Biochemical Pharmacology, Vol. 43, No. 10, pp. 2143–2150, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
Pergamon Press Ltd

EFFECTS OF SINGLE AND MULTIPLE INCREASING DOSES OF VIGABATRIN ON BRAIN GABA METABOLISM AND CORRELATION WITH VIGABATRIN PLASMA CONCENTRATION

ELSA M. VALDIZÁN and JUAN A. ARMIJO*

Clinical Pharmacology Service, "M. de Valdecilla" University Hospital; and Department of Physiology and Pharmacology, Faculty of Medicine, University of Cantabria, E-39008 Santander, Spain

(*Received* 24 *September* 1991; *accepted* 19 *February* 1992)

Abstract—The effects of increasing (50–1600 mg/kg/day) doses of vigabatrin (GVG) both as single doses and after 8 or 28 days of treatment have been studied in 19 groups of 10 adult Wistar rats. The parameters studied were brain γ-aminobutyric acid-transaminase (GABA-T) activity, GABA concentration and L-glutamate decarboxylase (GAD) activity. Single increasing doses of GVG progressively inhibited GABA-T activity, but a residual activity of about 40% was observed with the highest doses. GABA concentration increased in a dose-dependent manner but a ceiling was not reached. GAD activity was slightly inhibited at low doses and stimulated at high ones. When treatment was continued for 8 days, more marked effects of GVG on GABA-T and GABA, a more severe toxicity and higher GVG plasma concentrations were observed. GAD was inhibited instead of stimulated by high GVG doses. After 28 days of treatment the effects of GVG on GABA-T and GABA were similar to those after 8 days. However, toxic effects decreased and lower GVG plasma concentrations were found. In conclusion: (a) the more marked brain GABAergic effects observed after 8 days of treatment with GVG may explain the greater anticonvulsant effects observed by others in animals, and (b) GVG plasma concentrations correlate well with changes in brain GABA-T and GABA, and may partly explain changes in the effects of GVG related to the length of treatment.

γ-Aminobutyric acid-transaminase (GABA-T)† inhibitors produce a dose-dependent increase in brain GABA concentration [1] which in rodents is parallel to the increase in synapse [2] and seems to be related to their anticonvulsant effects [1].

Vigabatrin (γ-vinyl GABA, GVG) is a specific and suicide inhibitor of α-oxoglutarate aminotransferase (EC 2.6.1.19). GVG inhibits this enzyme in a dose-dependent way both *in vitro* and *in vivo* [3], and has anticonvulsant effects in animals and humans [4]. The relationship between either dose size or treatment length and the effects of this antiepileptic drug show some peculiarities: (a) In mice, multiple doses of GVG produce a greater anticonvulsant effect on audiogenic convulsions than single doses [1]. (b) High doses of GVG in epileptic patients do not seem to be more effective than low ones [5, 6]. In mice, the anticonvulsant effects of GVG were less at high rather than at low doses [7]. (c) Tolerance to the anticonvulsant action of GVG has been observed in mice [8] and gerbils [9], and was related to GVG dose size. Tolerance to brain GABA release has also described in rats [10].

The neurochemical basis for these peculiarities concerning the effects of GVG is none too clear. The ceiling in the anticonvulsant effect may be the result of a plateau in GABA increase or a reduction in L-glutamate decarboxylase activity (EC 4.1.1.15, GAD), which would prevent any further rise in brain GABA concentrations. The lower anticonvulsant effects of GVG at high doses and the development of tolerance may also be attributed to GAD inhibition [8]. In fact, although GVG did not inhibit GAD *in vitro* [11], its effects *in vivo* are variable [1, 2, 12, 13].

Pharmacokinetic mechanisms should also be taken into account. However, data on GVG plasma concentration related to dose size or length of treatment are scanty. A poor relationship between GVG plasma concentration and the effects of this antiepileptic drug has been observed in patients [14–16], but studies on the correlation between GVG plasma concentration and GVG effects in humans or animals are lacking.

The aim of this study was to analyse the effects of increasing (50–1600 mg/kg/day) doses of GVG administered as single doses, and for 8 and 28 days of treatment, on GABA-T activity, GABA concentration and GAD activity in brain, and to analyse their relationship with GVG plasma concentrations in rats.

MATERIALS AND METHODS

Animals and groups. The study was carried out in 19 groups of 10 adult, male Wistar rats (450–500 g) of similar age, housed at a temperature of 22° ± 2° under natural light/dark conditions, and allowed food and water *ad lib.*

* Corresponding author: Dr Juan A. Armijo, Servicio de Farmacología Clínica, Hospital "M. de Valdecilla", E-39008 Santander, Spain. FAX (34) 42-202655.

† Abbreviations: GABA, γ-aminobutyric acid; GVG, vigabatrin, γ-vinyl GABA; GABA-T, GABA-transaminase; GAD, L-glutamate decarboxylase.

Seven groups were treated with a single (9 a.m.) i.p. dose of saline (control group), and 50, 100, 200, 400, 800 and 1600 mg/kg/day of GVG in aqueous solution (1 mL/rat). Another seven groups were treated with the same doses for 8 days, but seven rats in the 800 mg/kg/day group and 10 rats in the 1600 mg/kg/day group died. Therefore, for the 28-day study only five groups were treated with doses of saline (control group), and 50, 100, 200 and 400 mg/kg/day of GVG.

Samples. Samples were obtained 4 hr after the last GVG dose, since the highest brain GABA concentrations and anticonvulsant effects have been observed at that time [1, 17]. Maximum GVG effects on GABA-T activity may be reached at 24 hr or later, but GVG plasma concentrations are practically undetectable at 24 hr [18]. The rats were anaesthetized with ether, 10 mL of blood being collected from each rat by heart puncture and placed into chilled glass tubes containing 1 mL of 5% EDTA to measure GVG plasma concentrations. The whole brain was immediately removed, sagittally divided and weighed. One half was homogenized in 5 mL of chilled water and an aliquot stored to analyse GAD activity. Homogenates were centrifuged at 2500 *g* for 10 min at 4° and the supernatant stored at −20° until brain GABA-T assay. The other half was homogenized in 5 mL cold methanol to measure the GABA concentration in the supernatant obtained as described. All samples were maintained at −20° until assay.

Assays. Brain GABA-T activity was measured by the radiometric method described by White and Faison [19] and modified as described previously [20]. Mean brain GABA-T activity in control groups was 624 pmol/min/mg of protein, close to the value of 750 found by White and Faison [19]. Quality control was assessed in five control samples assayed for 9 days in triplicate. Within-assay and between-assay coefficients of variation were 6.0 ± 0.9 and 12.4 ± 1.8, respectively.

Brain GABA concentration was assayed by the liquid chromatographic method of Turnell and Cooper [21] with some modifications: before assay, 50 μL of brain supernatant (or of 0.25, 0.5, 1 and 2.5 mM GABA calibrators), 50 μL internal standard (1 mM ethanolamine) and 50 μL of deproteinizing reactive (5% 5-sulfosalicylic acid) were vortex-mixed and centrifuged, all samples simultaneously, at 10,500 *g* and room temperature for 2 min and maintained in an ice bath. Just before injection, 50 μL of the supernatant were vortex-mixed with 100 μL of daily prepared *O*-phthalaldehyde-2-mercaptoethanol derivatizing reagent (50 mg of *O*-phthalaldehyde in 1 mL of methanol were added to 9 mL of 0.9 M borate buffer, pH 9.9, and 40 μL of 2-mercaptoethanol) and 10 μL immediately injected in a liquid chromatograph equipped with a 100 mm × 5 mm i.d. column packed with 5 μm "Lichrosphere" RP-18 and a Waters-420 fluorescence detector (338 nm excitation and 425 nm emission wavelength filters). The assay was performed at room temperature and under isocratic conditions using a mobile phase of methanol/phosphate buffer at 67 mM, pH 5.3, 25/75% by volume at a flow of 1 mL/min. Mean brain GABA concentration in control groups was 2.4 μmol/g of tissue. Within-assay and between-assay coefficients of variation in the five control samples were 2.3 ± 0.9 and 6.8 ± 3.4, respectively.

The brain GAD activity was assayed using the method of Lowe *et al.* [22]. Mean brain GAD activity in control groups was 80 μmol/hr/g of protein. Within-assay and between-assay coefficients of variation in the five control samples were 6.7 ± 0.9 and 11.2 ± 4.2, respectively.

Brain homogenate supernatant concentrations of protein were determined by the method of Lowry *et al.* [23], using human albumin as a standard.

GVG plasma concentrations were measured using the liquid chromatographic method of Eslami *et al.* [24] to assay plasma GABA concentration, modified as described previously [20]. Correlation between spiked GVG concentration (x) and GVG/ethanolamine peak height ratio (y) from 10 calibration curves was linear ($r = 1.00$; $y = 0.17 + 1.01x$). Plasma controls with 6.25 and 12.5 mg/L of GVG were assayed for 8 days in duplicate. Within-assay coefficient of variation were 4.8 and 11.6%, respectively. Between-assay coefficients of variation of 14% were found for both controls.

Data analysis. Maximum inhibition (I_{max}) of GABA-T and GVG dose which reduced GABA-T activity to 50% of control group (ID_{50}) could be estimated from the double-reciprocal plot of GVG dose against GABA-T inhibition using the following equation, where I means inhibition and D dose:

$$\frac{1}{I} = \frac{1}{I_{max}} + \frac{ID_{50}}{I_{max}} \times \frac{1}{D}.$$

Dose–effect curves were drawn by means of the following equation:

$$I = \frac{I_{max} \times D}{ID_{50} - D}.$$

Statistical analysis was carried out using SPSS/PC+ software. Comparison between the effects of increasing doses and differences between single doses, and 8 and 28 days of treatment for each GVG dose were assessed by analysis of variance (one way), followed by Newman–Keuls' test. Differences between single doses, and 8 and 28 days of treatment, as a whole, were assessed by analysis of covariance. Comparison between body weight changes at 8, 16 and 28 days of treatment was performed by paired Student's *t*-test. The correlation between GVG plasma concentration and either GABA-T inhibition or GABA increase was estimated by the minimum-square linear regression method. Data are expressed as mean ± SD in text and Tables and as mean ± SE in Figures. A significance of $P < 0.05$, two-sided test, was accepted throughout.

RESULTS

Effects on GABA-T

Single increasing doses of GVG inhibited brain GABA-T activity in a dose-dependent way, but inhibition with 800 and 1600 mg/kg was similar to that with 400 mg/kg, there remaining with these doses a residual GABA-T activity of about 40% in

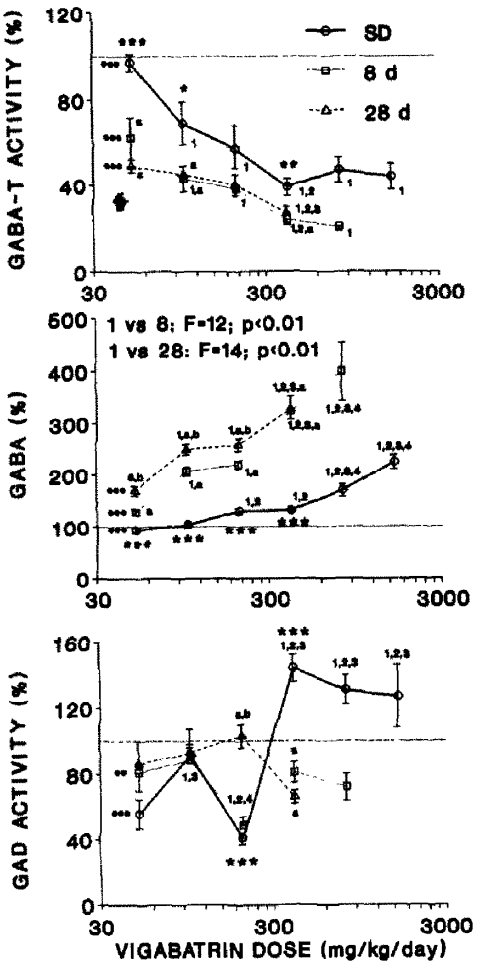

Fig. 1. Effects of GVG on the activity of GABA-T, the concentration of GABA and the activity of GAD in rat brains after single doses (SD), 8 days (8 d) and 28 days of treatment (28 d). Effects are expressed as percentages of the control groups. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ between single doses, and 8 and 28 days of treatment by analysis of variance; $^{\bullet\bullet}P < 0.01$ and $^{\bullet\bullet\bullet}P < 0.001$ between the effects of increasing doses by analysis of variance. Statistically different from [1]50 mg/kg, [2]100 mg/kg, [3]200 mg/kg and [4]400 mg/kg, and from [a]single doses and [b]8 days by Newman–Keuls' test. *F*, analysis of covariance for common doses of 50, 100, 200 and 400 mg/kg. Samples were obtained 4 hr after GVG administration. Data are means ± SE.

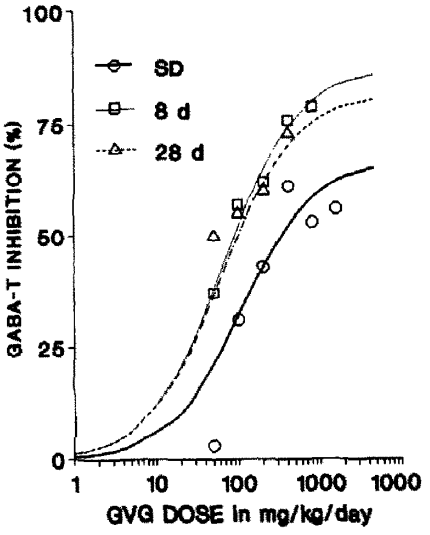

Fig. 2. Relationship between GVG dose and percentage of brain GABA-T inhibition with respect to the control groups after single doses (SD), and after 8 (8 d) and 28 days (28 d) of treatment, in rat brains. Samples were obtained 4 hr after GVG administration.

relation to the control group. Dose-dependent inhibition was also observed after 8 and 28 days of treatment. The ceiling observed after single doses could not be confirmed after multiple doses because of the death of some rats in the 8-day study and the lower dose range administered in the 28-day study (Fig. 1A).

GVG inhibition of brain GABA-T was more marked after 8 days of treatment than after single doses. GABA-T inhibition after 28 days of treatment with GVG was similar to that observed after 8 days. Although the effects of 8 and 28 days of treatment, as a whole, were not significantly higher than those of single doses by analysis of covariance, a significantly higher inhibition after multiple than single doses was observed with 50, 100 and 400 mg/kg/day of GVG by analysis of variance (Fig. 1A).

I_{max} increased from 65% after single doses to 87% and 82% after 8 and 28 days of treatment, respectively. ID_{50} decreased from 105 mg/kg after single doses to 65 and 62 mg/kg after 8 and 28 days of treatment, respectively. Dose–effect curves are shown in Fig. 2.

Effects on GABA

Progressively greater inhibition of GABA-T by increasing GVG doses was accompanied by a statistically significant dose-dependent increase in brain GABA concentration after single doses, and after 8 and 28 days of treatment. However, on this occasion, a ceiling for the effect of GVG on GABA was not observed. As described for GABA-T, the effects of 8 and 28 days of treatment with GVG on GABA were higher than after single doses, reaching on this occasion a high statistical significance by analysis of covariance (Fig. 1B).

Correlations between percentages of GABA-T inhibition and GABA increase with respect to each control group were statistically significant ($P < 0.001$) after single doses ($r = -0.53$), and 8 days ($r = -0.66$) and 28 days of treatment ($r = -0.64$). Data fitted better into a power curve ($r = -0.60$ after single doses and $r = -0.79$ after 8 days of treatment). As shown in Fig. 3, the analysis of data obtained after single doses, and 8 days and 28 days of treatment, as a whole, also fitted better into a power curve ($r = -0.70$) rather than into a line ($r = -0.61$).

Effects on GAD

Single doses of GVG reduced brain GAD activity

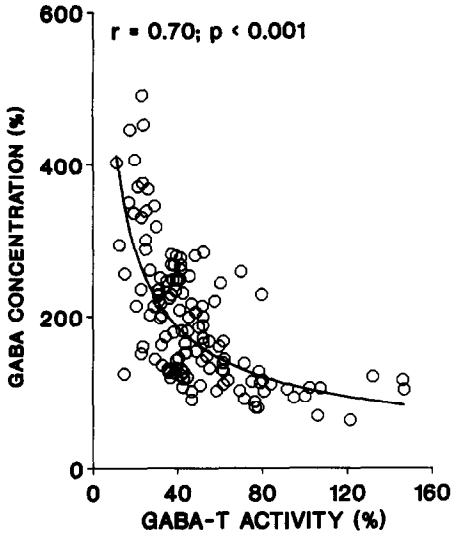

Fig. 3. Correlation between the inhibition of GABA-T activity and the increase in GABA concentration in rat brains produced by single doses (50, 100, 200, 800 and 1600 mg/kg), 8 days (50, 100, 200, 400 and 800 mg/kg/day) and 28 days of treatment with GVG (50, 100, 200 and 400 mg/kg/day). Effects are expressed as percentages of the control groups. Samples were obtained 4 hr after GVG administration. *r*, coefficient of correlation by power regression.

in a variable way at doses lower than 200 mg/kg of GVG, and increased it over the control with doses higher than 200 mg/kg (Fig. 1C). After 8 and 28 days of treatment, GAD inhibition was also observed at high GVG doses. A progressive dose-dependent inhibition of this enzyme with increasing doses of GVG, or a greater effect after multiple doses with respect to single doses was not demonstrated (Fig. 1C).

Toxic effects

Single doses of 1600 mg/kg of GVG produced diarrhoea and periocular and peribucal hemorrhagic ulcers. After 8 days of treatment, a syndrome characterized by sedation, ataxia, hunched posture, piloerection, food ingesta reduction and body weight loss could be observed. Seizures were not noticed. This syndrome was detected after 5–7 days of treatment with 400 mg/kg/day of GVG; with 800 mg/kg/day, it appeared earlier (3–4 days) and was more severe, since seven of the 10 rats died; in the 1600 mg/kg/day group all 10 rats died, but a significant loss of body weight was observed on the 4th day. In rats treated for 28 days the syndrome was observed after 12–15 days of treatment with 200 mg/kg/day and improved from 20 days of treatment onwards; after 400 mg/kg, toxic effects appeared after 5–7 days and decreased from 23–24 days on. In the 28-day experiment, a significant reduction in body weight was observed with 200 mg/kg/day on the 8th day, which was maintained until the 16th day and was practically reversed on the 28th day. With 400 mg/kg/day, body weight loss was greater than with 200 mg/kg/day on the 8th day, increased on the 16th day and partly reversed on the 28th day (Fig. 4).

GVG plasma concentration

Plasma GVG concentration increased in a dose-dependent manner both after single doses and after 8 days of treatment. As was pointed out for brain GABA-T inhibition and brain GABA increase, GVG plasma concentrations after 8 days of treatment were higher than after single doses, especially with doses of 200 mg/kg and higher. On the other hand, GVG plasma concentrations after 28 days of treatment with 200 or 400 mg/kg/day were lower than after 8 days and similar to those after single doses (Table 1).

The relationship between GVG plasma concentration and brain GABA-T inhibition fitted better to a power curve than to a line (Table 2). The

Table 1. GVG plasma concentration with increasing single doses and after 8 and 28 days of treatment

GVG dose (mg/kg)	GVG plasma concentrations (mg/L)			*F* (P)
	Single dose	8 days	28 days	
50	8.2 ± 3.8	6.2 ± 1.1	5.5 ± 3.7	2.1 (NS)
100	8.4 ± 3.5	11.1 ± 3.6	10.4 ± 6.1	1.0 (NS)
200	11.2 ± 4.9	35.2 ± 21.5‖¶	15.4 ± 10.5	8.3 (<0.05)
400	29.6 ± 10.5	77.7 ± 44.2*†‡‖¶	22.3 ± 17.4*†	11.5 (<0.001)
800	91.2 ± 51.4*†‡§	125.7 ± 51.1*†‡§	—	—
1600	115.5 ± 55.9*†‡§	—	—	—
F (P)	22.6 (<0.001)	19.7 (<0.001)	4.4 (<0.01)	

F, analysis of variance.
NS, not significant.
Statistically different from * 50 mg/kg, † 100 mg/kg, ‡ 200 mg/kg and § 400 mg/kg, and from ‖ single dose and ¶ 28 doses of treatment by Newman–Keuls' test.
Differences between single dose and 8 days of treatment (for the common doses of 50, 100, 200 and 400 mg/kg) were significant by analysis of covariance ($F = 8.0$, $P < 0.05$).

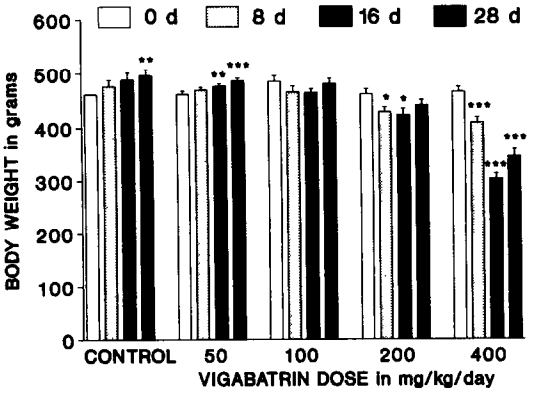

Fig. 4. Effects of increasing doses of GVG on rat body weight on 8th, 16th and 28th days of treatment. Samples were obtained 4 hr after GVG administration. *P < 0.05, **P < 0.01 and ***P < 0.001 with respect to the baseline values (0 d) by paired Student's *t*-test.

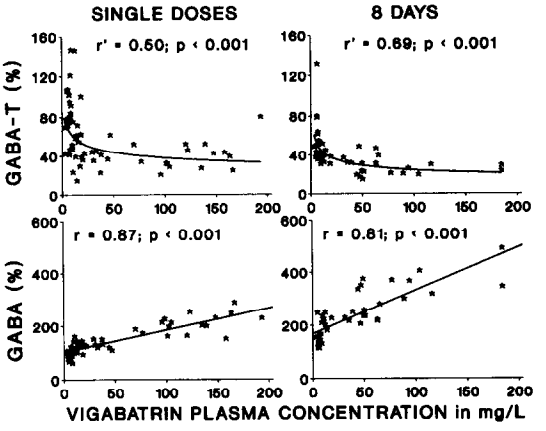

Fig. 5. Correlation between GVG plasma concentration and either GABA-T activity or GABA concentration, after single doses and after 8 days of treatment. The effects are expressed as percentages of the control groups. Samples were obtained 4 hr after GVG administration. *r*, coefficient of correlation by linear regression; *r'*, coefficient of correlation by power regression.

Table 2. Correlation between GVG plasma concentration and GABA-T activity, GABA concentration and GAD activity in rat brains

Length of GVG treatment	GABA-T activity (% of control)			GABA concentration (% of control)			GAD activity (% of control)		
	N	*r*	P	N	*r*	P	N	*r*	P
Linear regression ($y = a + bx$)									
Single dose	59	−0.38	<0.01	59	0.87†	<0.001	58	0.40	<0.01
8 days	43	−0.47	<0.001	42	0.81*	<0.001	43	0.09	NS
28 days	38	−0.45	<0.01	36	0.50	<0.01	37	0.08	NS
All	140	−0.30	<0.001	137	0.42	<0.001	138	0.22	<0.01
Power regression ($y = ax^b$)									
Single dose	59	−0.50	0.001	59	0.86†	0.001			
8 days	43	−0.69	0.001	42	0.84	0.001			
28 days	36	−0.33	NS	34	0.24	NS			
All	148	−0.42	0.001	135	0.45	0.001			

N, number of rats.
* P < 0.01 and † P < 0.001 vs GABA-T by Fisher's "z" transformation.
NS, not significant.

relationship between GVG plasma concentration and the increase in brain GABA fitted well to a line and the coefficients of correlation were significantly higher than those found with GABA-T, both after single doses and after 8 days of treatment (Fig. 5). A positive and significant correlation between GVG plasma concentration and effects on brain GAD activity was observed after single doses, but a negative correlation after multiple doses was not found (Table 2).

DISCUSSION

Single doses

Increasing single doses of vigabatrin reduced brain GABA-T activity in a dose-dependent manner, but the effects of 800 and 1600 mg/kg were similar to those of 400 mg/kg, suggesting that a ceiling of about 40% of the control group had been reached. This residual activity of brain GABA-T after single doses of GVG has been observed in mice [3] and rats [18] *in vivo*. However, GVG is able to inhibit completely GABA-T *in vitro* [25] and in some peripheral tissues such as the liver [18] and platelets [20]. Residual activity of GABA-T in the brain has been attributed to the protective effect of the increase in brain GABA concentration secondary to GABA-T inhibition [18].

Our results show a progressive increase in GABA concentration with increasing GVG doses. The increase in brain GABA concentration correlated with the degree of brain GABA-T inhibition, but the relationship between both parameters was curvilinear: the increase in GABA was more marked

when GABA-T activity was less than 60% of the control group. A ceiling in the effect of GVG on GABA was not observed. Therefore, the ceiling in GVG effects on brain GABA-T seems unable to prevent the increase in brain GABA concentration. Our results do not support the hypothesis that the weak antiepileptic efficacy of increasing GVG doses in clinical practice [5, 6] may be due to a ceiling in the increase in brain GABA. They support the hypothesis that the increase in brain GABA may be due also to other mechanisms [17].

The variability in the GAD response to low single doses of GVG confirms the small and variable effects described by other authors who did not observe changes [10] or a non-significant decrease [1, 3, 12]. On the other hand, the significant stimulation observed with high single doses of GVG contrasts with the results of other studies in which high doses of GVG reduced GAD activity [1, 3]. Nevertheless, an increase in brain and synaptosomal GAD activity has also been described [2, 13].

GVG inhibition of GAD activity is not observed *in vitro* [11]. The inhibition observed *in vivo* has been described as being secondary to the increase in GABA concentration [8, 26, 27]. In this case, GAD inhibition should increase with dose and should reduce the increase in brain GABA concentration, as has been described for ethanolamine-*O*-sulphate [26]. However, neither of these assumptions were true for our study. The GAD stimulation observed at 4 hr after high single doses of GVG in this study may explain why GABA concentrations continued to increase with increasing doses of GVG in spite of a ceiling in the inhibition of GABA-T.

Eight days of treatment

More marked effects on brain GABA-T and GABA were observed after multiple as compared with single doses of GVG. These greater effects of GVG may explain the greater anticonvulsant effects against audiogenic convulsions observed in mice after multiple doses of GVG as compared with single doses [1].

The higher GABAergic effects were accompanied by a higher GVG toxicity. In fact, doses of 800 and 1600 mg/kg/day, which were well tolerated as single doses, were lethal after 8 days of treatment. Toxic effects of GVG were similar to those described by Schechter and Trainer [28]. They are probably due to the increase in GABA concentration [8], but may also be attributed to the inhibitory effects of GVG on other brain enzymes [12]. The proconvulsant action found by other authors after multiple doses [29, 30] was not detected in this study. Nevertheless, the study was not specifically designed to detect this and, therefore, its presence cannot be excluded.

The more noticeable effects of repeated doses of GVG can be explained by pharmacokinetic mechanisms, such as plasma or tissue accumulation. Plasma accumulation was not expected because of the short half-life of GVG and the large interval of administration (24 hr) used in this study. However, significantly higher GVG plasma concentrations were found after 8 days of treatment than after single doses and, therefore, the greater effects on brain GABA-T and GABA of GVG multiple dose could be explained by this mechanism. The markedly higher GVG plasma concentrations were observed with GVG doses of 200 mg/kg and higher suggesting that they may be related to saturation of some mechanism of elimination at high doses.

Maximum inhibition of GABA-T was also higher after multiple doses, which would suggest that the high residual activity found after single doses may be due partly to a ceiling in the access of GVG to the brain at 4 hr of a single dose. GVG penetrates the blood–brain barrier poorly after single doses and brain accumulation may take place after multiple doses. In fact, an increase in cerebrospinal fluid GVG concentration after 1 month of treatment as compared with a single dose has been described in patients [31]. Differences in the maximum GABA-T inhibition achieved by other GABA-T inhibitors may also depend on their access to the brain. Also, because of the long-lasting inhibition of GABA-T, accumulation of GABA-T inhibition may occur when GVG is administered every day.

Twenty-eight days of treatment

When the treatment was extended over 28 days, the GVG effects on brain GABA-T activity and brain GABA concentration were similar to those of the 8-day treatment. Thus, our results do not explain the development of tolerance to the anticonvulsant effects of GVG found in mice [8] and gerbils [9], and do not confirm the lower brain GABA concentrations observed with 100 than with 50 mg/kg/day after 17 days of treatment with GVG by those authors [9]. Nevertheless, as we have only studied the effects of GVG after single doses, and after 8 and 28 days of treatment, development of tolerance within both the 1–8- and the 8–28-day periods cannot be excluded.

After multiple doses of GVG, GAD activity was inhibited instead of stimulated by high doses. This suggests that stimulation may be an immediate action, perhaps a direct one, only detectable in an acute experiment (i.e. 4 hr after a single dose), while inhibition may be a delayed effect, secondary to the increase in GABA concentration, which could mask and even reverse after multiple doses the stimulation shown after single doses. This hypothesis concerning an additive effect of a dual action of GVG on GAD could explain why there is no progressive inhibition of GAD activity with dose, parallel to the increase in GABA concentration, as well as the variable effects of GVG on GAD found in different studies.

The lack of a progressive reduction in GAD activity which prevents the progressive increase in brain GABA concentration does not rule out other possible mechanisms of tolerance: the inhibition of GAD can reduce the release of GABA in the synapse even when high brain GABA concentrations are detected [32]; GABA may be redistributed reaching high concentrations in glia at low synapse concentrations [10]; GABA release may also be reduced by GVG stimulation of presynaptic autoreceptors [33]; the increase of GABA can cause desensitization of GABA postsynaptic receptors [34], although changes in GABA receptors after

multiple doses of GVG have not been found in rats [35].

Our results add weight to other possible explanations for the decrease in GVG effects: GVG plasma concentrations after 28 days of treatment by an i.p. route were markedly lower than after 8 days and even slightly lower than after single doses. The mechanism of these low GVG plasma concentrations is not clear, since macroscopic alterations or drug deposits were not found in the peritoneal cavity. These lower concentrations may prevent a greater effect of GVG on brain GABA-T and GABA and may explain the decrease in toxic effects as found in this study. Furthermore, the reduction in food intake and in body weight observed in this study, or in drinking water intake found by others [26], may explain less effects of GABA-T inhibitors when they are administered by oral route [8].

Tolerance to the antiepileptic effect of GVG has not been found in patients [6, 36, 37]. This has been attributed to differences in GVG dosage [9] but may also be due to differences in GVG administration.

Correlation with GVG plasma concentration

If the effects of GVG depend on its plasma concentration, a relationship between them is only to be expected. In fact, a good correlation was observed between GVG plasma concentration at 4 hr after GVG administration and either brain GABA-T inhibition or brain GABA increase, both after single and after 8 days of treatment. However, neurochemical effects of GVG after 28 days of treatment were similar to those after 8 days, whereas GVG plasma concentrations were lower, suggesting that changes in GVG effects related with time also depend on other factors.

The good correlation between GVG plasma concentration and GVG effects found in this study suggests the possibility of using this accessible parameter as a reflection of the actions of GVG in the brain. However, our results contrast with the poor relationship between steady-state plasma concentration of GVG and GVG effects observed in patients [14–16]. Therefore, more specific studies on the relationship between GVG plasma concentration and effects are necessary.

In summary, our results show that single increasing doses of GVG progressively inhibit GABA-T activity, but a residual activity of 40% was observed at the highest doses. GABA concentrations also increased in a dose-dependent manner but a ceiling was not reached. When treatment was continued for 8 days more marked effects on GABA-T and GABA, a more severe toxicity, and higher GVG plasma concentrations were observed. The more marked effects of multiple doses of GVG may be attributed to the higher plasma concentrations found in this study and, in fact, a good correlation between GVG and either the inhibition of GABA-T and the increase in GABA concentration was demonstrated. After 28 days of treatment the effects of GVG on both parameters were similar to those after 8 days. Toxic effects decreased, however, and lower GVG plasma concentrations were found.

It is concluded that: (a) the more marked brain GABAergic effects observed after 8 days of treatment with GVG may explain the higher anticonvulsant effects observed by other authors in animals, and (b) GVG plasma concentrations correlated well with changes in brain GABA-T and GABA, and may partly explain changes in the GVG effects related to the length of treatment.

Acknowledgements—Vigabatrin was kindly supplied by the Marion Merrell-Dow Research Institute. The study was partly supported by a grant from the University of Cantabria and the Government of Cantabria. One of the authors (E.M.V.) has a fellowship from the Spanish Health Ministry (FISS 88/418).

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