Development of tolerance to the effects of vigabatrin (γ -vinyl-GABA) on GABA release from rat cerebral cortex, spinal cord and retina

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- 1 The effects of acute and chronic vigabatrin (γ -vinyl-GABA) (GVG) administration on γ -aminobutyric acid (GABA) levels and release in rat cortical slices, spinal cord slices and retinas were studied.
- 2 GVG (250 mg kg⁻¹ i.p.) administered to rats 18 h before death (acute administration) produced an almost 3 fold increase in GABA levels of the cortex and spinal cord and a 6 fold increase in retinal GABA. The levels of glutamate, aspartate, glycine and taurine were unaffected.
- 3 When GVG (250 mg kg⁻¹ i.p.) was administered daily for 17 days (chronic administration) a similar (almost 3 fold) increase in cortical GABA occurred but the increases in spinal and retinal GABA were reduced by approximately 40%.
- 4 Acute administration of GVG strikingly increased the potassium-evoked release (KCl 50 mM) of GABA from all three tissues. This enhanced evoked release was reduced by about 50% in tissues taken from rats that had been chronically treated with GVG.
- 5 Acute administration of GVG reduced GABA-transaminase (GABA-T) activity by approximately 80% in cortex and cord and by 98% in the retina. Following the chronic administration of GVG, there was a trend for GABA-T activities to recover (significant only in cortex). Acute administration of GVG had no effect on glutamic acid decarboxylase (GAD) activity in cortex or spinal cord. However, chronic treatment resulted in significant decreases in GAD activity in both the cortex and cord (35% and 50% reduction respectively).
- 6 The K-evoked release of glutamate, aspartate, glycine and taurine from cortical slices and the K-evoked release of glycine from spinal slices and retinas were not affected by either acute or chronic GVG treatment.
- 7 These experiments indicate that GVG treatment increases specifically the K-evoked release of GABA and that tolerance can develop to this enhancing effect of GVG on central GABA release. This tolerance may result from increased feedback inhibition of GAD with a consequent reduction of presynaptic GABA stores.

Introduction

γ -Vinyl-GABA (GVG) is an enzyme-activated irreversible inhibitor of γ -aminobutyric acid (GABA)-transaminase (GABA-T) (Lippert *et al.*, 1977) that has anticonvulsant activity in both animals (Meldrum & Horton, 1978); Schechter & Trainer, 1978) and epileptic patients (Gram *et al.*, 1983; Rimmer & Richens, 1984). The drug increases GABA levels in the brain and it is generally believed that it exerts its anti-convulsant effects by acting as an indirectly acting GABA-mimetic and increasing GABA release. This is supported by release experiments in animals where GVG has been shown to increase GABA release from the cerebral cortex (Abdul-Ghani *et al.*, 1980; 1981) and from mouse embryonic cultured neurones (Gram *et al.*, 1988). The evidence in human epileptics is less direct, but GVG increases the GABA concentration in the CSF and this probably reflects an increase in the central release of GABA (Grove *et al.*, 1981; Schechter *et al.*, 1984).

A problem with some anti-epileptics, notably the benzodiazepines, is that tolerance develops and recently tolerance to the antiseizure action of GVG in epileptic gerbils was described (Löscher & Frey, 1987).

The present study was undertaken to examine the mechanism underlying the development of tolerance to the antiseizure action of GVG. It was found that the increase in the K-evoked release of GABA produced by GVG was reduced when the drug was administered chronically. This decrease in evoked GABA release, which occurred in three areas of the

CNS, might explain the development of tolerance to the anti-seizure activity of GVG.

Methods

Preparation of tissues

Male Wistar rats (150–200 g) were killed by cervical dislocation. The eyes were enucleated and the retinas dissected in Krebs bicarbonate medium at room temperature. The brains were removed and slices of cerebral cortex (0.2 mm thick) were prepared with a McIlwain tissue chopper as described previously (Cunningham & Neal, 1981). The spinal cord was removed following an extensive laminectomy and transverse (0.3 mm thick) slices were again prepared with a McIlwain chopper (Hopkin & Neal, 1971).

Incubation of tissues

In order to measure the release of GABA from the tissues, slices of cortex (5–6 slices, weight 25 mg), spinal cord (5–6 slices, weight 25 mg), or two entire retinas (approx. 20 mg) were placed in a small perspex chamber (volume 0.5 ml) containing Krebs bicarbonate medium (250 μ l) at room temperature and were gently agitated by a stream of 95% O₂ plus 5% CO₂.

At 10 min intervals, the medium was drained off and replaced with fresh medium. The GABA and other amino acids in each of the resulting 10 min samples were measured

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by high performance liquid chromatography (h.p.l.c.) (Yazulla *et al.*, 1985).

Tissue amino acids

Tissue amino acids were measured as described previously (Yazulla *et al.*, 1985). Briefly, slices of cerebral cortex, spinal cord or entire retinas were homogenized in 1 M formic acid 15% and acetone 85% with a glass homogenizer (40 mg ml⁻¹). The homogenates were centrifuged at 3000 r.p.m. for 5 min at room temperature and the amino acids in the supernatant were measured by h.p.l.c.

High performance liquid chromatography

The samples were analysed on a Varian 5000 HPLC by pre-column derivatisation with *o*-phthalaldehyde (Turnell & Cooper, 1981).

Glutamic acid decarboxylase activity and GABA-transaminase activity

These were measured by the methods of Lowe *et al.* (1958) and Salvador & Alders (1959), respectively.

Acute GVG administration

Animals were given a single intraperitoneal injection of GVG (250 mg kg⁻¹) 18 h before death. Control rats were given an injection of saline 18 h before death.

Chronic GVG administration

Rats were given an injection of either saline (controls) or GVG (250 mg kg⁻¹) daily for 17 days. The last injections were given 18 h before death. In all cases injections were given between 16 h 00 min and 18 h 00 min.

Analysis of results

Differences in amino acid contents, and in the K-evoked release of GABA were tested for statistical significance by Student's unpaired *t* test.

Materials

Incubation medium Krebs-Ringer bicarbonate of the following composition was used (mM): NaCl 118, KCl 4.8, CaCl₂ 2.4, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, glucose 9.5. The medium was continuously gassed with 95% O₂ plus 5% CO₂. γ -Vinyl-GABA (vigabatrin) was a gift from Merrell-Dow Pharmaceuticals Ltd.

Results

Effect of GVG administration on tissue GABA levels

In the cerebral cortex both acute and chronic administration of GVG more than doubled the GABA levels (Figure 1). There was no evidence of tolerance developing to the effects of GVG on cortical GABA levels. Indeed, the GABA level in cortical tissue from the chronically treated rats was slightly higher than that in the tissue from rats receiving only one injection (2.7 and 2.5 fold increases respectively).

In contrast to the cerebral cortex, the increases in GABA levels caused by GVG in the spinal cord and retina were significantly smaller in the chronically treated animals (Figure 1). Thus, in the spinal cord, a single injection of GVG caused a

Figure 1 Effect of acute (single injection 18 h before death) or chronic (17 daily injections) treatment with saline (solid columns) or γ -vinyl-GABA (open columns) (250 mg kg⁻¹) on GABA content of cortex, spinal cord and retina. Each column is the mean and the bar s.e.mean of 5–10 experiments. There was a significant difference in GABA content between acutely and chronically treated animals, ** $P < 0.05$, * $P < 0.001$.

2.8 fold increase in GABA levels 18 h later, but with chronic administration this increase was reduced by 65%.

In retina GVG produced very large increases in GABA levels. Thus, acute treatment increased retinal GABA levels more than 6 fold, an increase that was reduced by approximately 40% by chronic treatment with GVG (Figure 1).

Effect of GVG administration on tissue glutamate, aspartate, glycine and taurine levels

Neither acute nor chronic administration of GVG affected the tissue levels of these amino acids (Table 1).

Effect of GVG administration on GABA-transaminase and glutamate decarboxylase activities

GABA-transaminase Acute treatment of animals with GVG produced large decreases in the activity of GABA-T when this was measured 18 h after drug administration (Table 2). In the cerebral cortex and spinal cord, GABA-T activity was reduced by approximately 80% but the most striking inhibition was seen in the retina where GABA-T activity was virtually abolished. When GVG was administered chronically, there was a trend in all three areas for the GABA-T activity to recover slightly but this was significant only in the cerebral cortex (Table 2).

Glutamate decarboxylase Acute treatment of animals with GVG had no effect on the activity of glutamate decarboxylase (GAD) in either the cerebral cortex or spinal cord. In contrast, after chronic treatment with GVG, the GAD activity in the cortex and spinal cord was reduced by approximately 35% and 50% respectively (Table 2).

Effect of GVG treatment on the release of GABA

Saline-injected controls When slices of cerebral cortex from control (saline-injected) animals were exposed to high-K (50 mM KCl) the release of GABA was increased approximately 4 fold (acute and chronic saline controls were similar). In contrast, when slices of spinal cord or entire retinas from control rats (acute or chronic) were exposed to high-K, there

Table 1 Effects of acute and chronic γ -vinyl-GABA (GVG) administration on amino acid levels

	Amino acid level ($\mu\text{mol g}^{-1}$ wet wt)		
	Cerebral cortex	Spinal cord	Retina
Glutamate			
Acute saline	11.49	3.28	4.23
Acute GVG	12.31	3.60	4.40
Chronic saline	11.70	3.84	4.69
Chronic GVG	12.15	3.82	4.11
Aspartate			
Acute saline	2.52	1.65	1.88
Acute GVG	2.46	1.71	1.85
Chronic saline	2.49	1.66	2.06
Chronic GVG	2.35	1.58	1.78
Glycine			
Acute saline	1.58	5.83	1.80
Acute GVG	1.58	5.86	1.85
Chronic saline	1.75	5.31	2.13
Chronic GVG	1.36	5.98	1.85
Taurine			
Acute saline	7.11	2.41	35.3
Acute GVG	6.32	2.65	35.3
Chronic saline	8.11	2.41	38.2
Chronic GVG	7.49	2.44	35.7

Rats were injected (i.p.) with either saline or GVG (250 mg kg^{-1}) once (acute treatment) or daily for 17 days (chronic treatment). Results are the mean of 8–10 measurements, s.e.means are omitted for clarity but were approximately 10%. Drug treatment did not affect the level of any of the amino acids examined.

was no significant increase in GABA release (not illustrated). These results confirm previous observations (Neal & Shah, 1989).

Acute GVG treatment Acute treatment of rats with GVG increased the spontaneous resting release of GABA (not illustrated) and strikingly increased the K-evoked release of GABA from all three tissues (Figure 2, open columns). Thus, in the spinal cord and retina the K-evoked releases were increased from undetectable to 4.5 fold and 2.8 fold the resting release respectively. In cortical slices from rats given one injection of GVG, the K-evoked GABA release was strikingly greater than that seen in tissue from saline-injected rats (Figure 2). These results are very similar to the effects of acute GVG administration on GABA release from cortex, spinal

Table 2 Effects of acute and chronic γ -vinyl GABA (GVG) administration on GABA-transaminase (GABA-T) and glutamate decarboxylase (GAD) activities

	Enzyme activity (% of control)			
	GABA-T		GAD	
	Acute	Chronic	Acute	Chronic
Cerebral cortex	23 $\pm$ 4.1	33.3 $\pm$ 4.5*	102 $\pm$ 8.5	64.5 $\pm$ 4.4†
Spinal cord	21.3 $\pm$ 3.6	29.2 $\pm$ 4.1	99.1 $\pm$ 7.2	48.3 $\pm$ 3.6†
Retina	2.3 $\pm$ 0.9	8.0 $\pm$ 3.8		

Rats were injected (i.p.) with either saline or GVG (250 mg kg^{-1}) once (acute) or daily for 17 days (chronic). The results are expressed as the % of the appropriate controls ($\pm$ s.e.mean, $n = 6$). Values obtained in saline-injected acute and chronic controls were not significantly different. Acute and chronic GVG significantly inhibited GABA-T activity ($P < 0.02$). Chronic significantly different from acute, * $P < 0.05$, † $P < 0.01$. In saline-injected controls, the specific activities in cortex, cord and retina of GAD ($\text{nmol h}^{-1}\text{ mg}^{-1}$ protein) were 85.9 ± 10.6 , 36.7 ± 10.9 and 94 ± 8 , respectively and GABA-T ($\mu\text{mol h}^{-1}\text{ mg}^{-1}$ protein) 1.52 ± 0.13 , 1.19 ± 0.12 and 2.27 ± 0.76 , respectively.

Figure 2 Release of GABA from cortex, spinal cord and retina depolarized with 50 mM KCl. Solid columns, saline-injected controls (acute and chronic were not different). Open columns, single injection of γ -vinyl GABA (GVG). Hatched columns, chronic administration of GVG. Each column is the mean and bar s.e.mean of 6–10 experiments. * Significant difference between evoked releases from acutely and chronically treated animals $P < 0.001$.

cord and retina that have recently been described in detail (Neal & Shah, 1989).

Chronic GVG treatment The chronic administration of GVG to rats had little effect on the resting release of GABA from any of the tissues when compared with the resting release from tissues taken from acutely treated rats (not illustrated). However, in all three tissues the K-evoked release of GABA was significantly less in tissues taken from chronically treated rats compared with that found in tissues taken from acutely treated rats (Figure 2). Thus, in all tissues the absolute amount of GABA released in the presence of high-K was reduced by more than 50% in tissues taken from rats treated chronically with GVG.

Effects of GVG treatment on the release of glutamate, aspartate, glycine and taurine from cortical slices

The K-evoked releases of glutamate, aspartate, glycine and taurine from cortical slices taken from rats treated acutely or chronically with GVG were not significantly different (not illustrated).

Effects of GVG treatment on the release of glycine from retina and spinal cord

The K-evoked release of glycine from retinas and spinal slices from rats treated acutely or chronically with GVG were not significantly different (not illustrated).

Discussion

The mechanism of action of anti-epileptic drugs remains unclear. However, there is reasonable evidence that the benzodiazepines and GABA-T inhibitors exert their antiseizure activity by mechanisms involving GABAergic synapses.

The benzodiazepines are potent anticonvulsants but their use is limited by the development of tolerance. There have not, to our knowledge, been any reports of tolerance developing to the anticonvulsant actions of GVG in epileptic patients. On the contrary, it is claimed that tolerance is not a problem in man (Remy & Beaumont, 1989). However, Löscher & Frey (1987) found that tolerance developed to the anticonvulsant action of GVG in gerbils which exhibited seizures in response to an air blast. In these experiments it was found that the daily administration of GVG at a dose that initially gave 60% seizure protection resulted in marked tolerance so that after 17 days, seizure protection was reduced to 13%. Tolerance to the anticonvulsant effects of the GABA-T inhibitors, γ -acetylenic-GABA and aminooxyacetic acid (AOAA), after prolonged treatment has also been demonstrated in both gerbils (Löscher, 1986) and mice (Löscher,

1982). GVG is believed to be an anticonvulsant because it increases central GABA release. In the present experiments a single injection of GVG caused large increases in GABA release from three areas of the CNS (cerebral cortex, spinal cord and retina). However, this enhanced release was strikingly reduced when GVG was given chronically and this could provide an explanation for the development of tolerance to the anticonvulsant action of the drug.

The mechanism that underlies this reduction in enhanced GABA release following chronic GVG administration is not altogether clear but it cannot be simply related to tissue GABA levels. Thus, the GABA levels in spinal cord and retinas taken from rats subjected to prolonged GVG treatment were significantly lower than in those given a single injection (although they were still above saline-injected controls). However, the GABA levels in cortical slices taken from chronically treated rats were actually higher than those in cortex taken from rats given in a single injection of drug.

It seems unlikely that changes in GABA-T inhibition with chronic GVG treatment are important. Although there was a trend for the enzyme inhibition to decrease slightly with chronic administration, this was only significant in the cerebral cortex. Furthermore, in the retina, the GABA-T activity was still only 8% of control values, even after chronic treatment.

A possible explanation for the present results is that the increase in presynaptic nerve terminal GABA stores produced by GVG is not completely maintained when the drug is given for a long period. This implies a reduction in GABA synthesis. In the present experiments, chronic GVG administration resulted in a substantial reduction in GAD activity in both the cortex and spinal cord. GAD is the rate limiting enzyme for GABA synthesis and is highly localized in presynaptic nerve endings (Neal & Iversen, 1969). GAD activity is reduced by high levels of GABA and chronic treatment of rats, mice or gerbils with GABA-T inhibitors has been shown previously to reduce the activity of the enzyme (Fletcher & Fowler, 1980; Löscher, 1982). This feedback inhibition of GABA synthesis in nerve endings offers an explanation for both the decrease in

GABA levels and release from cord and retina following prolonged treatment with GVG.

In the cerebral cortex the situation is more complicated because the total GABA level in the tissue was increased by prolonged GVG treatment, although tolerance still developed to the K-evoked release. These seemingly paradoxical results may result from a change in the cellular distribution of cortical GABA pools. Normally, GABA released into the synaptic cleft is inactivated by uptake systems in the nerve-endings and glial cells. The GABA taken up by the glia is quickly degraded and does not accumulate there. However, when the GABA-T is inhibited, the glia can accumulate substantial amounts of GABA. This redistribution of cellular GABA pools produced by GVG has been shown indirectly in biochemical experiments (Iadarola & Gale, 1981) and clearly demonstrated in the rat retina by use of immunocytochemical techniques (Neal *et al.*, 1989a). A possible explanation for the present results is that in cerebral cortex, prolonged GVG treatment results in a glial accumulation of GABA which outweighs the decrease in nerve-ending GABA. This causes an increase in tissue GABA together with a decrease in evoked release (high-K causes little GABA release from glial cells, Neal *et al.*, 1989b). In cord and retina the decrease in nerve-ending GABA produced by chronic GVG treatment may outweigh the increase in glial GABA and so there is a decrease in total GABA, together with a decrease in evoked release. The failure of increased GABA levels in various brain areas produced by GABA-T inhibitors to correlate with anticonvulsant actions has been shown previously (Löscher & Frey, 1987).

In summary, we have found in the present experiments that GVG administration increases the K-evoked release of GABA from the CNS, but this enhanced release is smaller when the drug is given chronically. This may be because the initially very high concentration of GABA in the nerve terminals results in feedback inhibition of GAD and a reduction in GABA synthesis.

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