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REGIONAL RAT BRAIN BENZODIAZEPINE RECEPTOR NUMBER AND γ-AMINOBUTYRIC ACID CONCENTRATION FOLLOWING A CONVULSION

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1 Following administration to rats of an electroconvulsive shock (ECS) which resulted in a major tonic-clonic seizure, no changes in [^{3}H]-diazepam binding characteristics were observed in cortex or hippocampus with either a well washed membrane preparation or a crude synaptosomal preparation.

2 No changes were observed in [^{3}H]-diazepam binding in any other brain region examined 30 min after an ECS.

3 Thirty min following an ECS, regional brain γ-aminobutyric acid (GABA) concentrations increased in hippocampus, cortex and hypothalamus. Only in the hippocampus did the increase occur within 5 min of the seizure.

4 Similar increases in GABA concentration were seen after a bicuculline-induced seizure but not after a seizure induced by flurothyl; both treatments produced a tonic-clonic seizure.

5 Pretreatment of the rats with (+)-propranolol 5 min before the ECS abolished the tonic extension and prevented the brain GABA concentration changes that occur 30 min after the seizure.

6 No increase in GABA concentration was seen in hippocampus, cortex or hypothalamus 30 min after the final ECS of a course of 10 ECS given once daily for 10 days. In contrast a marked increase in striatal GABA concentration was observed.

7 These changes in GABA biochemistry following a seizure are discussed in relation to the post-ictal rise in seizure threshold that is occurring at the same time.

Introduction

Following a single electroconvulsive shock (ECS) or a bicuculline-induced seizure, rats exhibit a rapid increase in seizure threshold lasting approximately 3 h to γ-aminobutyric acid (GABA) antagonist drugs (bicuculline, pentylenetetrazol and isopropylbicyclophosphate) but not to strychnine or quipazine. Inhibition of 5-hydroxytryptamine, catecholamine or prostaglandin synthesis or naloxone did not prevent the post-ictal rise in seizure threshold. These data suggest that specific neurochemical mechanisms are involved in the rise in seizure threshold following a convulsion and suggest that a change in GABA function is probably occurring (Nutt, Cowen & Green, 1981).

Benzodiazepines have been shown to enhance GABA function (see Costa, 1979). It therefore seemed possible that the rise in seizure threshold was associated with the reported increase in benzodiazepine receptor number following a convulsion (Paul & Skolnick, 1978). We have now examined both benzodiazepine receptor number and GABA concentration in various brain regions following seizures elicited both by drugs and ECS. A preliminary account of some of these findings has been given to the British Pharmacological Society (Bowdler & Green, 1981).

Methods

Animals and seizure induction

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

Electrically induced seizures (tonic-clonic) were produced by administration via earclip electrodes of a single ECS (125 V, 50 Hz sinusoidal for 1 s) using a Theratronics small animal electroplexy unit. Control rats were handled only. Bicuculline-induced seizures (tonic-clonic) were produced by administration of

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bicuculline (0.32 mg/kg) injected intravenously via a tail vein. Control animals received saline (pH3). Flurothyl-induced seizures were produced by placing rats in a desiccator jar (volume 10 l) and introducing 0.1 ml flurothyl, which rapidly vaporized and produced convulsions in about 15 s, starting with myoclonic jerks and followed rapidly by tonic-clonic convulsion. At this stage they were removed from the vapour and convulsions ceased within a further 15 s. Control rats were handled only.

Groups were also given ECS during halothane anaesthesia. Under these conditions the convulsion is modified, tonic extension not being seen (see Cowen, Nutt & Green, 1980). In these experiments, control groups received halothane.

When (+)-propranolol (30 mg/kg i.p.) was used it was given before the ECS and control rats received only (+)-propranolol (30 mg/kg).

Measurement of benzodiazepine receptor number

In experiments using a crude synaptosomal preparation, rats were killed by pneumothoracic stun and decapitation. The brain was rapidly removed and dissected on ice into various brain regions as described by Glowinski & Iversen (1966). Subsequent preparation and measurement of benzodiazepine receptor number (using [^{3}H]-diazepam as ligand) was as described by Paul & Skolnick (1978) except that the final pellet was suspended in 100 volumes of 0.05 M, Tris-HCl buffer, pH 7.4 rather than 50 volumes.

The measurement of benzodiazepine receptors was also performed in a well washed membrane preparation. Brain regions were homogenized in 20 vol ice cold 0.1 M Tris-citrate buffer, pH 7.1, and sonicated for 10 s. The membrane fragments were prepared by centrifugation at 38000 *g* for 20 min at 4°C. The pellets were washed by resuspension and sonication and centrifuged a further three times, using the original volume of cold Tris-citrate buffer each time. Finally, they were resuspended and frozen at −20°C for at least 16 h. The suspension was thawed, sonicated and recentrifuged and the pellet resuspended on 0.1 M Tris-citrate pH 7.1 buffer.

Aliquots of 0.1 ml (containing approximately 200 μg protein) were incubated on ice with various concentrations of [^{3}H]-diazepam (87.6 Ci/mmol, NEN) in a total volume of 1.0 ml. Specific binding was determined by displacement of [^{3}H]-diazepam with 3 μM clonazepam. Specific binding was always approx. 90% of total binding. Incubation was terminated after 30 min by the addition of 5 ml of ice cold 0.1 M Tris-citrate buffer, pH 7.1, and immediate rapid filtration through Whatman GF/B filters. The membrane fragments were rapidly washed with 3 × 5 ml ice cold 0.1 M Tris-citrate buffer, pH 7.1, and filters placed in counting vials containing 4 ml of 2-ethoxy ethanol overnight, after which 10 ml of Aqualuma (Lumac, Holland) was added for radioactive counting. All determinations were carried out in triplicate.

Specific binding was determined at a fixed concentration of [^{3}H]-diazepam (3.5 or 4.0 nM) or saturation binding using a concentration range of 1–30 nM at seven different concentrations with subsequent Scatchard analysis of the data, using linear regression analysis by the method of least squares.

Measurement of brain γ-aminobutyric acid concentration

Rats were killed by exposure of the head to a focused high intensity microwave beam of power density 70 W/cm^2 for 4 s, as described by Guidotti, Cheney, Trabucchi, Doteuchi, Wang & Hawkins (1974), and the brains dissected into 4 regions. Only tissue showing an even grey colour was taken for assay since this is indicative of effective microwave irradiation. Samples were homogenized in 0.002 M HCl, centrifuged and the supernatant assayed for GABA content by a modification of the method described by Baxter (1972). Samples were incubated in a buffered solution (total volume 90 μl) containing (final concentrations shown in parentheses) β-mercaptoethanol (0.02%), α-ketogluturate (2.87×10^{-3}M); NADP (2.87×10^{-4}M) and GABAse (GABA transaminase and succinic semialdehyde dehydrogenase obtained from Sigma) (0.086 mg) at room temperature for 35 min. Any remaining NADP was then destroyed by adding alkaline phosphate buffer and heating. The NADPH was converted to a fluorescent derivative by heating with 10 M NaOH containing H_2O_2 (0.15%). Fluorescence was measured at activation 370 nM, emission 450 nM (both uncorrected).

Measurement of protein concentration

Protein determinations were carried out by the method of Lowry, Rosebrough, Farr & Randall (1951).

Measurement of seizure threshold

Seizure susceptibility was measured by examining the dose of bicuculline infused via a tail vein necessary to elicit a seizure, as described by Nutt *et al.* (1981).

Statistics

All results are given as the mean and standard error of mean and statistical significance examined by use of Student's *t* test (unpaired).

Results

Effect of an ECS on benzodiazepine receptors in a well washed membrane preparation

Rats were given a single ECS or handled only and killed 30 min later. The seizure produced was a full tonic-clonic seizure of both fore- and hind-limbs lasting about 20 s. Well washed membrane preparations from the cortex and hippocampus were used for saturation binding and Scatchard analysis performed on the [^{3}H]-diazepam binding data obtained.

No change in either the dissociation constant (K_d) or maximum number of binding sites (B_{max}) was observed in either region following the seizure (Table 1).

To determine whether a change was occurring at some other period after the convulsion, specific binding of [^{3}H]-diazepam was examined in these regions 15, 60 and 120 min after the convulsion. ECS did not alter the specific binding at these times when compared with handled controls (Table 2). Nor was any change observed in the specific binding of [^{3}H]-diazepam in several other brain regions 30 min following a convulsion (Table 3).

Effect of ECS on benzodiazepine receptors in a crude synaptosomal preparation

We next attempted to replicate the data of Paul & Skolnick (1978) using, as they did, a crude synaptosomal preparation, since it seemed possible that the increase in B_{max} that they observed was due to the influence of endogenous compounds, not present in the well washed membrane preparation.

Rats were given a single ECS or handled, killed 30 min later and Scatchard analysis performed of [^{3}H]-diazepam binding in the synaptosomal preparation prepared from the cortex.

Table 1 Characteristics of specific bound [^{3}H]-diazepam 30 min after an electroconvulsive shock (ECS)

	Handled	*ECS*
Cortex		
K_d	8.67 ± 0.38 (3)	9.36 ± 0.60 (3)
B_{max}	2204 ± 136 (3)	2273 ± 77 (3)
Hippocampus		
K_d	8.13 ± 0.24 (3)	8.24 ± 0.21 (3)
B_{max}	1632 ± 93 (3)	1644 ± 62 (3)

Values expressed as mean ± s.e.mean with number of determinations in parentheses. K_d: nM. B_{max}: fmol/mg protein. Correlation coefficient (r) = 0.99 on every analysis.

No change in K_d was observed 30 min after a convulsion (control 4.25 ± 0.45 nM (7); ECS 4.51 ± 0.33 nM (7); mean ± s.e.mean, number of observations in parentheses). Nor was any change observed in B_{max}, either when the control data were compared with ECS-treated animals for each individual experiment or when data were subsequently pooled (Figure 1).

Regional brain γ-aminobutyric acid concentration following a single ECS

The above data on benzodiazepine receptors did not apparently provide any explanation for the rise in seizure threshold seen following a convulsion. Since GABAergic mechanisms nevertheless seemed to be involved in the rise in threshold we next examined brain GABA concentrations following a seizure.

Rats were given a single ECS and killed by focused microwave irradiation to the head at various times thereafter. The hippocampus, cortex, corpus striatum and hypothalamus were dissected and the GABA concentrations measured.

In the hippocampus the GABA concentration was raised 5 min after the ECS and had returned to

Table 2 Specific bound [^{3}H]-diazepam in cortex and hippocampus at various times after an electroconvulsive shock (ECS)

Treatment	*Time after convulsion*	*Brain Region* *Cortex*	*Hippocampus*
Handled	–	573 ± 8 (8)	375 ± 19 (7)
ECS	15	607 ± 8 (5)	382 ± 12 (10)
ECS	60	528 ± 19 (6)	366 ± 22 (5)
ECS	120	573 ± 17 (5)	366 ± 10 (10)

Results shown as mean ± s.e.mean of the specifically bound [^{3}H]-diazepam (fmol/mg protein) using a concentration of [^{3}H]-diazepam of 3.5 nM (cortex) and 4.0 nM (hippocampus).

Table 3 Specific bound [^{3}H]-diazepam in various brain regions 30 min after an electroconvulsive shock (ECS)

Brain region	*Specific bound [^{3}H]-diazepam* *Handled*	*ECS*
Pons/medulla	101 ± 4 (6)	114 ± 4 (6)
Cerebellum	187 ± 12 (6)	185 ± 11 (6)
Corpus striatum	187 ± 8 (6)	189 ± 11 (6)
Hypothalamus	238 ± 15 (5)	258 ± 12 (5)

Results shown as mean ± s.e.mean with number of observations in parentheses of the specifically bound [^{3}H]-diazepam (fmol/mg protein) using a concentration of [^{3}H]-diazepam of 4 nM.

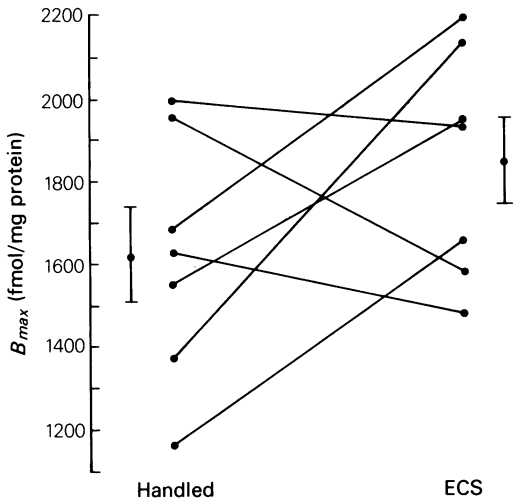

Figure 1 B_{max} values of [^{3}H]-diazepam binding in the cortex of control rats and the cortex of rats 30 min following a single electroconvulsive shock (ECS). Lines join control and experimental data obtained in each experiment performed at the same time.

control values by 2 h after the seizure. The concentration was also raised in the cortex although the change was not statistically significant until 30 min after the convulsion. In the corpus striatum a significant increase was seen both 45 min and 60 min after the convulsion, whilst an increase was only seen in the hypothalamus 30 min after the seizure (Figure 2).

Effect of a bicuculline-induced convulsion on brain γ-aminobutyric acid concentration

Rats were injected with either saline, pH 3.0, (controls) or bicuculline (0.32 mg/kg) via a tail vein. The bicuculline-treated rats invariably had tonic extension of both fore- and hind-limbs lasting around 20 s with clonic contractions lasting for another 90 s. Thirty min later both groups were killed and regional brain GABA concentrations measured.

The effect of immobilization and subsequent injection of saline was to produce an increase in GABA concentration in hippocampus, cortex and striatum compared to handled controls. However the bicuculline-treated rats showed a further marked increase in GABA concentration in hippocampus, cortex and hypothalamus (Table 4).

In contrast, animals in which a seizure had been produced by exposure to flurothyl showed no increase in regional brain GABA concentration (Table 4) despite the fact that the drug produced a seizure with tonic extension of hind- and fore-limbs and clonic contractions lasting for about 15 s after removal from the gas.

Seizure threshold following a flurothyl-induced convulsion

The dose of bicuculline required to elicit a seizure 30 min after a flurothyl-induced convulsion was examined. Rats that had been convulsed by exposure to flurothyl showed a marked increase in seizure threshold expressed as the dose of bicuculline (mg/kg) necessary to elicit a further seizure (control: 0.37 ± 0.3 (4); 0.60 ± 0.05 (5), $P < 0.01$).

Brain γ-aminobutyric acid concentration after ECS given during halothane anaesthesia or after (+)-propranolol treatment

When ECS was given to animals anaesthetized with halothane the seizure was modified with no tonic extension; clonic convulsions were produced lasting about 10 s. Despite this modification, a marked increase in GABA concentration occurred in hippocampus and hypothalamus (Table 5).

(+)-Propranolol (30 mg/kg, i.p.), the isomer without β-adrenoceptor antagonist activity, did not affect regional GABA concentration 35 min later (Table 5). However, when this drug was given 5 min before an ECS it abolished the tonic extension and prevented the brain GABA concentration changes that occured 30 min after a seizure (Table 5).

Table 4 Brain regional γ-aminobutyric acid (GABA) concentrations (μmol/g tissue) 30 min after a seizure elicited by bicuculline (0.375 mg/kg, i.v.) or exposure to flurothyl

Treatment	*Hippocampus*	*Cortex*	*Hypothalamus*	*Striatum*
Saline	5.79 ± 0.41 (8)	4.96 ± 0.24 (5)	8.20 ± 0.52 (4)	7.25 ± 0.61 (5)
Bicuculline	7.71 ± 0.57 (6)*	5.82 ± 0.20 (6)*	12.99 ± 0.98 (6)†	8.35 ± 0.40 (6)
Handled	4.70 ± 0.18 (20)	3.58 ± 0.09 (17)	8.38 ± 0.48 (13)	6.28 ± 0.26 (15)
Flurothyl	5.16 ± 0.22 (9)	3.72 ± 0.22 (8)	9.40 ± 1.05 (8)	6.06 ± 0.48 (9)

Results shown as mean ± s.e.mean with number of observations in parentheses.
Different from saline controls: *$P < 0.05$; †$P < 0.01$.

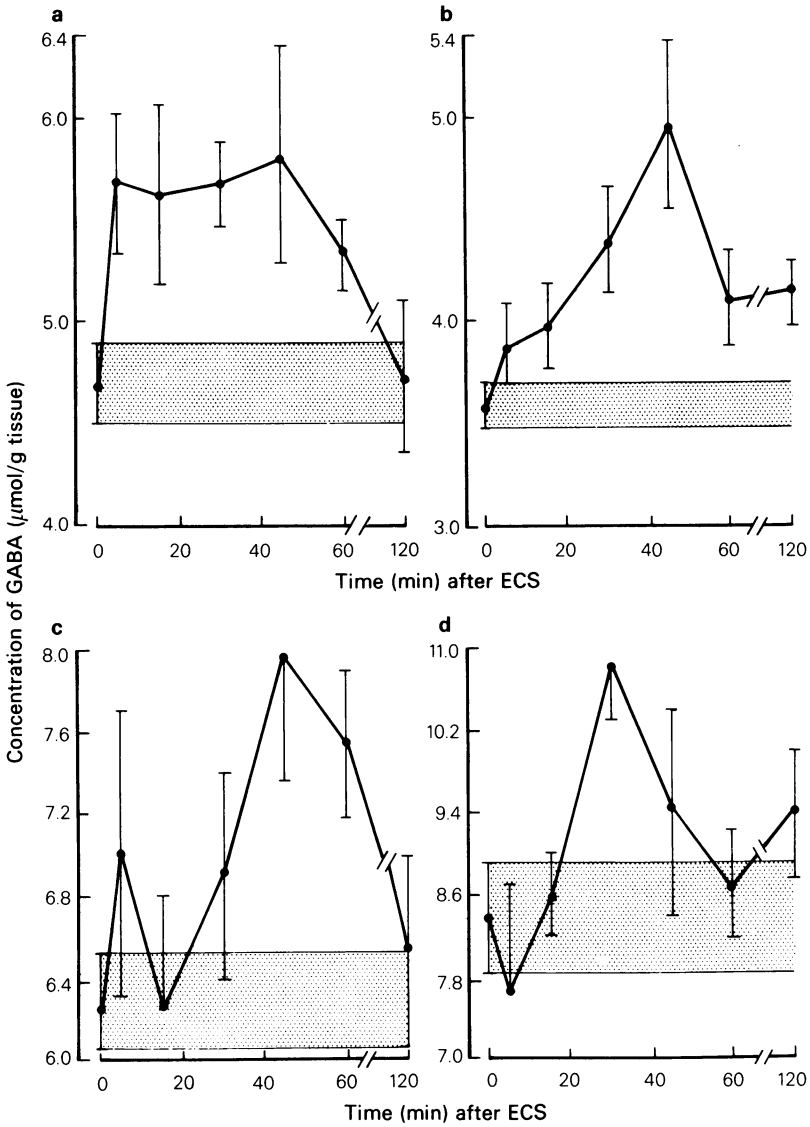

Figure 2 γ-Aminobutyric acid (GABA) concentration in (a) hippocampus, (b) cortex, (c) corpus striatum and (d) hypothalamus in the 120 min after an electroconvulsive shock (ECS). Shaded area shows projection of the mean ± s.e.mean of GABA concentration in the control (handled only) group. Statistically significant increases ($P < 0.05$ or better) hippocampus (5–45 min), cortex (30–120 min), striatum (45–60 min), hypothalamus (30 min).

Effect of repeated ECS on regional brain γ-aminobutyric acid concentration

Rats were either handled or given a single ECS once daily for 10 days. Thirty min after the final treatment they were killed and regional brain GABA concentrations measured.

In contrast to the effect of a single ECS, no increase in GABA concentration was seen in the hippocampus, cortex or hypothalamus of ECS-treated rats. There was a significant increase in GABA concentration in the corpus striatum (Table 6).

Discussion

Following a convulsion there is a rapid, marked increase in seizure threshold and this relative resistance to a further convulsion lasts for somewhat over 3 h. Data suggest that GABAergic mechanisms are involved (Nutt *et al.*, 1981, see Introduction).

Table 5 The effect of administering electroconvulsive shock (ECS) during halothane anaesthesia or (+)-propranolol (30 mg/kg, i.p.) pretreatment on the increase in brain γ-aminobutyric acid (GABA) concentration (μmol/g tissue) 30 min following an ECS

	Hippocampus	*Cortex*	*Hypothalamus*	*Striatum*
Halothane	4.75 ± 0.07 (9)	3.89 ± 0.17 (8)	8.40 ± 0.82 (8)	6.48 ± 0.36 (8)
Halothane/ECS	5.40 ± 0.21 (8)†	4.18 ± 0.26 (8)	10.60 ± 0.21 (8)*	6.62 ± 0.34 (8)
Propranolol	4.04 ± 0.10 (4)	3.03 ± 0.12 (4)	7.28 ± 0.94 (4)	5.37 ± 0.48 (4)
Propranolol/ECS	4.27 ± 0.23 (4)	3.03 ± 0.32 (4)	5.96 ± 0.78 (6)	4.82 ± 0.30 (4)

Results expressed as mean ± s.e.mean with number of observations in parentheses.
Different from handled controls: $^*P < 0.05$; $^\dagger P < 0.01$.

We were unable to confirm the report (Paul & Skolnick, 1978) that during this period of post-ictal increase in seizure threshold, there was any alteration in benzodiazepine receptor systems. No change in dissociation constant or maximum number of binding sites was observed either in well washed membrane preparations obtained for six brain regions or when a crude synaptosomal membrane preparation was used and assayed as described by Paul & Skolnick (1978). Whilst we are unable to explain why this discrepancy exists between their data and our own, reference to Figure 1 shows that in some of the Scatchard analyses performed from individual experiments a rise in B_{max} did occur and it is possible, therefore, that this inconsistent change was seen and reported.

Since we were unable to explain the rise in seizure threshold seen in rats following an ECS by a change in benzodiazepine receptor characteristics, we decided to examine whether there were changes in GABA concentration following a seizure. A change that was consistently observed following an ECS was a rise in GABA concentration. The GABA concentrations found in control rats were higher than in some other studies. Nevertheless they were only slightly elevated over those seen by some other groups (e.g. Patsalos & Lascelles, 1981). Currently we are obtaining values about 30% lower than those reported in this study, but the post-ictal changes are still seen. It seems unlikely that this increase is due to post-ictal hypoxia, since no change in GABA concentration was observed in rats convulsed by administration of flurothyl. The increase in the cortex is temporally rather similar to the benzodiazepine change described by Paul & Skolnick (1978). Certainly a change in GABA concentration can alter the characteristics of benzodiazepine binding, although it has been reported that K_d changes rather than B_{max} (Martin & Candy, 1980).

The change in GABA concentration in the hippocampus is most closely related temporally to the increase in seizure threshold; a marked increase occurring within 5 min and returning towards baseline by 60 min. Increased brain GABA concentrations can certainly inhibit the seizures produced by various treatments (see, for example, Kuriyama, Roberts & Rubinstein, 1966) and the hippocampus is a region generally considered to be involved in the aetiology of some seizure disorders since temporal lobe epilepsy can result from pathological changes in this region (see Scheibel, 1980).

A convulsion produced by bicuculline administration resulted in an increase in seizure threshold (Nutt *et al.*, 1981) and an increase in brain GABA concentration. Chapman, Meldrum & Siesjö (1977) have also reported a rise of the same order in brain GABA concentration 5 min after the start of a seizure induced by bicuculline administration, which results in a prolonged seizure. However, our data do not suggest a simple relationship between the severity of the seizure, the brain GABA concentration increase and the previously reported rise in seizure threshold. Following administration of ECS, bicuculline or flurothyl, the rats showed tonic extension of both fore- and hind-limbs, whilst there was no tonic extension when the ECS was given to rats during halothane anaesthesia. All these treatments increased seizure

Table 6 Regional brain γ-aminobutyric acid (GABA) concentrations (μmol/g tissue) 30 min after the final electroconvulsive shock (ECS) of a series of 10 ECS given once daily for 10 days

	Hippocampus	*Cortex*	*Hypothalamus*	*Striatum*
Handled × 10	5.03 ± 0.14 (9)	3.87 ± 0.13 (9)	7.88 ± 0.37 (9)	6.35 ± 0.36 (9)
ECS × 10	5.43 ± 0.26 (4)	4.03 ± 0.20 (4)	8.76 ± 1.00 (4)	8.03 ± 0.79 (4)*

Results expressed as mean ± s.e.mean with number of observations in parentheses.
Different from handled control: $^*P < 0.05$.

threshold. However, no post-ictal rise in hippocampal GABA concentration was seen in animals convulsed by exposure to flurothyl.

(+)-Propranolol pretreatment abolishes the ECS-induced tonic fore- and hind-limb extension and has been shown to inhibit the rise in seizure threshold, probably by a membrane stabilizing effect (Nutt, *et al.*, 1981). The change in brain GABA concentration following the seizure was also prevented (this paper).

While brain GABA concentration has returned to normal at 2 h, the seizure threshold is still somewhat elevated at 3 h both to pentylenetetrazol (Nutt *et al.*, 1981) and bicuculline (Bowdler, Cowen & Green, unpublished). A further indication that the increase in seizure threshold is not related simply to an increase in brain GABA concentration, was the observation that 30 min following the final of 10 ECS given once daily for 10 days, no change in GABA content was seen in cortex, hippocampus or hypothalamus and at this time seizure threshold was increased to GABA antagonist drugs (Nutt *et al.*, 1981). There was, however, an increase in GABA content in the corpus striatum. This increase has previously been observed 24 h after the final of 10 ECS using gas chromatography/mass spectrometry (Green, Peralta, Hong, Mao, Atterwill & Costa, 1978) and has been confirmed at this time using the methods of the current study (Bowdler & Green, 1982). It would therefore appear to be a longer term adaptive change following a daily convulsion for 10 days. This change has been examined further and is reported elsewhere (Bowdler & Green, 1982).

In conclusion, therefore, following an electrically or drug-induced convulsion, hippocampal GABA concentration rises at the same time as the post-ictal increase in seizure threshold. Furthermore, both can be prevented by (+)-propranolol pretreatment. The relationship is not straightforward since flurothyl-induced convulsions do not increase brain GABA concentration and the same GABA changes are not seen after repeated ECS, whilst both these treatments do increase threshold. In these cases it is possible that other mechanisms are involved (for example, the concentration of possible endogenous ligands for the benzodiazepine receptor). At present the mechanism of the increase in brain GABA concentration is unknown.

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References

BAXTER, C.F. Assay of γ-aminobutyric acid and enzymes involved in its metabolism. In *Methods in Neurochemistry*. ed. Fried, R. Vol. 3, pp. 1–73. New York: Marcel Dekker.

BOWDLER, J.M. & GREEN, A.R. (1981). Rat brain benzodiazepine receptor number and GABA concentration following a seizure. *Br. J. Pharmac.*, **74,** 814–815P.

BOWDLER, J.M. & GREEN, A.R. (1982). Regional rat brain GABA concentrations following repeated seizures. *Br. J. Pharmac.*, **75,** 89P.

CHAPMAN, A.G., MELDRUM, B.S. & SIESJÖ, B.K. (1977). Cerebral metabolic changes during prolonged epileptic seizures in rats. *J. Neurochem.*, **28,** 1025–1305.

COSTA, E. (1979). The role of gamma-aminobutyric acid in the action of 1,4-benzodiazepines. *Trends. Pharmac. Sci.*, **1,** 41–44.

COWEN, P.J., NUTT, D.J. & GREEN, A.R. (1980). Enhanced 5-hydroxytryptamine and dopamine mediated behavioural responses following convulsions. II: The effects of anaesthesia and current conditions on the appearance of enhanced responses following electroconvulsive shock. *Neuropharmacology*, **19,** 901–906.

GLOWINSKI, J. & IVERSEN, L.L. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H]-norepinephrine, [^{3}H]-dopamine and [^{3}H]-dopa in various regions of the brain. *J. Neurochem.*, **13,** 655–669.

GREEN, A.R., PERALTA, E., HONG, J-S., MAO, C.C., ATTERWILL, C.K. & COSTA, E. (1978). Alterations in GABA metabolism and met-enkephalin content in rat brain following repeated electroconvulsive shocks. *J. Neurochem.*, **31,** 607–611.

GUIDOTTI, A., CHENEY, D.L., TRABUCCHI, M., DOTEUCHI, M., WANG, C. & HAWKINS, R.A. (1974). Focused microwave radiation: a technique to minimise post mortem changes of cyclic nucleotides, dopa, and choline and to preserve brain morphology. *Neuropharmacology*, **13,** 1115–1122.

KURIYAMA, K., ROBERTS, E. & RUBINSTEIN, M.K. (1966). Elevation of γ-aminobutyric acid in brain with aminooxyacetic acid and susceptibility to convulsive seizures in mice: a quantitative re-evaluation. *Biochem. Pharmac.*, **15,** 221–236.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.L. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193,** 265–276.

MARTIN, I.L. & CANDY, J.M. (1980). Facilitation of specific benzodiazepine binding in rat brain membrane fragments by a number of anions. *Neuropharmacology.*, **19,** 175–179.

NUTT, D.J., COWEN, P.J. & GREEN, A.R. (1981). Studies on the post-ictal rise in seizure threshold. *Eur. J. Pharmac.*, **71,** 287–295.

PATSALOS, P.N. & LASCELLES, P.T. (1981). Changes in regional brain levels of amino acid putative neurotransmitters after prolonged treatment with the anticonvulsant drugs diphenylhydantoin, phenobarbitone, sodium valproate, ethosuximide, and sulthiamine in the rat. *J.*

Neurochem., **36,** 688–695.

PAUL, S.M. & SKOLNICK, P. (1978). Rapid changes in brain benzodiazepine receptor after experimental seizures. *Science*, **202,** 892–894.

SCHEIBEL, A.B. (1980). Morphological correlates of epilepsy: cells in the hippocampus. *Adv. Neurol.*, **27,** 49–61.

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