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Effect of single and repeated convulsions on glutamate decarboxylase (GAD) activity and [^{3}H]muscimol binding in the rat brain

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Previous work has shown that repeated electroconvulsive shock (e.c.s.) administered alone induces decreased γ-aminobutyric acid (GABA) turnover rates and increased GABA concentrations in rat nucleus accumbens and caudate nucleus 24 h postictally (Green et al 1978). It was thought that this may be functionally related to a decreased GABA release from nerve endings in these areas with possible alterations in postsynaptic GABA receptors. Therefore, [^{3}H]muscimol binding to crude synaptic membranes from striatum (and cerebral cortex) has been examined following both repeated e.c.s. and repeated administration of convulsant doses of the GABA antagonist, bicuculline. Specific [^{3}H]muscimol binding was used to assess the synaptic GABA receptor to which both muscimol and GABA have been shown to bind in a sodium-independent fashion (Williams & Risley 1979; Beaumont et al 1978; Snodgrass 1978).

Glutamate decarboxylase (GAD) activity was similarly measured in striatum following repeated ECS to see whether possible alterations in the activity of the rate-limiting enzyme might be causally related to the observed changes in GABA metabolism produced by electrically-induced convulsions. Previous studies have shown that many convulsants appear to affect brain GAD activity (immediately postictally) by interfering with the pyridoxal phosphate cofactor synthesis or function (see Löscher 1979). In this context, striatal GAD activity was also monitored immediately following an acute e.c.s. or bicuculline-induced convulsion. Long-term effects of drug or electrically-induced convulsions on enzyme activity are not yet known, however.

[Methylamine-^{3}H]muscimol (19 Ci mmol^{-1}; 1 mCi ml^{-1}) and L-[1-^{14}C]glutamic acid (50 mCi mmol^{-1}; 50 μCi ml^{-1}) were obtained from the Radiochemical Centre, Amersham, Bucks. Unlabelled GABA and (+)-bicuculline were obtained from the Sigma Chemical Co. Ltd.

Adult male Sprague-Dawley rats (120–140 g at start of experiment—chronic studies, 180–200 g—acute studies) were used (Charles River Ltd., Margate, Kent). Bicuculline convulsions were produced by injecting, via a tail vein, 0·375 mg kg^{-1} of (+)-bicuculline as a solution of 0·1 mg ml^{-1} in pH 3 0·9% w/v NaCl (saline). Controls received an equivalent volume of saline. E.c.s. was administered via ear-clip electrodes from a Theratronics small animal electroplexy unit (150 v, 50 Hz sinusoidal for 1 s) usually under halothane anaesthesia (controls received halothane only).

GAD activity was estimated radiometrically by monitoring [^{14}C]CO_2 evolution from [1-^{14}C]glutamate (see Roberts & Simonsen 1963). Striata were rapidly removed and homogenized (10 mg ml^{-1}) in a mixture of 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, and 0·5 mM pyridoxal-5′-phosphate. Portions (0·4 ml) were then pre-incubated at 37 °C for 15 min in a total volume of 5 ml. [1-^{14}C]Glutamate solution was then injected to a final concentration of 5 mM (0·5 μCi) and incubation continued for a further 30 min. Reactions were stopped with 1 ml of 5 M HCl and the acid-liberated [^{14}C]CO_2 trapped on Hyamine-soaked filters for 60 min. Blanks were determined in parallel by injecting the acid before substrate.

Fresh crude synaptic membranes (CSMs) were prepared from striata and cerebral cortices of treated rats by a procedure slightly different from that described previously (Zukin et al 1974; Beaumont et al 1978; Williams & Risley 1979). Tissue homogenates were centrifuged at 1000 *g* for 10 min (0—4 °C) and the resultant supernatant spun at 100 000 *g* for 20 min to give a combined crude synaptosomal + microsomal pellet (P_2 + P_3). After hypo-osmotic disruption of this fraction in 25 vol distilled water (0–4 °C), the 'shocked' suspension was frozen overnight (—20 °C), thawed, pelletted (100 000 *g* for 10 min) and the pellets resuspended in 25 mM HEPES-Tris buffer (pH 6·7; 37 °C) (Snodgrass 1978) containing 0·025% v/v Triton-X-100, and incubated after 37 °C for 30 min. Membranes were then collected by centrifugation as described, and washed twice by centrifugation through HEPES-Tris Buffer. Final pellets were stored at —20 °C.

Binding was measured using a filtration assay similar to that of Williams & Risley (1979). Frozen pellets were resuspended in 25 vol 50 mM HEPES-Tris buffer (pH 7·0, 2 °C). Assays were run in duplicate in a total volume of 2·0 ml containing HEPES-Tris buffer (pH 7·0, 2 °C), [^{3}H]muscimol (final concentration = 4 nM) and membranes (250 μl; approx. 400 μg protein). After incubation for 30 min, the samples were filtered under vacuum onto Whatman GF/C filters, washed with 2 × 5 ml ice-cold buffer and the filters counted for tritium by liquid scintillation spectrometry after the addition of 1 ml Soluene-350 (Packard Ltd.).

Specific [^{3}H]muscimol binding was determined as the

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Table 1. Effect of single and repeated convulsions on rat brain [^{3}H]muscimol binding. 'Test' animals received either e.c.s. (+ or − anaesthetic) or bicuculline and were compared in the same experiment with 'controls' receiving either only sham-e.c.s., halothane, or intravenous saline. Variations in 'control' binding data are due to inter-experimental variability, since all assays were not run contemporaneously. Comparing 'controls' treated with halothane to untreated preparations‡ no effect of anaesthetic could be deduced. The results were not significant by Student's 2-tailed *t*-test.

	Experimental Conditions	[^{3}H]Muscimol specific binding (4 nM) (pmol g^{-1} protein) (mean ± s.e.m.) Test	Control	n
(a)†	Striatum‡		106·65 ± 3·35‡	6
(b)	E.c.s. × 10 (24 h)* Striatum	130·62 ± 7·91	118·6 ± 4·11	5
(c)†	Bicuculline × 10 (24 h) Striatum	88·16 ± 9·04	79·55 ± 2·80	6
(d)	E.c.s. × 10 (24 h) C. cortex	172·67 ± 7·98	162·04 ± 8·97	6
(e)†	Bicuculline × 10 (24 h) C. Cortex	154·34 ± 4·48	163·95 ± 9·91	6
(f)†	E.c.s. × 1 (10 min) C. Cortex	199·14 ± 16·27	211·69 ± 12·83	6

* Postictal time; † No halothane given; ‡ Untreated striatal sample.

difference in ligand bound to filters in the absence and presence of 10 μM unlabelled GABA and represented approximately 80% of the total ligand bound.

Protein was analysed by the method of Lowry et al (1951). Bovine serum albumin was used as a standard and blanks were always run in parallel and subtracted from sample values to allow for interference by the HE PES-Tris buffer.

Binding equilibrium was reached in about 25 min and remained constant thereafter at ligand concentrations of 2 nM and 25 nM. Specific binding was linear up to a maximum tested protein concentration of approx. 600 μg/2 ml incubated (2 nM ligand). A Scatchard analysis of specific binding up to 25 nM [^{3}H]muscimol revealed a single population of binding sites (Kd (app) = 9 nM-; Bmax = 400 pmol g^{-1} protein), in agreement with several other studies employing similar experimental conditions to those described here (Beaumont et al 1978; Snodgrass 1978; Herschel & Baldessarini 1979), whereas, before Triton treatment, two binding sites were revealed one of high and one of low affinity by Beaumont et al (1978).

Central receptors for GABA have been shown to be able to alter their sensitivity after manipulation of the presynaptic input, as do central dopamine receptors. Thus, after striatal kainate lesions, supersensitivity of GABA receptors has been demonstrated in both striatum (Campochiaro et al 1977,—200% increase, Kuriyama et al 1980—100% increase) and substantia nigra (Waddington & Cross 1978,—80% increase) using both [^{3}H]GABA and [^{3}H]muscimol binding. In addition, a decrease in striatal [^{3}H]muscimol binding has recently been demonstrated following elevation of brain GABA concentrations by chronic aminooxyacetic acid treatment (Ferkany et al 1980). Hence it was surprising in view of the decrease in presynaptic GABA turnover/release by repeated e.c.s. treatment proposed by Green et al (1978), that, when compared with paired control brain preparations, neither repeated e.c.s. nor chronic convulsant treatment by bicuculline altered [^{3}H]muscimol binding in striatum or cortex (Table 1). There is some evidence, however, that [^{3}H]GABA and [^{3}H]muscimol may not label identical populations of binding sites in rat brain (Defeudis et al 1979; Herschel & Baldessarini 1979) and that displacement of [^{3}H]-muscimol with GABA may lead to an overestimate of GABA receptor density (Defeudis et al 1979).

A further consideration is that in the present study, e.c.s. treatment was given under anaesthetic whereas in the previous study (Green et al 1978) it was not. Preliminary experiments have shown that e.c.s. plus anaesthetic produces no change in GABA concentration (Cross et al 1979; Atterwill, unpublished) whereas chronic convulsions without anaesthetic do produce such a change (Liebowitz et al 1977; Green et al 1978). Future studies should perhaps include an examination of [^{3}H]GABA and [^{3}H]muscimol binding and the effects of repeated e.c.s. with and without anaesthetic.

Seizures induced in the rat by a single electroshock or injection of leptazol(pentylenetetrazol) have been shown to increase specific [^{3}H]diazepam binding to putative benzodiazepine receptor sites in cerebral cortical membranes in vitro (Paul & Skolnick 1978), whereas repeated e.c.s. appears to have no effect (Cross et al 1979). In contrast to the benzodiazepine receptor, no change in GABA receptor binding was seen 20 min after a single e.c.s. (Table 1e). Thus, even though central GABA and benzodiazepine receptors appear to be

Table 2. Effect of single and repeated convulsions on rat striatal GAD activity.

	Experimental Conditions	GAD specific activity (μmol h^{-1} g^{-1} wet wt mean ± s.e.m.) Test	Control	n	% Change
(a)	E.c.s. × 10 (24 h postictal)	31·48 ± 1·17	27·48 ± 0·98	15	14·5%*
(b)	E.c.s. × 1 (20–30 min postictal)	28·36 ± 0·97	27·17 ± 1·42	5	—
(c)†	E.c.s. × 1 (2 min postictal; No halothane)	27·20 ± 1·43	28·62 ± 1·24	5	—
(d)†	Bicuculline × 1 (2 min postictal)	28·06 ± 0·87	26·67 ± 1·36	5	5%

* Student's 2-tailed t-test $P < 0·02$; † Halothane not given to test or controls.

intimately linked (see Tallman et al 1978), common changes in these receptor populations do not seem to occur following experimental convulsions.

Depolarization-induced changes in striatal GABA-ergic activity in vitro lead to increases in GAD activity and it has been concluded that this enzyme activity resides in intrinsic, striatal GABA-ergic interneurons (Spehlmann et al 1977; Gold & Roth 1979) where the changes in GABA metabolism seen after repeated e.c.s. are thought to occur (Green et al 1978). A single e.c.s. given with or without anaesthetic (Table 2) produced no change in striatal GAD activity at 20 or 2 min (respectively) postictally. Similarly, a sustained bicuculline-induced convulsion produced no change in enzyme activity. This generally agrees with work where convulsions were elicited by administration of the anticholinesterase, soman (Lundy et al 1978), and contrasts with convulsants which cause an inhibition of GAD around the time of the convulsion by interfering with the enzyme cofactor, pyridoxal phosphate (see Horton et al 1978; Löscher 1979).

Repeated e.c.s. (× 10 days, with anaesthetic) produced a significant 15% increase in striatal GAD activity 24 h postictally (Table 2). This is not entirely consistent with a decreased GABA turnover rate, following repeated e.c.s. without anaesthetic (Green et al 1978), which might be expected to result from decreased enzyme activity. However, a more detailed comparison of the effects of repeated e.c.s. administered with and without anaesthesia on these parameters is now required.

In conclusion, it may be tentatively deduced from these data that the observed change in presynaptic GAD activity after repeated e.c.s. is not causally linked to alterations in GABA postsynaptic receptor sensitivity as measured by [^{3}H]muscimol binding.

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