Br. J. Pharmac. (1987), **92**, 13–18

Inhibition of GABA release from slices prepared from several brain regions of rats at various times following a convulsion

[1]A. Richard Green, [2]Michael C.W. Minchin & Nigel D. Vincent

MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE

1 A method is described for the measurement of the K^+-evoked release of endogenous γ-aminobutyric acid (GABA) from slices of rat cortex, hippocampus and striatum.

2 In tissue prepared 30 min following an electroconvulsive shock, K^+-evoked GABA release (above basal release) was inhibited by 45% in cortex, 50% in hippocampus and 75% in striatum. A similar inhibition of release was observed with slices prepared from rats in which a convulsion had been induced by flurothyl. There was no change in spontaneous (basal) release following either procedure.

3 An inhibition of K^+-evoked endogenous GABA release was also seen in tissue prepared 4 min post-ictally but not 2 h after the seizure.

4 No difference was observed in the release of [^{3}H]-GABA from preloaded cortical slices prepared from rats given a single electroconvulsive shock.

5 It is proposed that a convulsion results in an inhibition of GABA release and that this inhibition may in turn inhibit GABA synthesis as described in the preceding paper.

6 It is also proposed that changes in the endogenous releasable pool of GABA may not be detected by preloading slices with [^{3}H]-GABA.

Introduction

In the previous paper data were presented which showed that administration of a single electroconvulsive shock (ECS) or flurothyl-induced convulsion markedly inhibited the rate of GABA synthesis in regions of rat brain (Green *et al.*, 1987). It was also observed that while an ECS-induced convulsion increased regional brain GABA content, a seizure induced by flurothyl did not produce this change. To obtain further insight into the changes taking place at the presynaptic GABA terminal an investigation was made of the effects of a seizure on GABA release. Because of the indications that results obtained on the effects of treatments on neurotransmitter release are not always identical when examining endogenous transmitter release and release of ^{3}H-labelled transmitter from preloaded slices (see Discussion) both approaches were used in the current study. A method of measuring endogenous GABA release was therefore developed and is described here.

Some of these findings have been reported in preliminary form to the British Pharmacological Society (Green *et al.*, 1985).

Methods

Animals and ECS administration

Male Sprague-Dawley derived rats (Charles River, Kent) weighing 100–125 g were used. They were housed in groups in conditions of controlled temperature (21 ± 1°C) and lighting (light period 07 h 00 min–19 h 00 min) and food (modified 41B pellets) and tap water were available *ad libitum*.

Electroconvulsive shocks were administered through ear-clip electrodes using a Theratronics small animal electroplexy unit (120 V, 1 s, 50 Hz sinusoidal). Experimental animals were given a single ECS while control animals were handled, the ear-clips placed but no current passed.

[1] Author for correspondence; present address: Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

[2] Present address: Department of Pharmacology, Wyeth Laboratories, Taplow, Maidenhead, Berks SL6 OPH.

Production of flurothyl-induced convulsions

Rats were placed in a perspex box into which 0.3 ml of flurothyl (25 parts to a million) was injected. At the onset of full tonic-clonic convulsions they were removed to normal cages. Seizure activity was typically of 30–40 s duration.

Measurement of endogenous GABA release from slices from regions of rat brain

The preparation of brain slices for the assay was modified from the method of Iversen & Neal (1968). Rats were killed by thoracic stun and decapitation and brain regions dissected on an ice-cold surface. They were weighed and then chopped on a McIlwain tissue chopper in two directions at 45° at 200 μm intervals. The resulting prism-shaped slices were suspended in ice-cold freshly oxygenated buffer (composition: KCl 5 mM, $MgSO_4 7H_2O$ 1.2 mM, NaCl 0.15 M, $CaCl_2$ 1.2 mM, HEPES 20 mM, pH 7.4, $Na_2HPO_4 . 12H_2O$ 1.2 mM containing glucose 1 mg ml^{-1}). Dispersal of the slices was facilitated by use of a Pasteur pipette and immediately dispersed into aliquots, slices being washed 3 times before incubation at 37°C in a shaking water-bath for 20 min.

The supernatant from each sample was removed and fresh buffer added to bring the final tissue concentration to 50 mg ml^{-1}. Two ml of the tissue containing medium was then taken for each region and incubated for a further period of 10 min at 37°C after which 1.5 ml of supernatant was drawn off and acidified with 40 μl HCl (1 M) on ice. The remaining 0.5 ml of supernatant was discarded as this invariably contained a few stray slices from the incubate which however constituted an insignificant proportion of the total tissue. This first fraction represents the spontaneous efflux of GABA from the preparation.

To the tissue slices was then added 2 ml of high K^+-containing buffer freshly oxygenated, composition as before but with KCl (40 mM) and NaCl (0.115 M) for a further 10 min. From this second incubation period 1.5 ml of supernatant was added to 40 μl HCl (1 M) on ice for each region.

In any one assay, slices of cortex, hippocampus and striatum from a control animal would be run in parallel with the same regions from an animal which had received an electrically or chemically induced seizure.

Separation of GABA from the acidified supernatants was achieved by running them on columns containing 0.5 ml of an acidified Dowex-50 resin (8% cross-linking 100–200 mesh). These were washed with distilled water (10 ml) and 750 μl of ammonia solution (1 M). GABA from the columns was collected by elution with 2.5 ml ammonia solution (1 M) and the resulting eluate dried down under N_2 in a water-bath at 85°C. Recovery of GABA from the Dowex columns was estimated by running acidified standards in parallel. The residue for all samples was taken up in 50 μl HCl (0.002 M) and stored frozen overnight. Of the thawed sample 40 μl was subsequently taken for assay of GABA, as described in Green *et al.* (1987).

Initial studies confirmed that release was calcium-dependent, addition of the calcium channel antagonist methoxyverapamil (D-600, 100 μM) (Knoll, A.G. West Germany) totally abolishing the K^+-evoked release (data not shown).

Measurement of preloaded [^{3}H]-GABA release from slices of rat cortex

The method employed was modified from Raiteri *et al.* (1975). Rats were killed by thoracic stun and decapitation and the cortices from control and treated animals dissected and chopped at 200 μm intervals as described above. The slices were suspended in freshly oxygenated buffer (composition as before), containing glucose (1 mg ml^{-1}), and incubated for 10 min at 37°C in a shaking water-bath, at a final tissue concentration of 100 mg ml^{-1}. The suspension (500 μl) from control and treated animals was incubated with 2 μCi [^{3}H]-GABA (specific activity: 34 Ci $mmol^{-1}$; N.E.N.) in triplicate for a further 30 min at 37°C. Both the incubation and superfusion mediums contained amino-oxyacetic acid (AOAA; 10^{-5} M) to prevent metabolism of GABA. In these conditions, over 90% of the radioactivity recovered on filters or in superfusates is in the form of the original compound (Levi & Raiteri, 1973).

After incubation with [^{3}H]-GABA the slices were transferred to GF/A filters and washed under moderate vacuum within 30 s with the buffer at 37°C. The filters were placed in a six chamber superfusion system consisting of millipore filter holders (volume approximately 1 ml) connected to a multichannel peristaltic pump and perfused with glucose-containing oxygenated buffer medium maintained at 37°C by means of a heated water jacket. Perfuson for 20 min at a flow rate of 0.4 ml min^{-1} established a constant baseline efflux rate. Thereafter, effluent was collected at 3 min intervals for 9 min superfusing with buffer containing glucose and AOAA. The slices were then perfused with a high K^+ (40 mM) buffer (composition as before, containing glucose and AOAA) for 9 min, before superfusion for a further 9 min with normal medium to allow the efflux to return to baseline level. At the end of the experimental period, 10 ml scintillation fluid (Instagel) was added to the collected fractions and to the filters containing cortical slices. The radioactivity in each sample was expressed as the fractional rate constant, calculated as:

$$\frac{\text{d.p.m. in fraction}}{\text{d.p.m. in slice at start of collection} \times \text{interval (3 min)}}$$

All experiments were performed with paired control and treated rats.

Statistics

All data were compared by Student's *t* test (unpaired).

Results

The effect of a single ECS on K^+-evoked release of GABA 30 min post-ictally

Rats were handled or given a single ECS and killed 30 min later. Brains were dissected on an ice-cold surface into cortex, hippocampus and striatum and tissue slices prepared as described in the Methods.

Spontaneous GABA release from the tissue prepared from the three regions did not differ between control and ECS-treated groups. However 30 min after ECS there was a significant inhibition of K^+-evoked release of GABA in all regions examined (Table 1).

The inhibition of release calculated as the percentage difference in the K^+-evoked release above the spontaneous efflux in ECS-treated animals compared with controls was found to be 45% in cortex, 49% in hippocampus and 74% in striatum.

Following these initial experiments all subsequent studies including those on flurothyl (this paper) and studies following repeated ECS (Green & Vincent, 1987) produced data with higher values for basal efflux. The reason for this change is not known; however, to confirm that the inhibition observed previously could still be seen, an experiment on the effect of a single ECS on GABA release from the cortex was repeated. The same degree of inhibition of release was seen (Table 2). This inhibition was greater than that observed from tissue prepared 4 min post-ictally (Table 2).

No inhibition of K^+-evoked release was observed from tissue prepared from all three regions 2 h after a seizure (Table 3).

Table 1 The effect of a single electroconvulsive shock (ECS) on endogenous GABA release in tissue taken 30 min after convulsion

	GABA concentration (nmol g^{-1} h^{-1})	
	Spontaneous	*K^+* (40 mM)
Cortex		
Control	251 ± 36 (5)	772 ± 45 (5)
ECS	257 ± 41 (6)	543 ± 51 (6)*
Hippocampus		
Control	233 ± 39 (6)	701 ± 44 (6)
ECS	248 ± 17 (6)	486 ± 62 (6)*
Striatum		
Control	210 ± 57 (6)	648 ± 52 (6)
ECS	208 ± 43 (6)	321 ± 43 (6)*

Values are mean ± s.d. with the number of experiments in parentheses.
* Different from control (K^+-induced release): $P < 0.001$.

GABA content in tissues used to measure release of GABA

No difference was observed in the concentration of GABA in slices used to examine K^+-evoked release at either 30 or 120 min post ECS compared with tissue taken from handled control animals (Table 4).

Table 2 The effect of a single electroconvulsive shock (ECS) on the release of endogenous GABA from rat cortical slices taken 4 min and 30 min post-ictally

		Endogenous GABA released (nmol g^{-1} h^{-1})	
	Time of seizure	*Spontaneous*	*K^+* (40 mM)
Control		553 ± 28 (6)	959 ± 34 (5)
ECS	4 min	544 ± 31 (8)	846 ± 47 (8)*
ECS	30 min	531 ± 13 (3)	756 ± 19 (3)*

Values are mean ± s.d. with the number of experiments in parentheses.
* Different from control K^+-induced release: $P < 0.05$.

Table 3 The effect of a single electroconvulsive shock (ECS) on endogenous GABA release 2 h after convulsion

	Endogenous GABA released (nmol $g^{-1} h^{-1}$)	
	Spontaneous	K^+ (40 mM)
Cortex		
Control	532 ± 37 (5)	873 ± 44 (5)
ECS	579 ± 54 (5)	910 ± 36 (5)
Hippocampus		
Control	471 ± 37 (5)	820 ± 44 (5)
ECS	482 ± 28 (28)	776 ± 63 (5)
Striatum		
Control	447 ± 23 (5)	662 ± 51 (5)
ECS	485 ± 29 (5)	678 ± 32 (5)

Values are mean ± s.d. with the number of experiments in parentheses.
No statistical differences were observed between the 2 groups.

Effect of a flurothyl induced convulsion on the K^+-evoked release of GABA from rat brain slices

Rats were handled or had a seizure induced by exposure to flurothyl (Methods). After 30 min the rats were killed and slices prepared from the cortex, hippocampus and striatum as described earlier. No difference was observed in the spontaneous release from control or flurothyl-treated rats in any brain region. However, a similar degree of inhibition in K^+-evoked release was observed in the flurothyl-treated rats to that seen following ECS (Table 5).

Evoked release of [^{3}H]-GABA from superfused slices of cortex prepared from rats given a single ECS

Cortical slices were prepared from animals handled or given ECS and killed 30 min later. These were placed in the superfusion system (see Methods).

There was no difference in the basal efflux of [^{3}H]-GABA between control and ECS-treated animals and a high K^+ concentration (40 mM) evoked the same release of [^{3}H]-GABA in both groups (Table 6).

Discussion

The method detailed and presented in this paper is a novel means of analysing the evoked release of endogenous stores of GABA from brain slices, without the need for a superfusion system. The method is an easy and reliable means for collecting transmitter released into the incubating medium over two 10 min periods. The spontaneous efflux of GABA from the slices was readily measurable being in all studies between 0.2 μmol g^{-1} and 0.6 μmol $g^{-1} h^{-1}$ (3–10 nmol per incubate) well within the range of the enzymatic-fluorimetric assay employed. The method also permits the use of the same slice preparation for the measurement of K^+-evoked release of GABA, values for spontaneous and stimulated release being thus paired (if required). Release has been shown to be Ca^{2+}-dependent consistent with a previous study of

Table 4 The GABA concentration of brain slices from endogenous release experiments 30 and 120 min after a single electroconvulsive shock (ECS)

	GABA concentration in brain slices (μmol g^{-1} tissue)	
	30 min	*120 min*
Cortex		
Control	2.64 ± 0.18 (5)	2.92 ± 0.32 (5)
ECS	2.80 ± 0.22 (6)	3.15 ± 0.41 (5)
Hippocampus		
Control	2.98 ± 0.27 (6)	2.90 ± 0.25 (5)
ECS	3.08 ± 0.18 (6)	3.20 ± 0.36 (5)
Striatum		
Control	3.12 ± 0.12 (6)	3.04 ± 0.22 (5)
ECS	2.95 ± 0.17 (6)	3.22 ± 0.38 (5)

Values are mean ± s.d. with the number of experiments in parentheses. No statistical differences were observed between control and ECS-treated groups at either time point.

Table 5 The effect of a single flurothyl-induced convulsion on endogenous GABA release, 30 min post-ictally

	Endogenous GABA released (nmol $g^{-1} h^{-1}$)	
	Spontaneous	K^+ (40 mM)
Cortex		
Control	526 ± 44 (4)	881 ± 58 (4)
Flurothyl	535 ± 61 (5)	698 ± 32 (5)*
Hippocampus		
Control	489 ± 50 (5)	749 ± 45 (5)
Flurothyl	479 ± 23 (5)	622 ± 37 (5)*
Striatum		
Control	416 ± 36 (5)	651 ± 59 (5)
Flurothyl	435 ± 45 (5)	481 ± 42 (5)*

Values are mean ± s.d. with the number of experiments in parentheses.
* Different from control (K^+-induced release): $P < 0.01$.

Table 6 The effect of a single electroconvulsive shock (ECS) on the release of [^{3}H]-GABA from rat cortical slices 30 min post-ictally

	[^{3}H]-GABA release fractional rate content ($min^{-1} \times 10^{-3}$)	
	Spontaneous	*High K^+* (40 mM)
Control	5.4 ± 0.4 (7)	14.9 ± 2.2 (7)
ECS	5.6 ± 0.6 (7)	14.6 ± 2.8 (7)

Values are mean ± s.d. with the number of experiments in parentheses.
No statistically significant differences were observed between the two groups.

endogenous GABA release using tissue slices in a superfusion model (Szerb *et al.*, 1981).

Basal tissue levels of GABA measured from the slices after incubation were higher than values obtained from microwave irradiation (Green *et al.*, 1987) and the post-mortem rise in GABA levels is the probable explanation for the absence of any difference in basal GABA content between controls and ECS animals.

There was no difference in the spontaneous release of GABA following a single ECS. However 30 min after a single ECS there is a marked and significant reduction in the K^+-evoked release of endogenous GABA in all brain regions examined. This inhibition of stimulated release was greatest in the striatum (74%) with a 45% and 49% inhibition in the cortex and hippocampus respectively. There is thus an inhibition of Ca^{2+}-dependent, K^+-stimulated release at a time after ECS when inhibition of the synthesis of the neurotransmitter is also known to occur (Green *et al.*, 1987).

It is clear that this inhibition of K^+-evoked release occurs very rapidly after ECS. In experiments with slices from rat cortex taken 4 min post-ictally, a significant inhibition was also observed, approaching the order of that seen at 30 min. Inhibition of release can therefore be seen at a time before GABA concentrations are markedly elevated (Bowdler & Green, 1982; Green *et al.*, 1987). Nevertheless by 120 min post seizure, the K^+-stimulated release of GABA had returned to control values. At this time after ECS the rate of synthesis has also returned to normal.

The data showing inhibition of endogenous GABA release following ECS are clearly in marked contrast to those obtained on the effect of release of [^{3}H]-GABA from slices preloaded with [^{3}H]-GABA. There was no difference in the K^+-evoked GABA release from rat cortical slices 30 min after ECS, the basal efflux also being the same for both groups. This result clearly raises the question as to the extent and nature of the exchange in a preloaded preparation between the radiolabelled transmitter and any endogenous compartments occurring neuronally, and the precise location of the pools of transmitter with which the endogenous and exogenous methods for K^+-evoked release are concerned. Abe & Matsuda (1983) attempted to answer these questions by examining the K^+-evoked release of GABA that had either been preloaded or newly synthesized in slices. They concluded that newly synthesized GABA is located in a pool different from that of [^{3}H]-GABA taken up into the preparation and is not under the influence of GABA-transaminase. This conclusion is further corroborated by the work of Szerb *et al.* (1981) who demonstrated that inclusion of gabaculline in a superfusion system containing rat cortical slices augmented the depolarization-induced release of [^{3}H]-GABA but not that of endogenous GABA.

The release of endogenous GABA from the brain slices detailed in this paper would thus appear to be occurring from a small Ca^{2+}-dependent pool which is composed of newly synthesized GABA. It is this pool from which GABA is preferentially released and from which the release would appear to be inhibited 30 min following ECS. That this change is not seen in slices of cortex preloaded with GABA would suggest that the [^{3}H]-GABA was located elsewhere in the synaptic terminal.

It was found that 4 min after an ECS there was a significant inhibition of the K^+-evoked release of endogenous GABA from the cortex. This inhibition is therefore occurring at a time before any significant elevation of GABA concentration in the cortex (Bowdler & Green, 1982; Green *et al.*, 1987).

Clearly it is not only an electrically-induced seizure that results in an inhibition of GABA release since 30 min after a single seizure produced by flurothyl, a similar change was seen in all brain regions studied. As with a single ECS, the greatest inhibition of release was observed in the striatum. This inhibition of release with flurothyl is therefore present at a time after the seizure when synthesis is virtually attenuated, but with no concomitant change in GABA levels (Green *et al.*, 1987). It is clear, therefore, that flurothyl produced those changes in GABA transmission also demonstrated 30 min after a single ECS, with the exception of any alteration in GABA content.

The mechanism by which flurothyl (hexafluorodiethyl ether) produces seizures is unknown. It produces seizures when inhaled and these cease when exposure to the vapour is terminated. It is excreted unchanged by the lungs. What its use does make clear is that the changes in GABA release seen after ECS are due to the convulsion and not the passage of electrical current *per se*.

Data in Tables 1–3 suggest that a high percentage

of GABA can be released from the total pool (Table 4) which implies a high turnover rate for GABA. This proposal is in fact supported strongly by the measured rate of GABA synthesis in the brain regions in the preceding paper (Green *et al.*, 1987).

In conclusion these data suggest firstly that a convulsion markedly inhibits GABA release, a change which possibly initiates the inhibition of GABA synthesis that also occurs after a seizure and secondly that changes in release from the endogenous GABA pool many not be detected by techniques involving preloading slices with [^{3}H]-GABA.

We thank Professor Martin Adler (Temple University, Philadelphia) for supplies of flurothyl. N.D.V. held a M.R.C. studentship during this study.

References

ABE, M. & MATSUDA, M. (1983). On the existence of the GABA pools associated with newly synthesised GABA and with newly taken up GABA in nerve terminals. *Neurochem. Res.*, **8**, 563–573.

BOWDLER, J.M. & GREEN, A.R. (1982). Regional rat brain benzodiazepine receptor number and γ-aminobutyric acid concentration following a convulsion. *Br. J. Pharmac.*, **76**, 291–298.

GREEN, A.R., MINCHIN, M.C.W. & VINCENT, N.D. (1985). GABA release in rat brain following a seizure. *Br. J. Pharmac.*, **84**, 89P.

GREEN, A.R., METZ, A., MINCHIN, M.C.W. & VINCENT, N.D. (1987). Inhibition of the rate of GABA synthesis in regions of rat brain following a convulsion. *Br. J. Pharmac.*, **92**, 5–11.

GREEN, A.R. & VINCENT, N.D. (1987). The effect of repeated electroconvulsive shock on GABA synthesis and release in regions of rat brain. *Br. J. Pharmac.*, **92**, 19–24.

IVERSEN, L.L. & NEAL, M.J. (1968). The uptake of [^{3}H]-GABA by slices of rat cerebral cortex. *J. Neurochem.*, **15**, 1141–1149.

LEVI, G. & RAITERI, M. (1973). GABA and glutamate uptake by subcellular fractions enriched in synaptosomes: critical evaluation of some methodological aspects. *Brain Res.*, **57**, 165–185.

RAITERI, M., FEDERICO, R., COLETTI, A. & LEVI, G. (1975). Release and exchange studies relating to the synaptosomal uptake of GABA. *J. Neurochem.*, **24**, 1243–1250.

SZERB, J.C., ROSS, E.T. & GUREVICH, L. (1981). Compartments of labelled and endogenous γ-aminobutyric acid giving rise to release evoked by potassium or veratridine in rat cortical slices. *J. Neurochem.*, **37**, 1186–1192.

(Received September 17, 1986.
Revised April 13, 1987.
Accepted May 7, 1987.)