Br. J. Pharmacol. (1990), 101, 599–604

Acetylcholinesterase activity in regions of mouse brain following acute and chronic treatment with a benzodiazepine inverse agonist

[1]M.E. Appleyard, S.C. Taylor & [2]H.J. Little

Department of Pharmacology, South Parks Road, Oxford OX1 3QT

1 Chronic administration of the benzodiazepine inverse agonist FG 7142 has previously been shown to induce seizure activity in mice. In the present study we have investigated the effects of acute and chronic treatment with FG 7142 in mice on the levels of acetylcholinesterase activity in cortex, hippocampus, midbrain and striatum. We have also investigated the effects of acute and chronic stress in the form of handling (vehicle-injection) on acetylcholinesterase levels.

2 A single dose of FG 7142 produced a marked elevation of total acetylcholinesterase activities in the hippocampus and midbrain when compared with vehicle-injected control levels, but the levels were not different from those in unhandled animals.

3 Acute stress, in the form of vehicle-injection produced decreases in cortical and hippocampal soluble acetylcholinesterase activity but FG 7142 had no effect upon these stress-induced changes.

4 Total cortical and hippocampal acetylcholinesterase activities were increased by 56% and 16% respectively in the chronic FG 7142-treated mice that exhibited seizure activity (compared with vehicle-injected controls).

5 Soluble acetylcholinesterase activity in the midbrain was decreased to 82% of control levels only in animals that had undergone FG 7142-induced kindling. Smaller or no changes in acetylcholinesterase activity in the midbrain were observed in chronically FG 7142-treated animals that exhibited no seizure activity.

6 Mice that did not demonstrate seizure activity in response to chronic FG 7142 treatment showed alterations in the soluble acetylcholinesterase activities of the hippocampus and midbrain.

7 It is concluded that chronic treatment with the benzodiazepine inverse agonist FG 7142 produces alterations in the acetylcholinesterase activities of various brain regions, in a manner related to the kindling that can be produced by this treatment.

8 Chronic mild stress, in the form of repeated handling (vehicle injection), induced changes in brain activity with decreases in total activity occurring in the cortex and hippocampus, and an increase in soluble acetylcholinesterase activity occurring in the midbrain.

9 All these stress-induced changes appeared to be prevented by administration of FG 7142 at the time of the stress. It would appear therefore that FG 7142 can prevent the effects of chronic stress on brain acetylcholinesterase activity.

Introduction

Chronic administration of inverse agonists at the benzodiazepine receptor has been shown to produce chemical kindling (Little *et al.*, 1984; Morin, 1984) i.e. repeated administration of a dose of FG 7142 which is initially proconvulsant (but which does not itself produce convulsions) reliably produces generalised seizures in 60% of mice so treated. This effect persists for at least six months after cessation of treatment. The development of this chemical kindling phenomenon is prevented by concurrent administration of benzodiazepine antagonist Ro 15-1788 (Little *et al.*, 1984) indicating that FG 7142 kindling is mediated via the benzodiazepine receptor. Ro 15-1788 also prevents electrically kindled seizures (Robertson & Riives, 1983).

Chemical kindling can also be induced by repeated cholinergic stimulation of the limbic regions. The development and manifestation of this cholinergic kindling is prevented by atropine treatment (Wasterlain *et al.*, 1978; Olney *et al.*, 1983; Turski *et al.*, 1983). Similarly atropine also retards electrically-induced kindling (Arnold *et al.*, 1973) indicating that cholinergic hypersensitivity may be responsible for the development of seizures. Supersensitivity to endogenous acetylcholine (ACh) may also be a factor in cortical epileptogenesis: the chronically isolated cortex shows both spontaneous and prolonged epileptiform after discharges, and increased sensitivity to topically applied ACh after several weeks (Echlin & Battista, 1963). This increase in the sensitivity of the isolated cortex parallels a decrease in the acetylcholinesterase (EC 3.1.1.7; AChE) activity of the tissue (Hebb *et al.*, 1963; Rosenberg & Echlin, 1965; Chu *et al.*, 1971) prompting Girgis (1981) to propose that AChE may have a protective function in limbic structures prone to epileptiform activity.

Convulsions induced with a single electroconvulsive shock have previously been found to induce transient increases in the AChE activity of cortex and striatum, and sustained decreases in the AChE activity of the hippocampus and midbrain of rats (Appleyard *et al.*, 1986). We have therefore examined the AChE activities of various brain regions from mice that were kindled with chronic FG 7142 treatment. The effects produced by FG 7142 kindling were compared with those produced by a single dose of FG 7142. A preliminary report of some of these findings has been published as an abstract (Appleyard *et al.*, 1985).

Acute and repeated stress have also been reported to have marked effects upon the AChE activity of numerous brain nuclei. Increased levels of AChE activity have been observed in the cortex, thalamus and hypothalamus of rats that had been stressed by application of electric foot shocks (Singh *et al.*, 1979). Acute and chronic immobilization stress induced

[1] Present address and address for correspondence: Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF.

[2] Present address: Department of Pharmacology, The Medical School, University Walk, Bristol.

increases in the AChE activity of certain hypothalamic and brain stem nuclei (Romero, 1981). Repeated handling can be regarded as a form of mild chronic stress (Graham-Jones *et al.*, 1983; Stanford *et al.*, 1984) and has been shown to induce long-lasting changes in several cortical chemical parameters, such as adrenoceptor number. Therefore the effects of acute and chronic handling upon the AChE activity of various brain regions were also investigated by comparing vehicle-injected mice with handled animals.

Methods

Animals and seizure administration

Male CD1 mice (Charles River) weighing between 30–35 g were used for all experiments. They were housed in groups of 8–12 under conditions of controlled lighting and temperature, and with free access to food and water. Acute and chronic experiments were performed; in each case there were three experimental groups of mice consisting of FG 7142-treated, vehicle-injected (handled) and unhandled animals.

FG 7142 (A/S Ferrosan, Denmark) was suspended in distilled water with one drop of Tween 80 per 10 ml, and was administered in a volume of 10 ml kg^{-1}, i.p.

In the acute experiments mice received a single injection of 40 mg kg^{-1} FG 7142 i.p. and were killed seven days later by cervical dislocation. Vehicle-injected (handled) animals received a single injection of the Tween 80 vehicle and were also killed seven days later. Unhandled mice that arrived in the department with, and were housed under the same conditions as the experimental groups, but which received no treatments and remained unhandled during their stay in the department, were killed at the same time as the other groups.

In the chronic treatment experiments, a single dose of 40 mg kg^{-1} FG 7142 (i.p.) was administered daily at 10 h 00 min for 12 days. On days 10, 11 and 12 the mice were observed for 1 h after each injection in open top cages (30 cm × 60 cm). Observations were made of their behaviour during this time and the incidence of myoclonic jerks and full generalized seizures were recorded. A full generalized seizure was defined as clonic or tonic contractions of all limbs plus loss of posture (i.e. the mice fell onto their side or back). All injections and observations were made by the same observer and all injections were performed in the same room. Vehicle-injected (handled) animals received daily injections of the Tween 80 vehicle. On day 19, seven days after the last injection, all the mice were killed by cervical dislocation. Unhandled mice that had arrived in the department on the same day, and were housed under the same conditions, as the treatment groups were also killed by cervical dislocation at the same time as the other groups.

Measurement of acetylcholinesterase activity in brain regions

After the mice were killed by cervical dislocation their brains were removed and rapidly dissected at 0°C to obtain total cortex, hippocampus, midbrain and striatum. After weighing the dissected areas were stored at −20°C until analysis which was within four weeks.

Brain regions were homogenized in 20 volumes per wet weight of ice-cold 0.03 M sucrose, and homogenates were frozen and thawed once to liberate, as far as possible, the soluble AChE. Aliquots of this total homogenate were then centrifuged at 50,000 *g* for 120 min to sediment the membranes and their bound AChE.

Acetylcholinesterase activities of the total homogenate and supernatant were measured by a stopped assay version (Chubb & Smith, 1976) of the Ellman assay (Ellman *et al.*, 1961) using 1.0 mM acetylthiocholine as substrate, and the specific AChE inhibitor BW 284c51 to distinguish between AChE and non-specific cholinesterase activities. Protein was measured in the soluble and total fractions by the method of Lowry *et al.* (1951) after precipitation by 6% trichloroacetic acid.

Statistics

Results are expressed as mean ± s.e.mean and were analyzed by use of Student's two-tailed *t* test and Welch's d approximation where appropriate.

In order to determine the effects of FG 7142 treatment, levels in FG 7142-treated animals were compared with those found in vehicle-injected animals. The possible effects of stress (in the form of handling) were determined by comparing levels in the vehicle-injected mice with those found in unhandled mice.

Drug

FG 7142 (N-methyl-β-carboline-3-carboxamide) was obtained from A/S Ferrosan, Denmark.

Results

Acetylcholinesterase activities following acute administration of FG 7142

The total and soluble AChE activities per mg protein were determined in homogenates of cortex, hippocampus, midbrain and striatum seven days after administration of a single dose of FG 7142 or the Tween vehicle.

In the cortex there were no significant differences in the total AChE activity between these groups of mice (Figure 1).

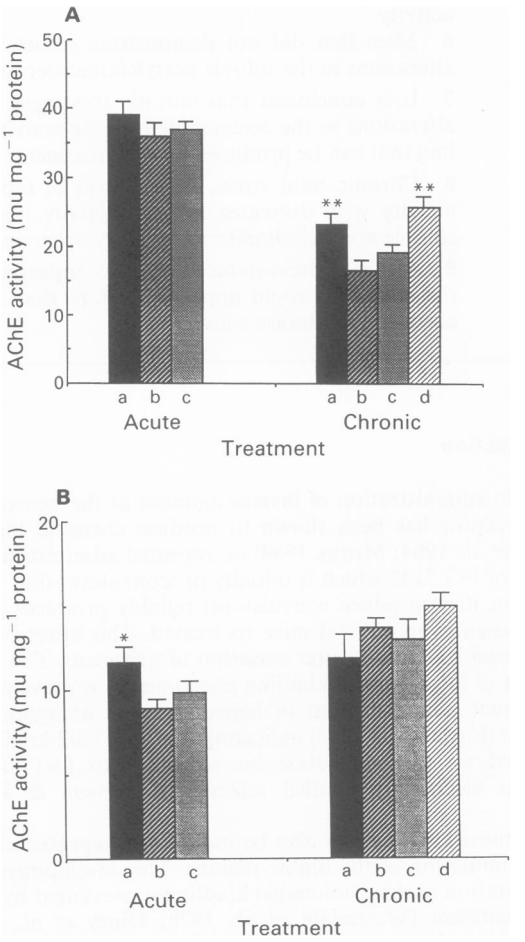

Figure 1 Acute and chronic effects of FG 7142 administration upon acetylcholinesterase activities in (A) the total homogenate (B) the soluble fraction of the cortex: (a) unhandled animals; (b) vehicle-injected controls; (c) FG 7142-treated animals not demonstrating seizure activity; (d) FG 7142-kindled animals. Acetylcholinesterase activity is expressed in nmol acetylthiocholine hydrolysed $min^{-1} mg^{-1}$ total or soluble protein, as appropriate. Data are presented as mean ± s.e.mean. Significantly different from controls with * $P < 0.05$ and ** $P < 0.001$.

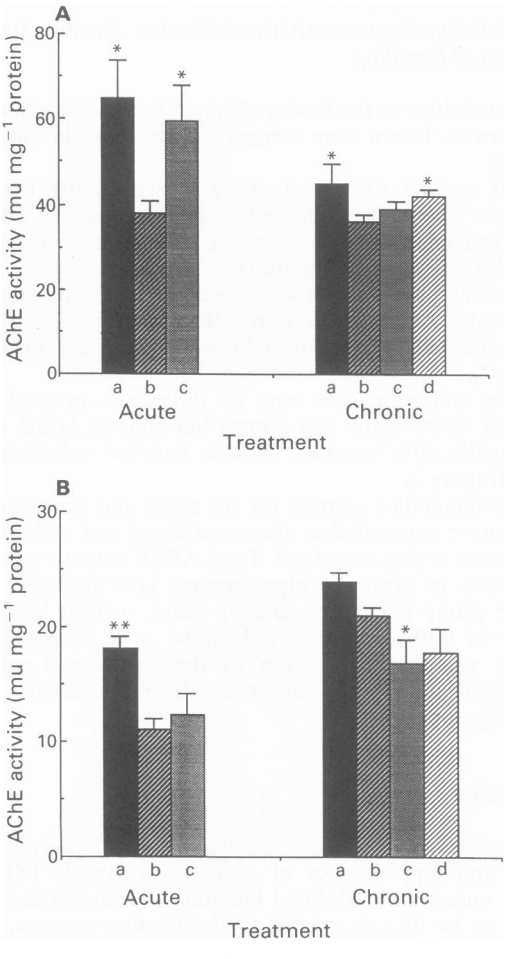

Figure 2 Acetylcholinesterase activities (expressed in nmol acetylthiocholine hydrolysed $min^{-1} mg^{-1}$ protein) in (A) the total homogenate and (B) the soluble fraction of the hippocampus following acute and chronic administration of FG 7142: (a) unhandled animals; (b) vehicle-injected controls; (c) FG 7142-treated animals not showing seizure activity; (d) FG 7142-kindled animals. Significantly different from vehicle injected controls with * $P < 0.05$ and ** $P < 0.005$.

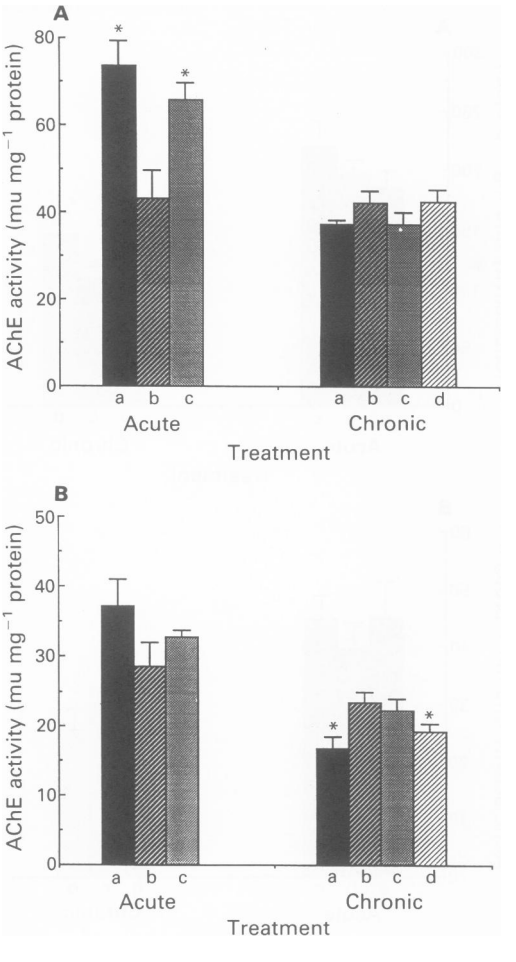

Figure 3 Acetylcholinesterase activities (expressed in nmol acetylthiocholine hydrolysed $min^{-1} mg^{-1}$ protein) in (A) the total homogenate and (B) the soluble fraction of the midbrain following acute and chronic administration of FG 7142; (a) unhandled animals; (b) vehicle-injected controls; (c) FG 7142-treated animals not showing seizure activity; (d) FG 7142-kindled animals. Significantly different from vehicle-injected controls with * $P < 0.05$ and ** $P < 0.005$.

The soluble AChE activity in the cortex of FG 7142-treated mice was not significantly different from that observed in the vehicle-injected animals (Figure 1).

In both hippocampal and midbrain regions the total AChE activities of mice treated acutely with FG 7142 were significantly higher (by 57% and 52% respectively) than those in the vehicle-injected group (Figures 2 and 3). In contrast, no changes in soluble AChE activity were observed in the hippocampus and midbrain of FG 7142-treated mice when compared to levels in vehicle-injected animals (Figures 2 and 3).

No differences in either total or soluble striatal AChE activity were observed between the two groups (Figure 4).

Acetylcholinesterase activities following acute stress in the form of handling

AChE activities found in the brains of vehicle-injected (handled) mice were compared with those found in unhandled mice.

In the cortex there were no significant differences in the total AChE activity, but the soluble AChE activity of the handled Tween-injected mice was significantly ($P < 0.05$) lower (by 24%) than that in the unhandled mice (Figure 1).

Total AChE levels in the hippocampus and midbrain were significantly lower (by 41% in both cases) in the vehicle-injected mice compared with levels in unhandled mice. Significantly lower levels (by 39%) of soluble AChE activity were also observed in the hippocampus of vehicle-injected mice (Figures 2 and 3).

No differences in either total or soluble AChE activity were observed between the two groups (Figure 4).

Acetylcholinesterase activities following chronic administration of FG 7142

The total and soluble AChE activities per mg protein were determined in several brain regions (cortex, hippocampus, midbrain and striatum) from mice that received a single daily injection of FG 7142 for 12 days and were killed seven days later. No behavioral effects were obvious after a single dose of FG 7142 but by day 5, animals showed brief myoclonic jerks of the head and neck in response to the FG 7142 injection. This progressed to generalized seizure activity with squeaking, and the number of mice convulsing increased throughout the treatment schedule, until by day 12 seizure activity was observed in 65% of the animals. The identity of mice that exhibited seizure activity was noted. In general, seizures were observed only once after each injection with a latency which tended to be constant for a given mouse but which varied between mice. The duration of the seizure was 10–20 s.

Total cortical AChE activity was significantly ($P < 0.001$) higher (by 56%) in the chronic FG 7142-treated animals that exhibited seizure activity (i.e. that had undergone kindling) compared with vehicle-injected animals. However, this effect

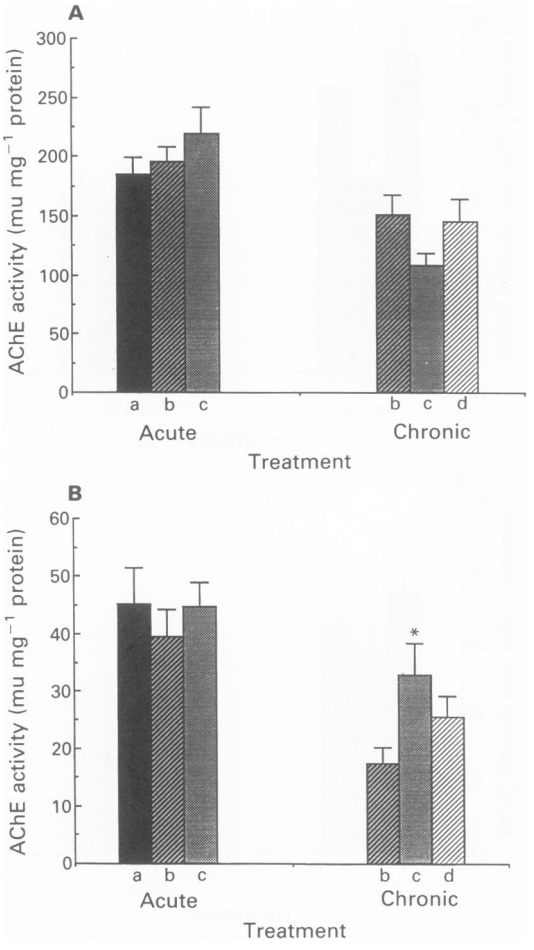

Figure 4 Acetylcholinesterase activities (expressed in nmol acetylthiocholine hydrolysed $min^{-1} mg^{-1}$ protein) in (A) the total homogenate and (B) the soluble fraction of the striatum following acute and chronic administration of FG 7142: (a) unhandled animals; (b) vehicle-injected controls; (c) FG 7142-treated animals not showing seizure activity; (d) FG 7142-kindled animals. Significantly different from vehicle-injected controls with $*P < 0.05$ and $**P < 0.005$ respectively.

was absent from the FG 7142-treated animals that did not exhibit seizure activity. In contrast, there were no differences in the soluble cortical AChE activity between any of the groups (Figure 1).

A similar phenomenon was also observed in the hippocampus, with a significant increase of 16% in the total AChE activity occurring in the kindled group compared with the vehicle-injected group but there was no difference in the mean total activity of vehicle-injected mice and FG 7142-treated mice that did not exhibit seizure activity. No changes in the soluble AChE activity of the hippocampus were observed, apart from a small (20%) but statistically significant decrease in the FG 7142-treated animals that did not exhibit seizure activity (Figure 2).

In the midbrain region there were no alterations in the total AChE activity induced by chronic FG 7142 treatment compared with the vehicle-injected group (Figure 3). However, significantly lower levels (by 18%) of soluble AChE activity were observed in the midbrain of FG 7142 kindled mice. No such difference was apparent in the FG 7142-treated animals that did not exhibit seizure activity (Figure 3).

No differences in the total AChE activity of the striatum were induced by chronic FG 7142 treatment, however, a significantly ($P < 0.02$) higher (by 90%) level of soluble striatal AChE activity was observed in the FG 7142-treated animals that did not exhibit seizure activity, but not in the FG 7142-kindled animals when compared with the vehicle-injected group (Figure 4).

Acetylcholinesterase activities following chronic stress in the form of handling

AChE activities in the brains of mice that had received daily injections of Tween were compared with those in unhandled animals.

Total cortical AChE activity was significantly lower (by 29%) in the Tween-injected mice compared with the unhandled mice, but there were no differences in the soluble cortical AChE activity (Figure 1).

A similar phenomenon was observed in the hippocampus where total AChE levels were also lower (by 20%) in the vehicle-injected mice, but soluble levels were unchanged (Figure 2).

In the midbrain there were no differences in total AChE activities between the two groups but soluble AChE activity was significantly elevated in the handled vehicle-injected group (Figure 3).

If the unhandled animals for the acute and chronic experiments are compared then there are significant differences in every brain region examined. Total AChE activity was markedly lower in both the hippocampus and midbrain of the chronic group (Figures 2 and 3) whilst cortical levels were similar in both groups of unhandled mice. Soluble AChE activity was markedly lower in the cortex and midbrain (Figures 1 and 3) but higher in the hippocampus of the chronic group.

Discussion

Increased levels of total AChE activity were observed in the cortex and hippocampus of only those chronic FG 7142-treated mice which exhibited kindling. Therefore these effects appear to be directly related to the kindling process, or the occurrence of seizures and are not a consequence simply of the chronic administration of FG 7142. However an increased level of hippocampal AChE activity was also observed following acute administration of FG 7142, a treatment which did not induce seizure activity. It is possible that such an effect reflected initiation of the kindling process.

A decrease in total hippocampal AChE activity has previously been observed in rats for up to 3 h immediately following a convulsion induced by acute electroconvulsive shock treatment (Appleyard *et al.*, 1986). It suggests that the changes in AChE activity which occur in the hippocampus are indeed related to the seizure process since susceptibility is decreased for a similar time period immediately following a single electroconvulsive shock (Nutt *et al.*, 1981) and increased (at least to FG 7142) following FG 7142 kindling. This could explain why the changes in AChE activity produced by these two treatments are in opposite directions. Cortical and midbrain changes in AChE activity may not be as important for seizure susceptibility since increased levels of total cortical activity and decreased levels of midbrain soluble AChE activity were observed following both FG 7142 kindling in mice and a single electroconvulsive shock in rats (Appleyard *et al.*, 1986) despite their opposite effects on seizure susceptibility. Species differences could contribute to this discrepancy. It should be noted however that FG 7142 kindling does not appear to be associated with a general decrease in seizure threshold since it produced less change in the seizure threshold to convulsant drugs such as pentylenetetrazol and bicuculline than the effects of benzodiazepine receptor ligands (Little *et al.*, 1986; 1987). Furthermore the changes seen in AChE activity after a single electroconvulsive shock are probably not causally related to the concurrent increase in seizure threshold since no such changes in AChE activity were observed following the last of a series of ten electroconvulsive shocks (Appleyard & Green, 1988) despite the rise in seizure threshold produced by this treatment. Hence, although the present study demonstrates changes in AChE activity in several brain regions which are specific to animals exhibiting kindling in response

to chronic FG 7142 treatment the precise contribution of these changes to the kindling process is at present unclear.

Changes in AChE activity that occurred only in animals chronically treated with FG 7142 but which did not exhibit seizure activity were also apparent, such as a decrease in soluble hippocampal activity. Similar changes have been observed in rats following a single electroconvulsive shock, and could reflect a compensatory mechanism that raises the seizure threshold, so preventing the development of kindling. No such changes in AChE activity were caused by acute administration of FG 7142.

Chronic mild stress, in the form of repeated handling, induced changes in the AChE activity of all three brain regions studied. Repeated handling makes animals tame and has been shown to decrease the emotional reactivity of rats in the open field test (Broadhurst, 1960; Coscina *et al.*, 1975). The changes in brain AChE activity observed in the handled animals could therefore be a direct result of the repeated stress, or could reflect a gradual adaptation to repeated stimulation of an initially stressful nature. Comparison of the effects of acute and chronic handling should distinguish between these two possibilities. A single injection of vehicle had no effect upon total cortical AChE activity, unlike repeated injections, so the changes in AChE activity observed in this brain region probably reflected adaptation to the repeated mild stress, as did changes in the midbrain. However, in the hippocampus, similar decreases in total AChE activity were observed following both a single injection of vehicle and repeated injections, and so this is probably a direct effect of the stress itself. It should be noted that there were marked differences in the AChE activity of all brain regions of unhandled animals for the acute and chronic experiments. These two groups of animals differed only in the amount of time they spent in the department.

Mild acute stress, in the form of a single vehicle injection induced long-lasting decreases in the AChE activity of both the hippocampus and the midbrain. Administration of a single dose of FG 7142 at the time of handling appeared to abolish this effect of stress since there was no difference between these FG 7142-treated mice and unhandled mice. Chronic FG 7142 treatment also appeared to prevent the appearance of chronic stress-induced changes in AChE activities. It is puzzling that FG 7142 appears to prevent the stress-induced changes in AChE activity, since its anxiogenic properties would be expected to intensify the effect of the stress. However, similar effects have been observed with adrenoceptors. Chronic mild stress in the form of repeated saline injections resulted in a decrease in cortical adrenoceptor binding in rats (Stanford *et al.*, 1984) whilst chronic FG 7142 treatment produced an elevation of cortical adrenoceptor binding over vehicle-injected levels in mice (Stanford *et al.*, 1986). It could be that these alterations in adrenoceptors and AChE reflect an adaptive process for coping with the stress, and that this is prevented by the anxiogenic agent.

What is the relevance of the alterations in AChE activity observed in animals kindled with FG 7142? The increases in total AChE activity that occurred in the cortex and hippocampus of kindled mice were due to increased levels of membrane-bound AChE, since levels of soluble enzyme remained unchanged. In cholinergic regions such as the cortex and hippocampus, such AChE would primarily function to terminate cholinergic transmission by hydrolysis of acetylcholine. Hence these altered levels of AChE activity could reflect, or result in, disruptions of cholinergic transmission that have been induced by, or contribute to, the kindling process. It is obviously important therefore to determine whether other cholinergic system markers are also affected by FG 1742 kindling. The influence of cholinomimetic drugs on the development of FG 7142 kindling should also be investigated.

In the midbrain, FG 7142 kindling led to decreased levels of soluble AChE. One of the main AChE-containing regions of the midbrain is the substantia nigra, a region where there is good evidence that soluble AChE is secreted and can affect the activity of pars compacta neurones (Greenfield, 1984; Greenfield *et al.*, 1988). Alterations of the level of soluble AChE could reflect, or result in, an altered secretion of the protein, and hence of nigral activity. Indeed, previous studies have shown that an increased secretion of AChE during a seizure (induced by electroconvulsive shock) (Appleyard *et al.*, 1987) resulted in decreased levels of midbrain AChE (Appleyard *et al.*, 1986). It has been suggested that the substantia nigra functions as a gate in the propagation of seizures (McNamara *et al.*, 1983; Garant & Gale, 1983). Obviously, if such a mechanism exists, any alteration of nigral activity could affect the incidence of seizure activity and so contribute to the kindling process.

References

APPLEYARD, M.E. & GREEN, A.R. (1988). Acetylcholinesterase activity in regions of rat brain following repeated administration of electroconvulsive shock. *J. Psychopharmacol.*, **1**, 211–215.

APPLEYARD, M.E., GREEN, A.R. & GREENFIELD, S.A. (1987). Acetylcholinesterase activity rises in rat cerebrospinal fluid post-ictally; effect of a substantia nigra lesion on this rise and on seizure threshold. *Br. J. Pharmacol.*, **91**, 149–154.

APPLEYARD, M.E., GREEN, A.R. & SMITH, A.D. (1986). Acetylcholinesterase activity in regions of the rat brain following a convulsion. *J. Neurochem.*, **46**, 1749–1793.

APPLEYARD, M.E., TAYLOR, S.C., LITTLE, H.J. & SMITH, A.D. (1985). Acetylcholinesterase activities in various regions of the mouse brain after kindling induced by a benzodiazepine contragonist. *Neurosci. Lett.*, Suppl., **24**, S57.

ARNOLD, P.S., RACINE, R.J. & WISE, R.A. (1973). Effects of atropine, 6-hydroxydopamine and handling on seizure development in the rat. *Exp. Neurol.*, **40**, 457–470.

BABINGTON, R.G. & WEDEKING, P.W. (1973). The pharmacology of seizures induced by sensitization with low-intensity brain stimulation. *Pharmacol. Biochem. Behav.*, **1**, 461–467.

BROADHURST, P.L. (1960). Applications of biomedical genetics to the analysis of behaviour. In *Experiments in Personality, Psychogenetics and Psychopharmacology* ed. Eysenck, H.J. pp. 1–102. London: Routledge and Kegan.

CHU, N.S., RUTLEDGE, L.T. & SELLINGER, O.Z. (1971). The effect of cortical undercutting and long-term electrical stimulation on synaptic acetylcholinesterase. *Brain Res.*, **29**, 323–330.

CHUBB, I.W. & SMITH, A.D. (1975). Isoenzymes of soluble and membrane-bound acetylcholinesterase in bovine splanchnic nerve and adrenal medulla. *Proc. R. Soc. B.*, **191**, 245–261.

COSCINA, D.V., GOODMAN, J., GODSE, D.D. & STANCER, H.C. (1975). Effects of handling before 6-hydroxydopamine treatment on subsequent emotionality and neurochemical changes in the rat. *Physiol. Psychol.*, **3**, 525–528.

ECHLIN, F.A. & BATTISTA, A. (1963). Epileptiform seizures from chronically isolated cortex. *Arch. Neuronl.*, **9**, 154–170.

ELLMAN, G.L., COURTNEY, D.K., ANDRES, V. & FEATHERSTONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 161–171.

GARANT, D.S. & GALE, K. (1983). Lesions of substantia nigra protect against experimentally induced seizures. *Brain Res.*, **273**, 156–161.

GIRGIS, M. (1981). *Neural Substrates of Limbic Epilepsy*. St Louis, U.S.A.: Warner H. Green Inc.

GRAHAM-JONES, S., FILLENZ, M. & GRAY, J.A. (1983). The effects of foot shock and handling on tyrosine hydroxylase activity in synaptosomes and solubilised preparations from rat brain. *Neuroscience*, **9**, 679–686.

GREENFIELD, S.A. (1984). Acetylcholinesterase may have novel functions in the brain. *Trends Neurosci.*, **7**, 364–368.

GREENFIELD, S.A., JACK, J.J.B., LAST, A.T.J. & FRENCH, M. (1988). An electrophysiological action of acetylcholinesterase independent of its catalytic site. *Exp. Brain Res.*, **70**, 441–444.

HEBB, C.O., KRNJEVIC, K. & SILVER, A. (1963). Effect of undercutting on the acetylcholinesterase and choline acetyltransferase activity in the cat's cerebral cortex. *Nature, Lond.*, **198**, 692.

LITTLE, H.J., NUTT, D.J. & TAYLOR, S.C. (1984). Acute and chronic

effects of the benzodiazepine receptor ligand FG 7142: proconvulsant properties and kindling. *Br. J. Pharmacol.*, **83**, 951–958.

LITTLE, H.J., NUTT, D.J. & TAYLOR, S.C. (1986). The effects of drugs acting at the $GABA_A$ receptor/ionophore after chemical kindling with the benzodiazepine receptor ligand FG 7142. *Br. J. Pharmacol.*, **88**, 507–514.

LITTLE, H.J., NUTT, D.J. & TAYLOR, S.C. (1987). Selective changes in the *in vivo* effects of benzodiazepine receptor ligands after chemical kindling with FG 7142. *Neuropharmacol.*, **26**, 25–31.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDAL, R.J. (1951). Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

McNAMARA, J.O., RIGSBEE, L.C. & GALLOWAY, M.T. (1983). Evidence that substantia nigra is crucial to neural network of kindled seizures. *Eur. J. Pharmacol.*, **86**, 485–486.

MORIN, A.M. (1984). β-Carboline kindling of the benzodiazepine receptors. *Brain Res.*, **321**, 151–154.

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

OLNEY, J.W., DE GUBANEFF, T. & LABRUYERE, J. (1983). Seizure-related brain damage induced by cholinergic agents. *Nature, Lond.*, **310**, 520–522.

ROBERTSON, H.A. & RIIVES, M.L. (1983). A benzodiazepine antagonist is an anticonvulsant in an animal model for limbic epilepsy. *Brain Res.*, **270**, 380–382.

ROMERO, E. (1981). Secretion of acetylcholinesterase from the central nervous system. *D. Phil. Thesis*, Oxford University.

ROSENBERG, P. & ECHLIN, F.A. (1965). Cholinesterase activity of chronic partially isolated cortex. *Neurology*, **15**, 700–707.

SINGH, H.C., SINGH, R.H. & UDAPA, K.N. (1979). Electric footshock induced changes in free, bound and total acetylcholine and acetylcholinesterase in different brain regions of rats. *Indian J. Exp. Biol.*, **17**, 304–306.

STANFORD, C., FILLENZ, M. & RYAN, E. (1984). The effect of repeated mild stress on cerebral cortical adrenoceptors and noradrenaline synthesis in the rat. *Neurosci. Lett.*, **45**, 163–167.

STANFORD, C., LITTLE, H.J., NUTT, D.J. & TAYLOR, S.C. (1986). Effects of chronic treatment with benzodiazepine receptor ligands on cortical adrenoceptors. *Eur. J. Pharmacol.*, **129**, 181–184.

TURSKI, W.A., CZUCZWAR, S.J., KLEINROK, Z. & TURSKI, L. (1983). Cholinomimetics produce seizures and brain damage in rats. *Experientia*, **39**, 1408–1411.

WASTERLAIN, C.G., JONES, V. & HOLM, S.J. (1978). Cholinergic kindling: a transynaptic phenomenon requiring the activation of muscarinic receptors. *Neurology*, **28**, 346.

(Received April 12, 1990
Revised June 26, 1990
Accepted July 2, 1990)