the clinical history of the patients was similar for responders and non-responders; and factors such as aetiology or duration of the disease or seizure frequency do not explain the differences in clinical response between the two groups. The plasma levels of other anticonvulsant medication remained constant during the GVG treatment, and it is improbable that other anticonvulsant medication could be involved in the clinical and biochemical changes found in this study.

Homocarnosine is synthesized from GABA and histidine. Its physiological significance in regulation of the seizure threshold is largely unknown. Our study suggests that homocarnosine can be used as a marker of GABA levels in the brain. However, its participation in the anticonvulsant effects of GVG needs further clarification.

The results of the present study suggest that elevation of inhibitory neurotransmission in the epileptic brain depresses the seizures in 55% of the epileptic patients with complex partial seizures. On the other hand, there is a relationship between the clinical response and elevated CSF levels of GABA during GVG treatment. Furthermore, analysis of the biochemical markers of GABAergic neurotransmission in CSF during drug administration may help to explain the mechanisms of drug action and even provide reasons for the poor response in some patients.

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