Br. J. clin. Pharmac. (1989), **27**, 13S–17S

Experimental studies of the influence of vigabatrin on the GABA system

L. GRAM[1], O. M. LARSSON[2], A. JOHNSEN[3] & A. SCHOUSBOE[4]
[1]University Clinic of Neurology, Hvidovre Hospital, [2]Department of Biology, Royal Danish School of Pharmacy, [3]Department of Clinical Chemistry KK, Rigshospital and [4]Department of Biochemistry A, Panum Institute, Copenhagen, Denmark

1 Studies of the influence of clinically relevant concentrations of vigabatrin on GABA-transaminase and on the release of endogenous GABA were performed in selectively cultured astrocytes and neurons. In addition, the two stereoisomers of vigabatrin were investigated separately.
2 The results indicated a preferential inhibition of neuronal GABA-transaminase by vigabatrin.
3 Only the (S)-form of vigabatrin seems to inhibit GABA-transaminase. This finding corresponds to observations in epileptic animals that the (R)-form exhibits no anticonvulsant effect.
4 Resynthesis of GABA-transaminase, following withdrawal of vigabatrin showed that maximum enzyme activity was obtained within 6 days. This finding corresponds to the persistent effect after withdrawal of the drug in patients, observed in clinical trials.
5 At a concentration of 25 μM, vigabatrin caused a significant increase in the release of endogenous GABA from cultured GABAergic neurons. Although no data on brain levels of the drugs are currently available, judging from vigabatrin blood concentrations in man and from information of brain levels in animals, following chronic treatment, it is conceivable that a sufficiently high concentration of the drug in human brain is obtained to augment GABA release.

Keywords vigabatrin γ-vinyl GABA GABA-transaminase GABA release

Introduction

γ-aminobutyric acid (GABA) has been established as the major inhibitory transmitter in the central nervous system (Roberts *et al.*, 1976). For several years impaired inhibition has been considered to play a prominent role in epileptogenesis, since inhibiting the synthesis of GABA as well as administration of GABA receptor antagonists results in seizures.

From a theoretical point of view several possibilities for correcting suboptimal GABAergic inhibition may be conceived: stimulating GABA synthesis, administering GABA agonists or prodrugs, inhibiting the metabolism of GABA, increasing GABA release or inhibiting GABA uptake. Currently the most promising approach seems to be inhibition of GABA metabolism, by inhibiting the principal GABA metabolising enzyme, GABA-transaminase (GABA-T).

Vigabatrin (γ-vinyl GABA, GVG) was synthesized as a rational approach to manipulate the GABA system and turned out to be a selective and irreversible inhibitor of GABA-T (Lippert *et al.*, 1977). In the clinic GVG has demonstrated excellent antiepileptic properties (Gram, 1987).

Correspondence: Dr L. Gram, Dianalund Epilepsy Hospital, DK-4293 Dianalund, Denmark

The aims of the present series of studies were to investigate the influence of GVG (including the two stereoisomer forms) on neuronal and glial GABA-T, separately, and to study the effect of clinically relevant concentrations of GVG on evoked release of endogenous GABA.

Methods

GABA-T investigations

The studies were performed in separately cultured neurons and astrocytes.

Interneurons from cerebral cortex of 15 day old mouse embryos were cultured as previously described (Dichter, 1978; Larsson *et al.*, 1981; Yu *et al.*, 1984) with the modifications described by Meier & Schousboe (1982). After 48 h in culture the cells were exposed to cytosine arabinoside, which leads to the disappearance of astroblasts (Dichter, 1978; Yu *et al.*, 1984). Such cultured neurons exhibit characteristics resembling those of GABAergic neurons (Dichter, 1978, 1980; Snodgrass *et al.*, 1980; Larsson *et al.*, 1981; Yu *et al.*, 1984; Drejer *et al.*, 1987).

Astrocytes were obtained from cortex of newborn mice and prepared as described by Hertz *et al.* (1982).

The activity of GABA-T was assayed using a modification (Schousboe *et al.*, 1977) of the method described by Hall & Kravitz (1967). The concentrations of GABA (^{14}C-labelled, specific radioactivity 0.1 μCi nmol^{-1}) and α-ketoglutaric acid were 1 and 0.5 mM, respectively. The reaction was allowed to take place for 10 min at 37° C, during which period the progress curve was linear. When determining the IC_{50} values for inhibition of GABA-T, the following concentrations of GVG were used: 3, 10, 30, 100 and 300 μM. In experiments investigating the resynthesis of GABA-T, the assay condition was slightly modified. The concentration of GABA (^{14}C-labelled, specific radioactivity 0.04 μCi nmol^{-1}), α-ketoglutaric acid and GVG were 25, 4 mM and 100 μM, respectively. The concentrations of GVG and (R)-GVG, when present in the assay mixture were 50, 100, 1000, 5000 and 10000 μM and in the case of (S)-GVG 10, 50, 100, 500, 1000 and 5000 μM. Protein measurements were done by the method of Lowry *et al.* (1951), using bovine serum albumin as the standard.

GABA release

Evoked release of endogenous GABA was investigated in neurons, cultured separately as described above. Release experiments were performed by the superfusion system described by Drejer *et al.* (1983, 1987). The cells were washed once with HEPES buffered saline (HBS) and placed in the superfusion chamber. The system comprised a peristaltic pump, continuously delivering thermostated (37° C) superfusion medium, 2 ml min^{-1}. The medium was continuously collected from the lower part of the slightly tilted Petri dish and delivered to a fraction collector. In order to wash out excess radioactivity the cells were superfused with HBS for 14 min, before connecting the fraction collector. The collection periods were 1 min and the cells were stimulated twice for 1 min, changing the superfusion medium from HBS to a corresponding medium, containing 55 mM of KCl. Isotonicity was maintained by removal of 50 mM NaCl from the medium. In some experiments the calcium dependence of the stimulated GABA release was studied employing a medium containing $CoCl_2$ in place of $CaCl_2$.

GABA was measured by ion exchange chromatography and post-column derivatization with *o*-phthaldialdehyde in the presence of mercaptoethanol on an LKB amino acid analyzer 4400 (Na-citrate, three buffer system). The fluorescence was detected with a Waters fluorometer 420. The detection level was better than 2 pmol.

Results

The IC_{50} values for inhibition of GABA-T by GVG appear in Table 1. A significant difference between IC_{50} values for inhibition of neuronal and glial GABA-T was demonstrated ($P < 0.005$), neuronal GABA-T being more sensitive to GVG than glial GABA-T. Culturing the cells in the presence of GVG increased the potency of the drug in both cell types (Table 2) with an unaltered statistically significant ($P < 0.001$) differential inhibitory effect between the two types of GABA-T.

Table 3 demonstrates the inhibitory effect of the two stereoisomers of GVG on GABA-T. It is seen that (R)-GVG was essentially without

Table 1 The IC_{50} values for inhibition of GABA-T by GVG present during incubation

Tissue	*IC_{50} (μM)*
Cerebral cortex	145 (131–162)
Cultured neurons	89 (83–95)
Cultured astrocytes	132 (120–145)

IC_{50} values have been calculated from log-probit analyses using linear regression. Ranges represent ± 2 s.e. mean of the logarithmic IC_{50} values. Modified from Larsson *et al.* (1986), with permission.

Table 2 The IC_{50} values for inhibition of GABA-T by GVG present during cell cultivation

	IC_{50} (μM)
Astrocytes	89 (69–116)
Neurons	24 (22–26)

The activity of GABA-T in the homogenates was determined without (RS)GVG being present in the GABA-T assay buffer. IC_{50} values have been calculated from log-probit analyses using linear regression. Ranges represent ± 2 s.e. mean of the logarithmic IC_{50} values. From Larsson *et al.* (1986), with permission.

Table 3 The IC_{50} values for inhibition of GABA-T by the stereoisomers of GVG.

	IC_{50} (μM)	
	(S)GVG	(R)GVG
Cerebral cortex	51 (44–59)	5453 (3969–7493)
Cultured neurons	43 (41–45)	5162 (4662–5717)
Cultured astrocytes	91 (82–192)	4678 (4212–5196)

IC_{50} values have been calculated from log-probit analyses using linear regression. Ranges represent ± 2 s.e. mean of the logarithmic IC_{50} values. From Larsson *et al.* (1986), with permission.

Table 4 Effect of GVG on cellular GABA content and release

Concentration of GVG (μM)	GABA content (% of controls)	GABA release (pmol min^{-1} mg^{-1})
0	100 (77.6–125.5) (7)	293.5 (257.3–306.1) (5)
10	359.1 (259.4–433.9)* (4)	—
25	—	628.2 (604.0–714.4)* (3)
30	563.4 (495.6–585.5)* (4)	—
100	336.0 (276.5–445.1)* (3)	436.8 (421.2–611.0)* (3)
300	319.6 (282.7–491.2)* (3)	—

The values represent medians with ranges and number of experiments in parentheses. Values for GABA content are expressed as percent of controls 19.2 (14.9–24.1) (7) nmol mg^{-1} cell protein. Asterisks indicate statistically significant differences from the controls ($P < 0.05$, Mann-Whitney test). Modified from Gram *et al.* (1988), with permission.

influence, with regard to neuronal as well as glial GABA-T.

Resynthesis of GABA-T, following withdrawal of GVG, was investigated in the cultured neurons (Figure 1) and GABA-T activity had reached the level of control values within 6 days after removal of GVG.

The effect of 24 h exposure to GVG on GABA content in the neurons and on evoked release of endogenous GABA is shown in Table 4. It is seen that concentrations of GVG as low as 25 μM were sufficient to increase evoked GABA release significantly ($P < 0.05$). In addition, a correlation exists between cellular GABA content and the magnitude of GABA release. The calcium dependence of the release process was confirmed (results not shown).

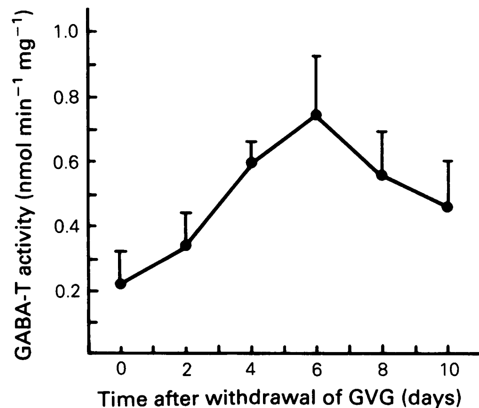

Figure 1 Biosynthesis of GABA-T in cultured neurons as a function of time after withdrawal of GVG, 100 μM, from the culture media. From Larsson *et al.* (1986), with permission.

Discussion

The findings of the studies presented seem to substantiate a direct GABAergic mechanism of

action of GVG. From a theoretical point of view inhibition of neuronal GABA-T seems far more important than inhibition of glial GABA-T for augmenting subnormal GABA inhibition. The observed preferential inhibitory influence of GVG on neuronal GABA-T speaks in favour of the drug being able to influence the amount of synaptically released GABA. The selective effect of (S)-GVG on GABA-T also lends support to a GABAergic mechanism of the drug since, in animals, it has been shown that only this enantiomer exhibits an anticonvulsant effect (Meldrum & Murugaiah, 1983). The rate of biosynthesis of GABA-T, following withdrawal of GVG, correlates with observations of a protracted clinical effect of the drug after withdrawal in patients, lasting for approximately 1 week (Gram *et al.*, 1983; Schechter *et al.*, 1984).

Studies of clinically relevant doses in man have demonstrated pronounced increases in the GABA content in CSF following treatment with the drug (Grove *et al.*, 1980; Pitkänen *et al.*, 1987). However, this is a somewhat indirect measure of what is taking place in the GABA system in the brain. It might be speculated that an increased GABA concentration in brain tissue, propagating into the CSF, could reflect an increased synaptic GABA release. However, no definite proof of such a correlation exists. Recently we have obtained data for valproate, which do not lend support to the assumption of a direct correlation between cellular GABA content and the release of GABA (Gram *et al.*, 1988). Consequently, it seemed worthwhile to perform investigations of the effect of clinically relevant GVG concentrations on the release of endogenous GABA. Previous animal studies have demonstrated a GVG-induced increase in the release of endogenous GABA, following single-dose treatment (Abdul-Ghani *et al.*, 1980, 1981; Grove *et al.*, 1982). However, no measurements of GVG brain or blood levels were performed in these experiments, rendering it difficult to evaluate their clinical relevance. Currently no information is available regarding GVG brain concentrations in man. Consequently, in our release investigation, we had to extrapolate from information on blood levels obtained in patients, being in the range of 50–150 μM (Gram *et al.*, 1983; Rimmer & Richens, 1984), and recent information of GVG brain levels in mice, demonstrating a range of 30–50 μM (Seiler *et al.*, 1987), following chronic treatment with anticonvulsant doses of the drug. Comparing these levels with the obtained result, that a GVG concentration as low as 25 μM caused a more than 100% increase in the amount of released GABA, it must be assumed that sufficient concentrations of GVG are obtained in the brains of patients to augment GABA release.

Finally, the apparently rather specific action of GVG with regard to GABA-T inhibition (Schechter *et al.*, 1984; Pitkänen *et al.*, 1987) further substantiates a direct GABAergic mechanism of action of the drug.

This work has been supported by grants from The Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands and Greenland, from The Danish State Medical Research Council and from The Foundation of Experimental Research in Neurology.

References

Abdul-Ghani, A. S., Coutinho-Netto, J. & Bradford, H. F. (1980). The action of γ-vinyl GABA and γ-acetylenic GABA on the resting and stimulated release of GABA *in vivo*. *Brain Res.*, **141**, 471–481.

Abdul-Ghani, A. S., Norris, P. H., Smith, C. C. T. & Bradford, H. F. (1981). Effect of γ-acetylenic GABA and γ-vinyl GABA on synaptosomal release and uptake of GABA. *Biochem. Pharmac.*, **30**, 1203–1209.

Dichter, M. A. (1978). Rat cortical neurons in cell culture – culture methods, cell morphology, electrophysiology and synapse formation. *Brain Res.*, **149**, 279–283.

Dichter, M. A. (1980). Physiological identification of GABA as the inhibitory transmitter for mammalian cortical neurons in cell culture. *Brain Res.*, **190**, 111–121.

Drejer, J., Honore, T. & Schousboe, A. (1987). Excitatory amino acid induced release of ^{3}H-GABA from cultured mouse cerebral cortex interneurons. *J. Neurosci.*, **7**, 2910–2916.

Drejer, J., Larsson, O. M. & Schousboe, A. (1983). Characterization of uptake and release processes for D- and L-aspartate in primary cultures of astrocytes and cerebellar granule cells. *Neurochem. Res.*, **8**, 231–243.

Gram, L. (1987). Vigabatrin. In *Epilepsy: Progress in treatment*, eds Dam, M., Johannessen, S. I., Nilsson B. & Sillanpäa M., pp. 263–268, Chichester: John Wiley & Sons.

Gram, L., Larsson, O. M., Johnsen, A. & Schousboe, A. (1988). Effect of valproate, vigabatrin and aminooxyacetic acid on release of endogenous and exogenous GABA from cultured neurons. *Epilepsy Res.*, **2**, 87–95.

Gram, L., Lyon, B. B. & Dam, M. (1983). Gamma-vinyl GABA: A single-blind trial in patients with epilepsy. *Acta Neurol. Scand.*, **68**, 34–39.

Grove, J., Gardner, C. R. & Richards, M. H. (1982).

Measurements of release of endogenous GABA and catabolites of (^{3}H)GABA from synaptosomal preparations using ion-exchange chromatography. *Neurochem. Res.*, **7**, 589–599.

Grove, J., Tell, G., Schechter, P. J., Koch-Weser, J., Warter, J. M., Marescaux, C. & Rumbach, L. (1980). Increased CSF gamma-aminobutyric acid after treatment with gamma-vinyl GABA. *Lancet*, **ii**, 647.

Hall, Z. W. & Kravitz, E. A. (1967). The metabolism of γ-aminobutyric acid (GABA) in the lobster neuron system. I. GABA-glutamate transaminase. *J. Neurochem.*, **14**, 45–54.

Hertz, E., Juurlink, B. H. J., Fosmark, H. & Schousboe, A. (1982). Astrocytes in primary culture. In *Neuroscience approached through cell culture*, ed. Pfeiffer, S. E., Vol. 1., pp. 175–186. Boca Raton, Florida: CRC Press.

Larsson, O. M., Gram, L., Schousboe, I. & Schousboe, A. (1986). Differential effect of gamma-vinyl GABA and valproate on GABA-transaminase from cultured neurones and astrocytes. *Neuropharmacology*, **25**, 617–625.

Larsson, O. M., Thorbek, P., Krogsgaard-Larsen, P. & Schousboe, A. (1981). Effect of homo-β-proline and other heterocyclic GABA analogues on GABA uptake in neurons and astroglial cells and on GABA receptor binding. *J. Neurochem.*, **37**, 1509–1516.

Lippert, B., Metcalf, B. W., Jung, M. J. & Casara, P. (1977). 4-Aminohex-5-enoic acid, a selective catalytic inhibitor of 4-aminobutyric acid aminotransferase in mammalian brain. *Eur. J. Biochem.*, **74**, 4411–445.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurements with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

Meier, E. & Schousboe, A. (1982). Differences between GABA receptor binding to membranes from cerebellum during postnatal development and from cultured cerebellar granule cells. *Dev. Neurosci.*, **5**, 546–553.

Meldrum, B. S. & Murugaiah, K. M. (1983). Anticonvulsant action in mice with sound-induced seizures of the optical isomers of gamma-vinyl GABA. *Eur. J. Pharmac.*, **89**, 149–152.

Pitkänen, A., Halonen, T., Ylinen, A. & Riekkinen, P. (1987). Somatostatin, β-endorphin, and prolactin levels in human cerebrospinal fluid during the γ-vinyl-GABA treatment of patients with complex partial epilepsy. *Neuropeptides*, **9**, 185–195.

Rimmer, E. & Richens, A. (1984). Double-blind study of γ-vinyl GABA in patients with refractory epilepsy. *Lancet*, **i**, 189–190.

Roberts, E., Chase, T. N. & Tower, D. B., eds (1976). *GABA in nervous system function*, 554 pp. New York: Raven Press.

Schechter, P. J., Hanke, N. J. F., Grove, J., Huebert, N. & Sjoerdsma, A. (1984). Biochemical and clinical effects of γ-vinyl GABA in patients with epilepsy. *Neurology*, **34**, 182–186.

Schousboe, A., Hertz, L. & Svenneby, G. (1977). Uptake and metabolism of GABA in astrocytes cultured from dissociated mouse brain hemispheres. *Neurochem. Res.*, **2**, 217–229.

Seiler, N., Spraggs, H. & Danne, G. (1987). Interrelationship between ornithine, glutamate and GABA. I. Feed-back inhibition of ornithine aminotransferase by elevated brain GABA levels. *Neurochem. Int.*, **10**, 391–397.

Snodgrass, S. R., White, W. F., Biales, B. & Dichter, M. (1980). Biochemical correlates of GABA function in rat cortical neurons in culture. *Brain Res.*, **190**, 123–138.

Yu, A. C. H., Hertz, E. & Hertz, L. (1984). Alterations in uptake and release rates for GABA, glutamate and glutamine during biochemical maturation of highly purified cultures of cerebral cortical neurons, a GABAergic preparation. *J. Neurochem.*, **42**, 951–960.