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The effect of repeated electroconvulsive shock on GABA synthesis and release in regions of rat brain

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1 The release of endogenous γ-aminobutyric acid (GABA) from slices of rat cortex, hippocampus and striatum prepared both 30 min and 24 h after the last of a series of electroconvulsive shocks (5 seizures given spread out over 10 days) has been investigated.

2 No change in spontaneous (basal) release was observed. However, 30 min after the last convulsion, K^+-evoked GABA release above basal release was inhibited in both hippocampus (20%) and striatum (33%) but not in the cortex. Release was still inhibited in striatum (22%) 24 h after the last seizure.

3 In confirmation of an earlier report, chronic electroconvulsive shock was found to increase basal GABA content in striatum and inhibit synthesis by 34%. The synthesis rate was also inhibited in the hippocampus (44%) but not in the cortex.

4 Glutamic acid decarboxylase activity was unchanged in all regions after repeated electroconvulsive shock treatment.

5 It is proposed that repeated electroconvulsive shocks lead to a substantial inhibition of release in the striatum and hippocampus and a long-term inhibition of GABA synthesis in these regions. Such changes may be associated with the altered monoamine biochemistry and function observed after repeated electroconvulsive shock and with the mechanism of the antidepressant action of electroconvulsive therapy.

Introduction

Green *et al.* (1978) observed that following administrtion to rats of an electroconvulsive shock (ECS) daily for 10 days the γ-aminobutyric acid (GABA) concentration rose in the n. accumbens and n. caudatus and the rate of GABA synthesis decreased in these two regions. The results were obtained by use of the technique of gas chromatography-mass spectrometry (mass fragmentography, Bertillson & Costa, 1976); however, methodological strictures including the precursor-product assumptions inherent in measuring turnover by infusion of labelled precursors (see Bertillson & Costa, 1976) precluded the use of the method in examining turnover in brain areas such as the cortex and hippocampus. A later study by Bowdler *et al.* (1983) confirmed and extended the observations that the GABA concentration changes in various brain regions following repeated ECS, but did not measure the synthesis rate of GABA.

The current studies have demonstrated that a single ECS or flurothyl-induced convulsion can induce marked changes in both GABA synthesis (Green *et al.*, 1987a) and release (Green *et al.*, 1987b) in regions of rat brain. An investigation has therefore been made of the effect of repeated seizures on GABA release in regions of rat brain and on the rate of GABA synthesis in various brain regions, the latter study being designed to try to confirm and extend an earlier investigation (Green *et al.*, 1978) by use of a different methodology. In addition a study has been made of the activity of glutamic acid decarboxylate activity in rat brain both in the absence and presence of added cofactor (pyridoxal 5-phosphate), following repeated ECS since there is one report that activity is increased following repeated seizures (Atterwill *et al.*, 1981). Such a change although reportedly small (Atterwill *et al.*, 1981) is difficult to reconcile with a decreased rate of GABA synthesis.

Some of these findings have been presented in

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preliminary form to the British Pharmacological Society (Green & Vincent, 1985).

Methods

Animals and ECS administration

Animals were purchased and housed as described in the preceding papers (Green *et al.*, 1987a, b). ECS was administered 5 times over 10 days (Monday, Wednesday, Friday, Monday and Wednesday) through ear-clip electrodes (120 V 1s, 50 Hz) from a Theratronics small animal electroplexy unit. Control animals were handled and the electrodes placed, but no current passed.

Measurement of GABA, GABA synthesis and release, and GAD activity

Measurement of GABA, of GABA synthesis and release, and of glutamic acid decarboxylase (GAD) activity were carried out as described in detail in the two preceding papers (Green *et al.*, 1987a, b).

Mathematical methods and statistics

Lines for the elevation of GABA levels over the time period were fitted by linear regression and subsequently analysed for co-variance (Snedecor & Cochran, 1971). GABA concentration and release values were analysed by Student's *t* test (unpaired).

Results

The effect of repeated ECS treatment on the release of GABA from brain slices, 30 min after the final seizure

Rats were either handled or received ECS 5 times over 10 days, and killed 30 min after the final treatment. Tissue slices were prepared from the cortex, hippocampus and striatum of paired control and electroconvulsive shock (ECS)-treated animals as described previously (Green *et al.*, 1978b). No difference was observed in the spontaneous efflux of endogenous GABA in slices prepared from two groups in the three regions examined (Table 1). However, there was significant inhibition of the K^+-evoked release of endogenous GABA from hippocampal and striatal slices of chronic ECS-treated rats. This inhibition was approximately 20% in the hippocampus and 30% in the striatum (expressed as the percentage inhibition of the K^+-evoked release of GABA above spontaneous efflux compared with controls). No change was seen between cortical slices prepared from the two groups of animals (Table 1).

Table 1 The effect of repeated electroconvulsive shocks (ECS) on endogenous GABA release 30 min after treatment

	GABA concentration (nmol g^{-1} h^{-1})	
	Spontaneous	K^+ (40 mM)
Cortex		
Control	546 ± 32 (5)	950 ± 52 (5)
ECS	562 ± 28 (5)	943 ± 40 (5)
Hippocampus		
Control	473 ± 15 (6)	776 ± 23 (6)
ECS	452 ± 24 (6)	697 ± 30 (6)*
Striatum		
Control	437 ± 34 (5)	685 ± 42 (5)
ECS	452 ± 20 (5)	618 ± 28 (5)*

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

The effect of repeated ECS treatment on the release of GABA from brain slices 24 h after the final ECS

Rats were handled or received ECS 5 times over 10 days, and killed 24 h after the final treatment. Tissue slices were prepared and GABA release examined as before. The spontaneous efflux of GABA was the

Table 2 The effect of repeated electroconvulsive shocks (ECS) on endogenous GABA release 24 h after treatment

	GABA concentration (nmol g^{-1} h^{-1})	
	Spontaneous	K^+ (40 mM)
Cortex		
Control	464 ± 25 (5)	965 ± 36 (5)
ECS	474 ± 40 (5)	992 ± 22 (5)
Hippocampus		
Control	466 ± 24 (6)	848 ± 36 (6)
ECS	482 ± 38 (6)	882 ± 52 (6)*
Striatum		
Control	488 ± 23 (6)	677 ± 44 (6)
ECS	460 ± 41 (6)	608 ± 38 (6)*

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

same for ECS-treated and chronically handled control animals. While a significant inhibition (20%) of the K^+-evoked release of GABA was still observed in the striatum of ECS-treated animals, no significant difference was now seen in the K^+-evoked release of GABA from slices prepared from the hippocampus. Cortical GABA release was again unaltered by repeated ECS at this time (Table 2).

The effect of repeated ECS on the regional synthesis rate of GABA, 24 h after the final ECS

Rats were handled or received ECS 5 times over 10 days and were injected with amino-oxyacetic acid (AOAA, 10 mg kg^{-1}) 24 h after the final treatment. These animals were killed by microwave irradiation to the head at 0, 20, 40 and 60 min following injection of AOAA and brain regions were analysed for GABA content. The concentration of GABA at time 0 is the basal GABA concentration, that is the concentration of GABA following the ECS (or sham)-treatment but before administration of AOAA. The administration of AOAA produced a linear accumulation of GABA in both groups of animals in the three regions examined for up to 1 h after injections (Figures 1, 2 and 3).

Chronic ECS treatment produced a significant rise in basal GABA concentrations in the striatum compared with controls ($P < 0.05$), as well as a significant inhibition of the accumulation of GABA in this area

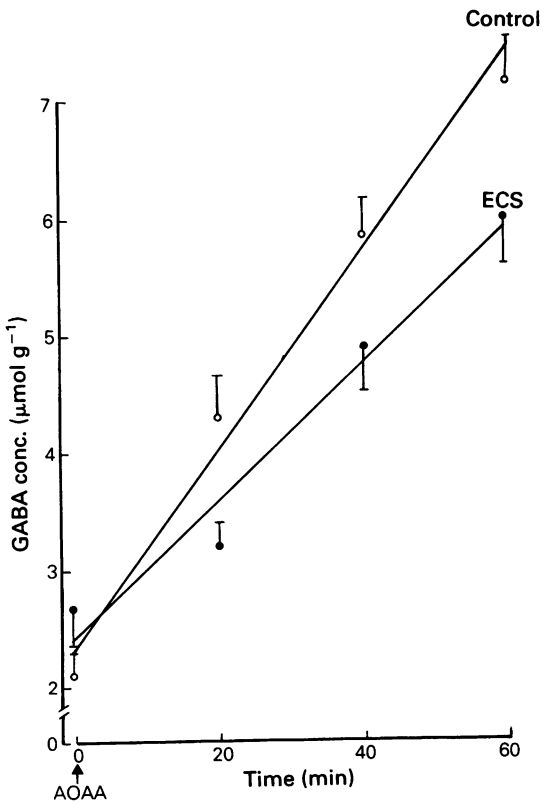

Figure 1 The effect of repeated electroconvulsive shock (ECS) administration on the accumulation of GABA produced by amino-oxyacetic acid (AOAA) in rat striatum. Values are means from 6 experiments with s.d. shown by vertical lines. ECS group different from handled controls: $P < 0.05$ (see Figure 4 for synthesis rate values). Chronic ECS treatment also produced a significant rise in basal GABA concentration compared with controls ($P < 0.05$).

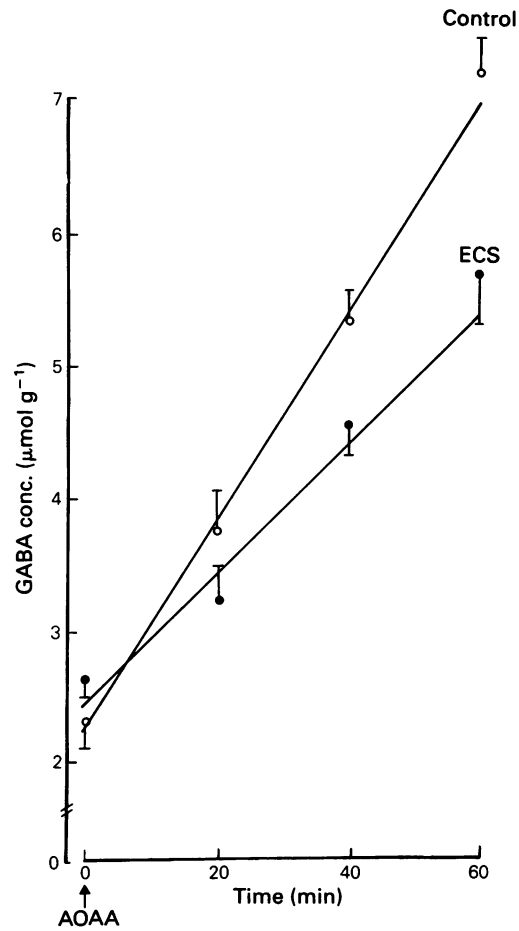

Figure 2 The effect of repeated electroconvulsive shock (ECS) administration on the accumulation of GABA produced by amino-oxyacetic acid (AOAA) in rat hippocampus: Values are means of $n = 6$; s.d. shown by vertical lines. ECS group different from handled controls: $P < 0.05$ (see Figure 4 for synthesis rate values).

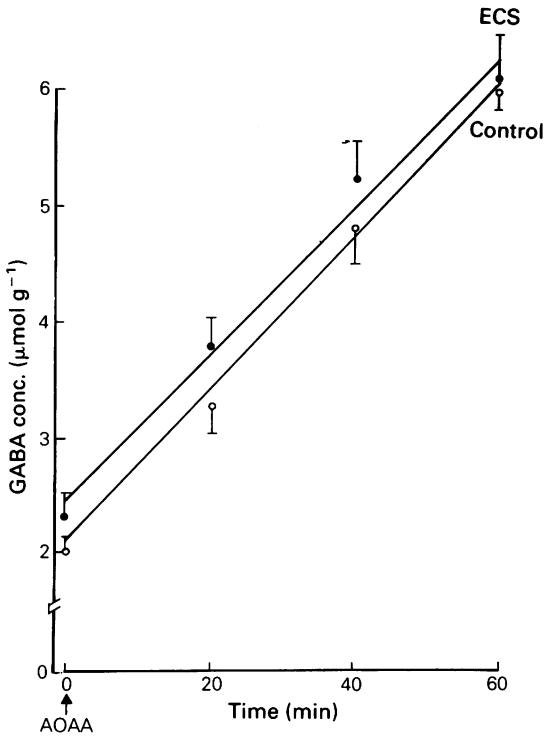

Figure 3 The effect of repeated electroconvulsive shock (ECS) administration on the accumulation of GABA produced by amino-oxyacetic acid (AOAA) in rat cortex. Values are means of $n = 6$; s.d. shown by vertical lines. No statistically significant differences were observed between the two groups.

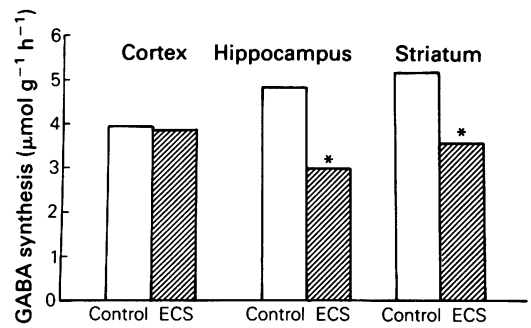

Figure 4 The effect of repeated electroconvulsive shock (ECS) administration on the regional synthesis of GABA in rat brain, 24 h after final treatment. Values calculated from linear regression of 4 time points (each the mean of 6 observations) over 1 h following amino-oxyacetic acid (AOAA), administered 24 h after the final ECS of a chronic regimen. $*P < 0.05$ compared with handled controls. No statistical differences were observed in the cortex between handled control and ECS-treated animals.

compared with handled controls ($P < 0.05$) (Figure 1) following injection with AOAA indicating a decrease in the rate of GABA synthesis following ECS. Synthesis rates were calculated and are shown in Figure 4.

The inhibition in GABA synthesis rate following AOAA was also observed in the hippocampus of ECS-treated rats compared with control animals (Figure 2) again indicating an inhibition in synthesis rate (Figure 4). There was no difference in the basal tissue content of the transmitter between ECS-treated and handled controls in this area (Figure 2).

The accumulation of GABA produced by AOAA was the same in the cortex of control and ECS-treated rats (Figure 3); synthesis rate of GABA was thus similar in both groups (Figure 4). There was also no statistically significant difference in the basal tissue concentration of GABA in this area between the two groups (Figure 3).

Chronic handling did not produce a significant alteration of the synthesis rate for GABA in the cortex, hippocampus and striatum compared with rats which had received a single handling treatment (Data not shown).

The effect of repeated ECS on regional GAD activity in the absence and presence of pyridoxal phosphate

Rats received repeated treatment with ECS (given 5 times over 10 days) and were killed 24 h after the last seizure. Control animals were handled 5 times over 10 days and killed 24 h after the last handling. GAD activity was assayed in the cortex, hippocampus and striatum of these animals in the absence and presence of co-factor. There was no change in the GAD activity of the ECS-treated animals compared with handled controls in any of these regions (Table 3).

Discussion

In tissue taken from rats given a single ECS it was observed that there was a marked reduction in K^+-evoked release of endogenous GABA. However, using a superfusion system for the estimation of release of GABA from slices preloaded with [^{3}H]-GABA did not demonstrate any change (Green *et al.*, 1987b) suggesting that release from preloaded slices might not be measuring release from the endogenous pool. The endogenous GABA release system was therefore chosen to investigate the release of GABA at two time points after chronic ECS treatment.

It was found that 30 min after the last seizure of the

Table 3 The effect of repeated electroconvulsive shocks (ECS) on glutamic acid decarboxylase (GAD) activity in the rat

	GAD activity (μmol h^{-1} g^{-1} wet weight tissue)	
	Without Pyr-p	*With Pyr-p*
Cortex		
Control	4.95 ± 0.47 (5)	12.80 ± 0.36 (5)
ECS	5.16 ± 0.32 (5)	13.08 ± 0.64 (5)
Hippocampus		
Control	6.60 ± 5.50 (6)	19.92 ± 0.47 (6)
ECS	6.74 ± 0.16 (6)	18.22 ± 0.85 (6)
Striatum		
Control	8.10 ± 0.70 (5)	24.90 ± 1.12 (5)
ECS	8.60 ± 0.87 (5)	27.60 ± 1.50 (5)

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

chronic regimen, the K^+-evoked release of GABA from slices of rat cortex was not significantly decreased in ECS-treated animals compared with handled controls. This is a marked contrast to the inhibition of evoked release (around 45%) seen in this area 30 min after a single ECS (Green *et al.*, 1987b). However, the K^+-evoked release of GABA was significantly reduced in the hippocampus and striatum taken from chronically treated animals 30 min after final treatment. Nevertheless the degree of inhibition was considerably less than that observed 30 min after a single ECS (hippocampus 20% compared with 50% following a single ECS, striatum 30% compared with 75%). Chronic handling and chronic ECS treatment produced no significant difference in the spontaneous efflux of endogenous GABA from brain slices compared with the single convulsion studies.

The marked inhibition of the K^+-evoked release of endogenous GABA seen 30 min after a single ECS was therefore no longer occurring in the cortex of chronically treated animals, and considerably reduced in the hippocampus and striatum; 24 h later it was still apparent only in the striatum.

These studies nevertheless point to a sustained inhibition of GABAergic function in the striatum with chronic ECS treatment, for although the inhibition of release observed 30 min after final treatment in the chronic regimen is less than that 30 min after a single ECS, it nevertheless clearly persists for over 24 h after the last convulsion. In contrast, release has returned to control values 2 h after a single ECS (Green *et al.*, 1987b). This prolonged inhibition of striatal function following chronic ECS may reflect the very large inhibition of evoked GABA release seen with a single ECS, being of the order of a 75% inhibition of the evoked release of GABA over spontaneous efflux from the slices (compared with control values), and larger than any other area examined. The GABA release in the striatum also showed the greatest inhibition 30 min after a flurothyl-induced convulsion compared with other brain regions (Green *et al.*, 1987b). The sustained inhibition of release in the striatum after chronic ECS treatment may explain the elevated concentrations of transmitter also seen at this time compared with control animals.

Why ECS should produce a smaller effect on release in the hippocampus and striatum following repeated treatment is unknown. However, GABA synthesis is decreased in these regions (see below) so the releasable pool size may be diminished. Even less explicable is the total lack of effect in the cortex, other than a standard explanation of 'adaptation'.

Justification for the use of the method for GABA synthesis measurement has been presented in a preceding paper (Green *et al.*, 1987a). However two points can immediately be made with regard to the synthesis rate data obtained in the striatum. First, the results confirm the results obtained by Green *et al.* (1978) who used the entirely different technique of mass-fragmentography (see Introduction). Second, this confirmation allows confidence in the use of the present method of measurement of GABA synthesis rate and the results obtained by this method to study synthesis rates following a single ECS (Green *et al.*, 1987a). The degree of synthesis inhibition observed in the striatum in the current study is, in percentage terms, remarkably similar to that observed by the mass fragmentographic approach and extends the previous study by showing that a similar inhibition occurs in the hippocampus.

What has not been examined in this study is the rate of GABA synthesis 30 min after the last seizure. What is clear however, is the very sustained inhibition that occurs after repeated ECS. The synthesis rate of GABA in the hippocampus and striatum were back to normal 2 h after single ECS but still markedly decreased 24 h after the last of a series of ECS given over 10 days. Thus in common with many of the behavioural changes observed after ECS (see Green & Nutt, 1987, for review) ECS appears to have induced a change which is consolidated and sustained by repeated administration. Of particular interest in this respect is the earlier observation on haloperidol-induced catalepsy. A brief attenuation of this response was seen after a single ECS while a sustained attenuation was seen after repeated ECS. This attenuation of the response was interpreted, in the light of other data as being due to a reduction in GABA function (Green *et al.*, 1979) which is consistent with the interpretation

of this current data.

An earlier study (Green *et al.*, 1978) using mass fragmentography concluded that the elevated GABA concentration and decreased turnover data suggested that a decrease in GABA release was occurring in the striatum. This suggestion is supported by the current observations on K^+-evoked release and it could be that it is the inhibition of release which is the change that initiates the other changes. It has been reported by Porter & Martin (1984) that GABA synthesis can be inhibited by feedback inhibition of the GAD enzyme. Unfortunately such a change in enzyme activity would probably not be detected by the *in vitro* assay system used in the current study although it seemed reasonable to make the attempt.

It is also quite possible that the changes in GABA function which occur after repeated ECS are involved in the enhanced 5-hydroxytryptamine (5-HT) and dopamine-mediated behaviour as has been proposed elsewhere (Green, 1986a, b). This proposal is given strength by the observations that prevention of the GABA antagonists with ECS also prevented the enhancement of the monoamine-mediated behaviour that is normally seen (Green *et al.*, 1983). One GABA system that does change after repeated ECS is the $GABA_B$ receptor in the frontal cortex (Lloyd *et al.*, 1985; Gray & Green, 1986) and changes in the function of this receptor can be shown to alter the 5-HT-mediated behaviour (Gray *et al.*, 1986). However, the failure to observe changes in GABA synthesis or release in the whole cortex after repeated ECS does make it unlikely that the change in $GABA_B$ receptor number is associated with a change in GABA biochemistry occurring in this region.

N.D.V. held an M.R.C. studentship during this study.

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