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Inhibition of the rate of GABA synthesis in regions of rat brain following a convulsion

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1 The rate of synthesis of γ-aminobutyric acid (GABA) in the cortex, hippocampus and striatum of rat brain was assessed by measuring the linear rate of accumulation of GABA following injection of amino-oxyacetic acid (AOAA).

2 Five min after a single electrically induced seizure there was a rise in GABA content in these brain regions and an almost total inhibition of the rate of synthesis.

3 Five min after seizure induced by the inhalant convulsant flurothyl there was no rise in GABA content in these brain regions but a similar marked degree of inhibition of GABA synthesis.

4 Two hours after the convulsion the rate of GABA synthesis had returned to control values in all three brain regions.

5 A single convulsion did not alter the glutamic acid decarboxylase activity in these brain regions either in the absence or presence of added co-factor (pyridoxal phosphate).

6 Evidence for an inhibition of GABA release following a convulsion which may be associated with the inhibition of GABA synthesis is presented in the following paper.

Introduction

Following a single electroconvulsive shock there is a rise in the concentration of γ-aminobutyric acid (GABA) in several brain regions (Bowdler & Green, 1982). Seizure threshold also rises at this time (Nutt *et al.*, 1981; Tacke *et al.*, 1984) but experiments with the inhalent convulsant flurothyl suggest that there is no simple relationship between these two phenomena, since seizure threshold rises following administration of this drug but brain GABA content does not alter (Bowdler & Green, 1982).

In order to try and clarify the mechanisms involved in the post-ictal rise in both brain GABA content and seizure threshold, an investigation has now been made of the rate of GABA synthesis in several brain regions following a single electrically or chemically evoked seizure.

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The method chosen for assessment of synthesis rate was that of measurement of the rate of accumulation of GABA in the brain following blockade of its metabolism by injection of the GABA-transaminase inhibitor amino-oxyacetic acid. This approach has been used by several other groups (e.g. Bernasconi *et al.*, 1982) and in the current studies (Green & Vincent, 1987) has been found to produce comparable data to that obtained using the technique of gas chromatography – mass spectrometry (mass fragmentography) coupled with infusion of [^{13}C]-glucose (Bertilsson & Costa, 1976). Some of the findings have been reported in preliminary form to the British Pharmacological Society (Green *et al.*, 1985).

Methods

Animals

Adult male Sprague-Dawley derived rats (Charles, River, Kent) weighing 100–125 g were used. They were housed in groups of 6 in conditions of controlled temperature (21°C) and lighting (light periods 08 h 00 min – 20 h 00 min) and given a diet of 41B pellets and tap water *ad libitum*.

Seizure induction by electroconvulsive shock or flurothyl

Seizures were induced in rats by administration of an electroconvulsive shock through ear-clip electrodes using a Theratronics small animal electroplexy unit (120 V, 1 s, 50 Hz sinusoidal). Control animals were handled, the earclips placed but no current passed. Typically a tonic-clonic seizure was induced of duration 20–30s.

Flurothyl-induced seizures were produced by placing rats in a perspex box into which 0.3 ml of flurothyl (25 parts per million) were introduced. 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 GABA in discrete brain regions

Animals were handled or given a single electrically or chemically induced convulsion. They were killed at various times thereafter by exposure of the head to a focussed high intensity microwave beam of power density 70 W cm^{-2} for 4 s. Only tissue showing an even grey colour was used for assay since this is indicative of effective denaturation. Samples were then assayed for GABA by a slight modification of the enzymatic-fluorimetric assay of Baxter (1972) as described by Bowdler & Green (1982) which has been found to be both sensitive (measuring concentrations as low as 2 nmol per tube) and reproducible.

Estimation of GABA synthesis rate

Various methods exist for the measurement of GABA synthesis ranging from mass fragmentography (Bertilsson & Costa, 1976) to the measurement of GABA accumulation following GABA transaminase inhibition (e.g. Walters *et al.*, 1978; Berasconi *et al.*, 1982). The former is complex and requires a gas chromatograph-mass spectrometer while the latter is relatively simple but possibly less accurate. Nevertheless the technique of GABA transaminase inhibition does give a good indication of changes in GABA synthesis rate following drug administration (Walters *et al.*, 1978) since GABA transaminase is the major degradation pathway for the transmitter (Löscher, 1980). Bernasconi *et al.* (1982) demonstrated that amino-oxyacetic acid (AOAA) was arguably the best drug to use, being a more rapid inhibitor than gabuculline and further suggested, in contrast to the data of Walters *et al.* (1978), that maximum GABA accumulation occured at an AOAA dose of 10 mg kg^{-1}. This view was enhanced by our current data showing that AOAA administration at a dose of 10 mg kg^{-1} resulted in the same accumulation of GABA as a dose of 30 mg kg^{-1} (Table 1). Higher doses of AOAA can inhibit glutamic acid decarboxylase (GAD) (Walters *et al.*, 1978; Bernasconi *et al.*, 1982) so the lower dose was considered preferable.

To determine regional values of the synthesis rate of GABA, therefore rats were injected with AOAA (10 mg kg^{-1}, i.p.) and killed as above at 0, 20, 40 and 60 min after administration. Brain samples were assayed for tissue GABA content. The synthesis rate for the transmitter in cortex, hippocampus and striatum was calculated as the increase in GABA concentration above basal tissue concentration in 1 h following AOAA.

Estimation of glutamic acid decarboxylase (GAD) activity

GAD activity was estimated radiometrically by monitoring [^{14}C]-CO_2 evolution from [1-^{14}C]-glutamate by a method adapted from Roberts & Simonsen (1963). Brain regions were rapidly removed from rats killed by thoracic stun and decapitation and homogenised at a concentration of 10 mg ml^{-1} in the following mixture (hippocampi were pooled from two animals): potassium phosphate buffer (50 mM), pH 6.4 containing 0.025% w/v EDTA; 0.1% v/v 2-mercaptoethanol; 0.5% v/v Triton-X-100; with or without pyridoxal phosphate (Pyr-p, 0.5 mM).

Portions of this homogenate (0.4 ml) were pre-incubated at 37°C for 15 min in a total volume of 5 ml, in a 25 ml conical flask closed with a 'Suba-seal' bung. [^{14}C]-glutamate (specific activity 50 mCi $mmol^{-1}$) was injected to a final concentration of 5 mM (0.5 μCi) and the incubation continued for a further 60 min. Reactions were stopped by injection of 1 ml of H_2SO_4 (5 M) and liberated [^{14}C]-CO_2 trapped on Hyamine-soaked filters (present in a central separate well in the flask) for a further 60 min. Blanks were determined in parallel by injection of the acid before substrate. The filters were extracted at the end of the incubation time and added to 10 ml of Aqualuma scintillation fluid before radioactive counting.

Results

Regional brain GABA concentration following AOAA

Rats were injected with AOAA (10 mg kg^{-1}, i.p.) and killed by microwave irradiation at 0, 20, 40 and 60 min after administration. The cortex, hippocampus and striatum of each animal were dissected and assayed for GABA content. In all regions there was a linear accumulation of GABA following AOAA above basal tissue content (see Figures 1,2 and 3). The percentage increase in GABA levels over basal content for individual regions was as follows: cortex 200%, hippocampus 290%, and striatum 140%. Mean synthesis rates for GABA in these regions were calculated as the

Table 1 The effect of two doses of amino-oxyacetic acid on the concentration of GABA in regions of rat brain 40 min after administration

	Amino-oxyacetic acid (dose) (10 mg kg^{-1})		(30 mg kg^{-1})	
	GABA concentration (μmol g^{-1} tissue)			
Cortex	4.12 ± 0.53	(6)	3.73 ± 0.46	(5)
Hippocampus	5.67 ± 0.89	(6)	5.13 ± 0.73	(5)
Striatum	5.82 ± 0.62	(6)	6.00 ± 0.77	(5)

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

increase in transmitter content in 1 h, and expressed as μmol GABA g^{-1} tissue h^{-1} are: cortex, 3.40; hippocampus, 5.60; striatum, 4.00.

Administration of a higher dose of AOAA (30 mg kg^{-1}, i.p.) produced an elevation in brain GABA content which was comparable with controls which had received the lower dose of 10 mg kg^{-1} (see Table 1).

The effect of a single ECS on the accumulation of GABA following AOAA

Rats were given a single ECS and injected with AOAA (10 mg kg^{-1}, i.p.) 5 min later. Control animals received ECS and were injected with saline 5 min post-ictally. Both groups were killed at time points up to 1 h after injection by microwave irradiation and the cortex, hippocampus and striatum of each animal assayed for GABA content. In the control group ECS produced a rise in GABA concentration in the cortex (Figure 1), hippocampus (Figure 2) and striatum (Figure 3) above basal values which persisted for the hour after the seizure. The elevation of concentration was statistically significant 5 min after the seizure in the hippocampus ($P < 0.01$; compared with basal tissue levels), and reached significance in all areas for all subsequent time points.

Administration of a single ECS attenuated the elevation normally seen after injection of AOAA in the cortex (Figure 1), hippocampus (Figure 2) and striatum (Figure 3). This attenuation was observed for the hour following ECS (Figures 1,2 and 3).

The effect of ECS on the accumulation of GABA produced by AOAA 120 min after a seizure

Rats were handled or given a single ECS, killed 120 min later, and brain regions assayed for GABA content. No statistically significant difference in the regional GABA content was seen between controls

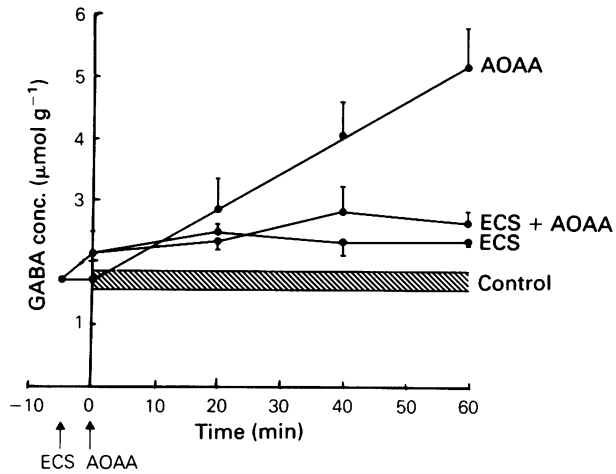

Figure 1 The effect of an electroconvulsive shock (ECS) on the concentration of GABA in the cortex and the effect of amino-oxyacetic acid (AOAA; 10 mg kg^{-1}) administration. ECS was given at −5 min as marked and 5 min later rats were injected with either saline or AOAA. Control animals were handled but not given the ECS. Regional GABA content shown as mean with s.d. as bars. Shaded area shows mean ± 1 s.d. for control group.

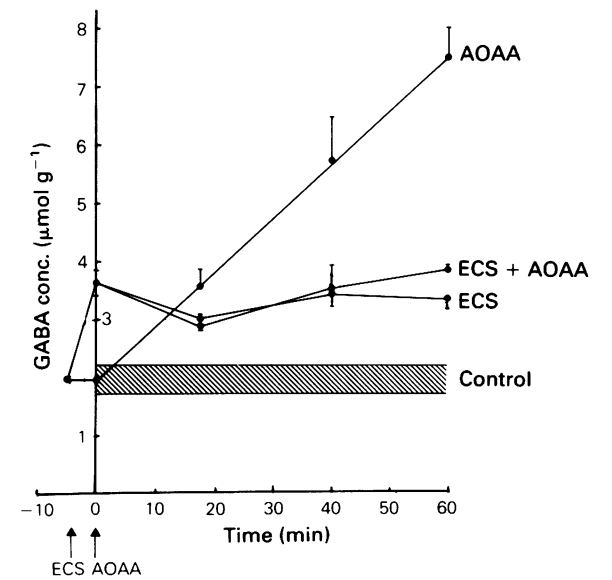

Figure 2 The effect of an electroconvulsive shock (ECS) on the concentration of GABA in the hippocampus and the effect of amino-oxyacetic acid (AOAA; 10 mg kg^{-1}) administration. ECS was given at −5 min as marked and 5 min later rats were injected either with saline or AOAA. Control animals were handled but not given the ECS. Regional GABA content shown as mean with s.d. as bars. Shaded area shows mean ± 1 s.d. for control group.

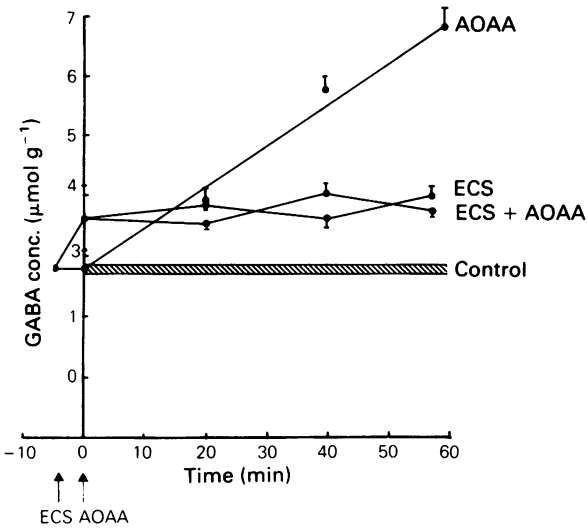

Figure 3 The effect of an electroconvulsive shock (ECS) on the concentration of GABA in the striatum and the effect of amino-oxyacetic acid (AOAA 10 mg kg^{-1}) administration. ECS was given at −5 min as marked and 5 min later rats were injected with either saline or AOAA. Control animals were handled but not given the ECS. Regional GABA content shown as mean with s.d. as bars. Shaded area shows mean ± 1 s.d. for control group.

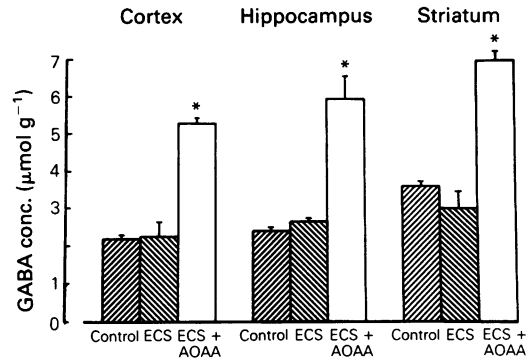

Figure 4 The rise in GABA concentration in regions of rat brain 60 min after administration of amino-oxyacetic acid (AOAA, 10 mg kg^{-1}) 2 h following a seizure. Results expressed as mean of 4–6 observations with s.d. shown by vertical lines. *Different from electroconvulsive shock (ECS) alone group, $P < 0.001$, not significantly different from rise seen in control animals given AOAA (data shown in Figures 1–3). ECS alone group not significantly different from handled (control) group.

and ECS-treated animals 2 h post-ictally (Figure 4). Other rats were given a single ECS and 120 min later injected with AOAA (10 mg kg^{-1}, i.p.) and killed 40 min after injection. The elevation of GABA produced by AOAA 2 h after ECS was similar to that seen in control rats given AOAA in the three regions examined (compare Figure 4 with values in Table 1).

The effect of a flurothyl-induced convulsion on regional GABA concentration 30 min post-ictally

Animals killed 30 min after a seizure elicited by flurothyl showed no significant change in the concentration of GABA in the cortex, hippocampus and striatum compared with handled controls (Figure 5).

The effect of a flurothyl-induced convulsion on the accumulation of GABA caused by AOAA (10 mg kg^{-1}, i.p.)

Rats were injected with AOAA (10 mg kg^{-1}, i.p.) 5 min after a convulsion elicited by flurothyl, and killed 30 min later. Handled controls were also killed 30 min following administration of AOAA. In control animals which received AOAA there was a significant elevation of GABA concentration in the cortex, hippocampus and striatum above basal tissue levels (Figure 5). However, in those animals treated with flurothyl the elevation of GABA concentration produced by AOAA was attenuated in all areas examined (Figure 5).

Measurement of GAD activity following a convulsion in the absence and presence of added co-factor

GAD activity was determined in the cortex, hippocampus and striatum of rat brain in the absence and presence of pyridoxal phosphate (Pyr-p; 0.5 mM). The addition of Pyr-p (0.5 mM) resulted in a large increase of GAD activity in all these brain areas (Table 2) and the resulting difference in activity enabled an estimation of the degree of enzyme saturation in each area, the degree of saturation being defined as:

$$\frac{\text{activity in the absence of Pyr-p}}{\text{activity in the presence of 50 } \mu\text{M Pyr-p}} \times 100$$

From these results regional GAD saturation was calculated as cortex, 39%; hippocampus, 37%; striatum, 30%.

Thirty minutes after a single ECS there was no difference in the GAD activity of ECS-treated animals compared with controls in the cortex, hippocampus or striatum (Table 2) when assessed either in the presence or absence of Pyr-p.

GAD activity was also determined in the hippocampus 5 min after administration of a single ECS and again no change was found in the enzyme activity of this region (data not shown).

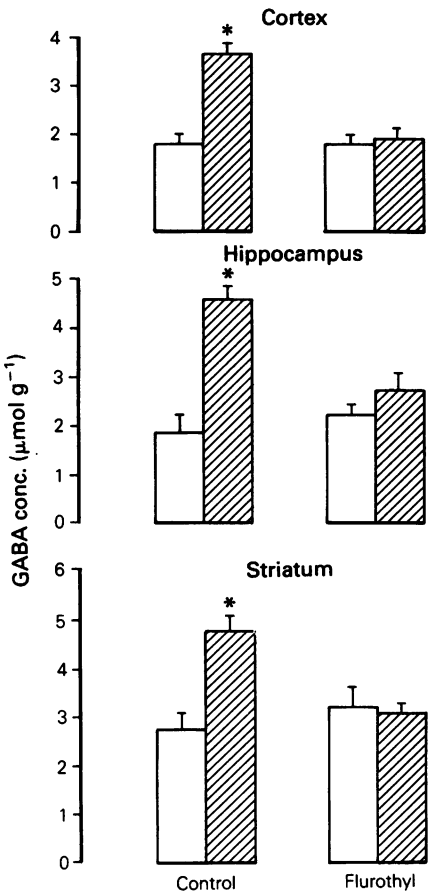

Figure 5 Regional rat brain GABA content following a flurothyl-induced convulsion and 30 min following injection of amino-oxyacetic acid (AOAA; 10 mg kg^{-1}) shown by shaded columns. *Increase in handled group given AOAA different from saline-injected group, $P < 0.01$. No significant increase in GABA content following a flurothyl seizure or in flurothyl-treated group following AOAA injection.

Discussion

The results obtained using AOAA injection and measurement of GABA accumulation as an index of GABA synthesis rate give confidence in the value of the technique in that accumulation was linear and the degree of elevation of GABA content was of the order of that seen in other studies in mice and rats. Thus the degree of elevation and values for the synthesis rate of GABA in different brain regions were similar to those obtained by Collins (1972), Pericic *et al.* (1977), Wood *et al.* (1980), Carmona *et al.* (1980) and Bernasconi *et al.* (1982). Furthermore the administration of a dose of AOAA (30 mg kg^{-1}, o.p.), three times higher than the dose used in this study, produced no further increase in GABA accumulation in either cortex, hippocampus or striatum 40 min after injection compared with the lower dose, thereby confirming that the lower dose of 10 mg kg^{-1} employed throughout, caused maximal inhibition of GABA-transaminase (GABA-T) activity and thus virtually complete attenuation of the catabolism of GABA.

Table 2 The effect of a single electroconvulsive shock (ECS) on glutamic acid decarboxylase (GAD) activity in regions of rat

Treatment	*GAD activity* (μmol h^{-1} g^{-1} wet weight tissue) *Without Pyr-p*		*With Pyr-p*	
Cortex				
Control	5.14 ± 0.67	(6)	13.18 ± 0.96	(6)
ECS	5.20 ± 0.72	(6)	11.60 ± 0.68	(6)
Hippocampus				
Control	6.43 ± 0.67	(5)	18.40 ± 0.24	(5)
ECS	6.60 ± 0.25	(5)	18.18 ± 0.45	(5)
Striatum				
Control	7.50 ± 0.67	(6)	24.80 ± 0.45	(6)
ECS	8.50 ± 0.73	(6)	25.45 ± 0.81	(6)

Values are mean ± s.d. with the number of experiments in brackets. No statistical differences were observed between the two groups. Pyr-p = pyridoxal phosphate.

A final reason for confidence in the current technique for evaluation of GABA synthesis is that using this method essentially identical data were obtained on GABA synthesis rates following repeated seizures (Green & Vincent, 1987) as those obtained with a gas chromatographic-mass spectrometric technique (Green *et al.*, 1978).

The present study confirmed that a single dose ECS elevates GABA levels as previously reported by Bowdler & Green (1982). The most significant increase in concentration occurred in the hippocampus 5 min after the seizure, with levels elevated from 1.95 μmol g^{-1} to 3.63 μmol g^{-1}. The duration of this elevation was also in agreement with the former study, being still significantly higher 1 h after the convulsion but returning to control values after 2 h in all regions.

In sharp contrast to the accumulation of GABA observed in control animals upon inhibition of metabolism with AOAA, was the almost complete abolition of accumulation in the ECS group given AOAA. The attentuation of the effect of AOAA in the ECS group was measurable from the time of injection of the drug 5 min after the seizure. It persisted for the whole hour after convulsion in all brain regions

examined. While it cannot entirely be ruled out that a pharmacokinetic explanation may account for the changes in the accumulation of GABA following AOAA in ECS-treated rats, such an explanation seems very unlikely. Data over many years have demonstrated an increased blood-brain barrier permeability following ECS and a resultant increase in-drug penetration (see Bolwig, 1984). It would therefore be likely that there would have been a facilitated entry of AOAA to the brain after ECS. Increased delivery of drug cannot explain the inhibition of synthetic activity, and thus an apparent inhibition of synthesis, because our studies have shown that administration of a dose three times that used in this study had no further effect on synthetic activity *per se*.

It therefore seems reasonable to suggest that the data indicate that there is a virtually complete inhibition of transmitter synthesis following ECS.

The synthesis rate for the transmitter had returned to control values in all regions by 2 h after the convulsion as the elevation of GABA concentration with AOAA administered 2 h post-ictally and measured 40 min later was the same for ECS and control groups. The inhibition of synthesis with time would thus appear to be the same as those changes in concentration: GABA levels remained elevated for an hour after ECS and returned to control values by 2 h (Bowdler & Green, 1982).

Flurothyl ($CF_3CH_2OCH_2CF_3$) a fluorinated ether is a volatile inhalant convulsant which is excreted unchanged by the lungs. It produces EEG changes in chick which are similar to those seen after electrically induced seizures (Herz *et al.*, 1970) but the mechanism by which it produces seizures is unknown. A similar change in GABA synthesis rate was observed after a seizure induced pharmacologically by administration of this compound. A flurothyl-induced seizure also produced an almost total inhibition of GABA synthesis (as indicated by the lack of rise of GABA following AOAA injection). However in agreement with the earlier work of Bowdler & Green (1982) regional brain GABA concentrations did not rise after a seizure induced by flurothyl.

Thus the inhibition in GABA synthesis is not apparently causally related to the rise in the GABA content. Why GABA concentration should be changed by an electrically induced seizure but not a chemically induced seizure is unclear. Both treatments increase seizure threshold (Nutt *et al.*, 1981; Bowdler & Green, 1982) and both treatments lead to an inhibition of GABA release (Green *et al.*, 1987). It could be therefore that the change in GABA content is of little functional significance and the reason for its occurrence, or not, is the relative speed of the combined changes in inhibition of release (Green *et al.*, 1987) and inhibition of synthesis (this paper).

No change in GAD activity was detected *in vitro* in tissue taken from ECS-treated rats. However, feedback inhibitory changes occurring *in vivo* would almost certainly not be detected by such a method. Since it has recently been reported that GABA synthesis can be inhibited by means of feedback inhibition at the level of the GAD enzyme (Porter & Martin, 1984), at present the most parsimonious explanation for the inhibition of GABA synthesis following a convulsion is that there is an inhibition of GABA release following a seizure (see Green *et al.*, 1987) which rapidly leads to an inhibition of synthesis. A flurothyl-induced seizure may induce a more rapid 'switch off' of synthesis so that the modest rise of GABA in the tissue is not seen.

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