Br. J. Pharmac. (1987), **91**, 149–154

Acetylcholinesterase activity rises in rat cerebrospinal fluid post-ictally; effect of a substantia nigra lesion on this rise and on seizure threshold

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1 The activity of acetylcholinesterase (AChE) in the cerebrospinal fluid (CSF) of rats increased by 53% following an electroconvulsive shock (ECS) while non-specific cholinesterase (nsChE) activity was unchanged.

2 A flurothyl-induced seizure failed to elicit a change in the AChE activity of CSF.

3 A bilateral lesion of the substantia nigra pars reticulata abolished the rise in AChE activity in the CSF but did not diminish the increase of seizure threshold which follows a convulsion.

4 These data suggest that AChE is released from the substantia nigra following a seizure but indicate that the change is not associated with the rise in seizure threshold which occurs.

Introduction

Following a single electroconvulsive shock (ECS) there is a marked increase in seizure threshold to convulsant drugs (Nutt *et al.*, 1981; Tacke *et al.*, 1984) lasting over 2 h following the seizure. Recently it has been observed that acetylcholinesterase (AChE) activity showed a sustained decrease over the same period in hippocampus and midbrain (Appleyard *et al.*, 1986).

There is good evidence that in several brain regions (and particularly the substantia nigra) AChE has a role that is independent of cholinergic neurotransmission (Greenfield, 1984). It has been shown, for example, to be secreted from dopamine-containing dendrites in the substantia nigra (Greenfield *et al.*, 1983a,b) and furthermore that this secretion is not directly related to the firing rate of the cells (Weston & Greenfield, 1986).

It seemed possible therefore that the change in AChE activity observed after a seizure was in some way related to an altered rate of AChE secretion and possibly to the change in seizure threshold occurring at the same time. The current study has investigated these possibilities both by measuring AChE activity in cerebrospinal fluid and examination of the consequences of a bilateral nigral lesion on post-ictal seizure threshold.

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Methods

Animals and seizure induction

Female Wistar rats (Charles River, Kent) (150–180 g) were used in all experiments. They were kept in conditions of controlled temperature and lighting and fed a diet of modified 41B pellets and tap water *ad libitum*. All rats had been in the department for 7 days before commencement of the experiments.

Electrically-induced seizures were produced by administration of a single ECS (45 mA for 1.5 s, 50 Hz) through earclip electrodes; control animals had the electrodes placed but no current passed. A full tonic-clonic seizure of about 20 s duration was produced by application of ECS. 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 20 s, starting with myoclonic jerks and rapidly followed by a tonic-clonic seizure. At the onset of the convulsion the rats were removed from the desiccator and allowed to recover in their home cage. Convulsions normally

lasted for about 15 s following removal from the desiccator jar. All seizures were produced at the same time of day (10 h 00 min).

Measurement of acetylcholinesterase activity of cisternal cerebrospinal fluid

At various times (30 min and 120 min) following the convulsion, rats were anaesthetized with urethane (1.75 g kg^{-1} i.p.) in the presence of atropine methyl nitrate (6 mg kg^{-1} i.p.). The rat was then placed in a headholder so that the occipital bone was maximally flexed from the atlas vertebra. A hypodermic needle (23G × 1.25 mm 63/100) mounted in a micromanipulator and attached to plastic tubing was then inserted into the cisterna magna to such a depth that the whole of the bevel was covered by the dura, and cerebrospinal fluid (CSF) was allowed to flow under its own pressure. A slight negative pressure was applied by means by a 1 ml syringe attached to the other end of the tubing in order to prime the flow of CSF if this did not occur immediately. Sampling of approximately 250 µl of clear CSF with no visible traces of red blood cells after centrifugation at 1,000 *g* for 10 min provided evidence of a successful cisterna magna puncture. All blood-contaminated samples were discarded.

AChE and non-specific cholinesterase (nsChE) activities of the cisternal CSF samples were measured by a stopped assay modification of the Ellman assay (Ellman *et al.*, 1961) using 1.0 mM acetylthiocholine as substrate at pH 7.0, 30°C, and the specific AChE inhibitor BW 284C51 (1,5-bis-(4 allyl dimethylammonium phenyl)-pentane-3-one dibromide).

Lesions of the substantia nigra

Rats weighing 150–160 g were anaesthetized with

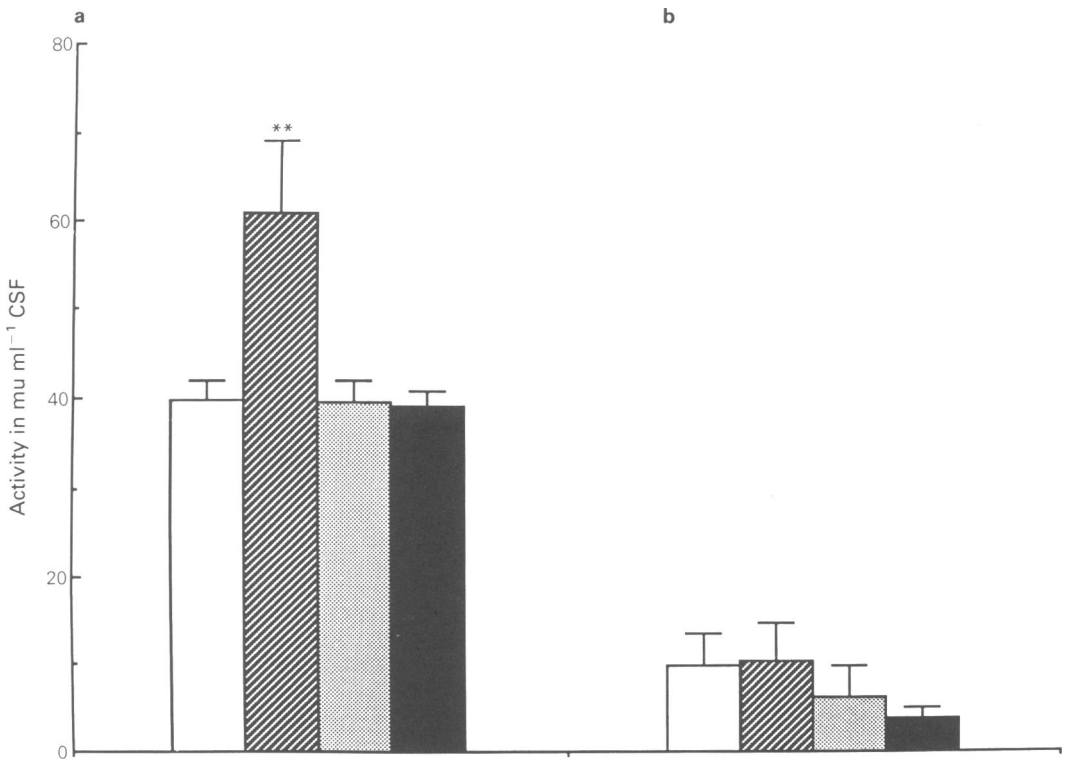

Figure 1 Cholinesterase activities of cisternal cerebrospinal fluid in sham-shocked control animals (open columns, $n = 8$); 30 min following a single electroconvulsive shock (ECS) (hatched columns, $n = 8$); 120 min following a single ECS (stippled columns, $n = 8$); 30 min following a flurothyl-induced convulsion (solid columns, $n = 13$). (a) Acetylcholinesterase and (b) non-specific cholinesterase activities are expressed as nmol acetylthiocholine hydrolysed per ml cerebrospinal fluid (CSF). **Significant difference from control level $P < 0.001$ (Student's t test).

chloral hydrate (0.5 g kg^{-1} i.p). Bilateral electrolytic lesions of the substantia nigra pars reticulata were performed via a stainless steel insect pin 0.62 mm in diameter insulated with nail varnish with a 0.5 mm exposed tip. This electrode was inserted at the following stereotaxic co-ordinates according to the atlas of Konig & Klippel (1967): AP + 1.95 from the interaural line; L ± 1.80 from midline; DV − 8.40 from skull, with upper incisor bar 2.4 mm below interaural line; and the lesion made, using a constant current lesion maker (1 mA for 15 s duration). The animal was left to recover for a minimum of two weeks before administration of electroconvulsive shock and subsequent sampling of CSF or, in other groups of animals, measurement of seizure threshold. Control sham-lesioned animals had the electrodes placed at the above-co-ordinates, but no current passed, and were also allowed to recover for at least two weeks.

Histology

The extent and position of the nigral lesions were verified histologically by examination of cresyl violet stained sections (40 μm).

Measurement of seizure threshold

The seizure thresholds of rats with bilateral nigral lesions and of sham-lesioned rats were determined 30 min after administration of an ECS or sham-shock.

Rats were lightly restrained in a whole rat holder and the convulsant drug pentylenetetrazol (Ptz; 10 mg ml^{-1}) infused via a 25 gauge 'butterfly' inserted into a tail vein. The time to first myoclonic twitch (which occurs synchronously with the first EEG discharge (Nutt *et al.*, 1981)) was recorded and the dose per kg required to produce the seizure calculated. Animals were only ever used once in the study.

Statistics

Results are expressed as mean ± s.e.mean and analysed by use of Student's two-tailed *t* test.

Results

Effect of ECS on AChE activity in cerebrospinal fluid

Cerebrospinal fluid was collected from the cisterna magna of urethane-anaesthetized rats either 30 min following a convulsion induced by either a single ECS or flurothyl exposure, or 120 min following a single ECS. Cerebrospinal fluid was also collected from control (sham-treated) rats 30 min later.

Thirty min following an ECS the activity of AChE per unit volume of CSF was significantly elevated by 53% compared to that from sham-shocked animals (Figure 1). In contrast, non-specific cholinesterase (nsChE) levels of the cisternal CSF were not significantly different from controls (Figure 1). Acetylcholinesterase activity of the CSF had returned to control levels 120 min after the convulsion (Figure 1). A flurothyl-induced convulsion failed to produce a change in the AChE activity of cisternal CSF 30 min later (Figure 1).

Effect of a single convulsion upon the AChE activity of cerebrospinal fluid from animals with bilateral nigral lesions

Rats were given a single ECS (45 mA, 1.5 s duration) two weeks following bilateral lesions of the substantia nigra pars reticulata, or sham operations; control animals were sham-shocked. Thirty min later, CSF was sampled from the cisterna magna under urethane anaesthesia.

A single ECS produced a full tonic-clonic seizure both in sham-operated animals and in animals with bilateral lesions of the substantia nigra. Convulsions normally lasted for about 20 s in sham-operated animals, but were of longer duration (approximately 35 s) and appeared to be more severe in animals with bilateral nigral lesions. In the lesioned animals the convulsion was followed by a period of catalepsy lasting for 5–10 min while no such effect was observed in sham-operated or unoperated animals.

Thirty min following ECS there was a significant increase (64%) in the AChE activity of cisternal CSF from sham-operated animals (Table 1), comparable to that observed in unoperated animals. However, there was no increase in the AChE activity of cisternal CSF in animals with bilateral lesions of the substantia nigra (Table 1).

The placement and extent of the lesion sites was determined by examination of Nissl stained sections.

Table 1 Effect of a single electroconvulsive shock (ECS) on acetylcholinesterase (AChE) activity in cisternal cerebrospinal fluid following bilateral lesions of the substantia nigra

Treatment	*Control*	*ECS*
Sham-operated	26.2 ± 1.0 (4)	42.8 ± 1.0 (4)*
Lesioned	33.2 ± 5.8 (4)	31.4 ± 1.3 (6)

Acetylcholinesterase activity (nmol acetylthiocholine hydrolysed min^{-1} per ml CSF) presented as mean ± s.e.mean (with number of observations in parentheses) in sham and bilateral substantia nigra lesioned rats. *Significantly different from control group $P < 0.001$ (Student's *t* test).

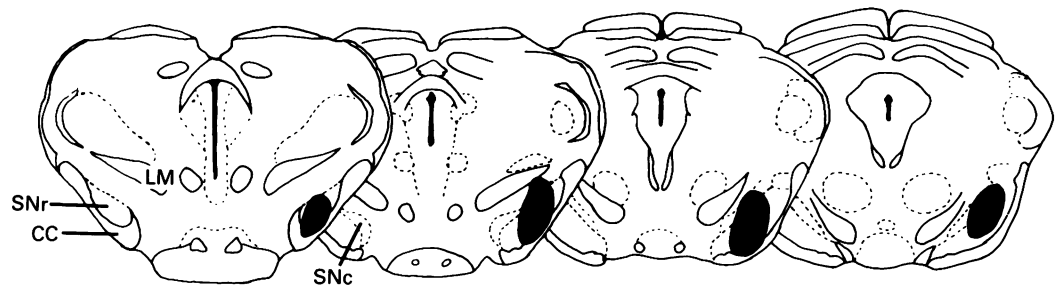

Figure 2 Line drawings of coronal sections of rat brain, based on the atlas of Konig & Klippel (1967). The area shaded in black is a composite area indicating the position and extent of the lesions of the substantia nigra pars reticulata observed in 3 experiments; although bilateral lesions were produced in each case, the lesion is shown on one side only for clarity. SNr substantia nigra pars reticulata; SNc substantia nigra pars compacta; CC crus cerebri; LM lemniscus medialis.

In each case a bilateral lesion of the substantia nigra had been successfully performed. All the lesions were restricted to the substantia nigra and did not involve any adjacent structures. However, the lesions were not complete, with only 50% to 75% of the substantia nigra reticulata being destroyed. In some animals there was also a slight involvement of the pars compacta. An example of a lesion site is shown in Figure 2.

Effect of a bilateral lesion of the substantia nigra on seizure threshold and the change in threshold following a convulsion

Seizure threshold was measured by tail-vein infusion of the convulsant drug pentylenetetrazol (Ptz) 30 min following administration of a single ECS to sham-lesioned animals or to rats with bilateral lesions of the substantia nigra; control rats were sham-shocked.

Thirty min following ECS the seizure threshold to Ptz was significantly increased to 165% of control values in rats that had undergone sham operations (Table 2). An ECS also induced a somewhat larger increase (to 181%) in the seizure threshold of animals with bilateral lesions of the substantia nigra (Table 2).

Discussion

The AChE activity of cisternal CSF was found to be increased 30 min following a single ECS, indicating an increased secretion of AChE from the brain. Previous experiments, examining the effects of electrical stimulation of brain regions upon the AChE activity of continuously sampled cisternal CSF (Greenfield & Smith, 1979), showed delays ranging from 15–85 min from the onset of stimulation to the appearance of an increased AChE activity in the cisternal CSF. Such latencies were considered to reflect the time taken for the enzyme to be distributed homogeneously throughout the CSF pool. Hence, the increased levels of AChE activity in cisternal CSF observed 30 min following ECS could reflect an increased secretion of AChE that occurs during the convulsion.

Secretion of AChE from the substantia nigra has previously been demonstrated (Greenfield *et al.*, 1983a,b; Cuello *et al.*, 1981) and lesioning studies have indicated that much of the AChE in cisternal CSF originates from the substantia nigra (Greenfield & Smith, 1979). It seemed possible, therefore, that the increased levels of cisternal AChE observed following a convulsion reflected an increased secretion of AChE

Table 2 Effect of bilateral lesion of the substantia nigra on seizure threshold

Treatment	*Control*	*ECS*	*% increase after ECS*
Sham-operated	25.9 ± 2.0 (4)	42.6 ± 2.3 (4)*	65
Lesioned	28.6 ± 2.2 (6)	51.7 ± 3.2 (6)**	81

Seizure threshold 30 min after handling or an electroconvulsive shock (ECS) expressed as dose of pentylenetetrazol (mg kg^{-1}) required to elicit seizure. Values are presented as mean ± s.e.mean (with number of observations in parentheses). Significantly different from handled controls, $*P < 0.002$; $**P < 0.001$.

from the nigra. The absence of an ECS-induced increase in the level of CSF AChE following bilateral substantia nigra lesions is consistent with this proposal. An increased tissue secretion of AChE from the substantia nigra might therefore have resulted in the lowered tissue content of AChE in the midbrain as previously observed after a convulsion (Appleyard *et al.*, 1986). The AChE content of the hippocampus was also lowered after a seizure (Appleyard *et al.*, 1986). However, the abolition of the CSF AChE change after a nigral lesion does suggest that any alteration in the AChE content of hippocampal tissue is not reflected by a change in the CSF. The hippocampus can secrete AChE (Appleyard & Smith, 1985), but any change produced by a convulsion might be masked by sampling at the level of the cisterna magna because of the contribution to CSF of AChE from several other brain regions. Therefore, the observation that cisternal AChE originates primarily from the substantia nigra (Greenfield & Smith, 1979) is reinforced. The lack of change in nsChE following a seizure shows that the observed changes in AChE have not resulted from contamination by blood in the CSF.

The increased secretion of AChE from the substantia nigra following ECS is probably not related to the post-ictal rise in seizure threshold, since levels had returned to normal two hours later, a time at which the threshold is still elevated (Nutt *et al.*, 1981). Furthermore abolition of the secretion of AChE from the nigra, achieved by lesions of this structure, did not prevent the post-ictal elevation of the seizure threshold; indeed a more pronounced elevation was observed. Further indication that AChE secretion is not involved in the seizure threshold change was the observation that a flurothyl-induced seizure did not alter AChE activity in the CSF, since there is a rise in seizure threshold following a flurothyl-induced convulsion (Bowdler & Green, 1982). Interestingly, however, flurothyl exposure, in contrast to an ECS, fails to induce a rise in γ-aminobutyric acid (GABA) concentration in various brain regions (Bowdler & Green, 1982; Green, Metz, Minchin & Vincent, unpublished observations), which suggests a possible association between changes in GABA biochemistry and AChE secretion. Such a suggestion is supported by the finding that GABA prevents K^+-induced secretion of AChE from the substantia nigra *in vitro* (Cuello *et al.*, 1981).

Bilateral lesions of the pars reticulata have previously been found to protect against experimentally-induced seizures (Garant & Gale, 1983; McNamara *et al.*, 1984), and so would presumably produce an increase in seizure threshold. However, in the present study, no change in the seizure threshold to Ptz was observed following bilateral nigral lesions (Table 2). Indeed the animals appeared to exhibit seizures of increased severity in response to ECS administration, since they were of longer duration and were followed by a prolonged period of catalepsy. The production of a more severe seizure by ECS administration in the lesioned animals is also suggested by the more pronounced post-ictal increase in the seizure threshold to Ptz. Bicuculline produces a more prolonged seizure than that seen after ECS, and also a greater elevation of seizure threshold than does ECS (Nutt *et al.*, 1981). It has previously been observed that any form of damage to the brain results in an increased susceptibility to seizures, and an increased severity of seizures produced by convulsants and electroconvulsive shock (Nutt, 1982).

A probable reason for the apparent discrepancy between our data, on the effect of bilateral nigral lesions upon the susceptibility to seizures, and those of Garant & Gale (1983) and McNamara *et al.* (1984) are the differences in the lesion produced. Examination of their histological records (McNamara *et al.*, 1984) shows that very large lesions were produced in these animals and it was stated that almost the whole of the pars reticulata had to be destroyed in order to achieve protection against seizures (Garant & Gale, 1983). In the present study only half to three-quarters of the pars reticulata was involved in the lesion.

It is concluded that a single ECS-induced convulsion evokes a transient increase in the secretion of AChE into the CSF, probably from the substantia nigra. However, this altered rate of AChE secretion is not common to all seizures and is probably not involved in the post-ictal rise in seizure threshold. The physiological consequences of such an increased secretion of AChE within the nigra, and its relevance to seizure production/propagation are not yet understood and should certainly form the basis of future investigation.

We thank Prof. Martin Adler (Temple University, Philadelphia) for generous supplies of flurothyl. The work was supported by a grant from the E.P. Abraham Cephalosporin Trust. M.E.A. held an M.R.C. Training Scholarship and an ICI Educational Trust Postgraduate Scholarship, M.E.A. is currently a Tilleard-Cole Research Fellow.

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(Received November 6, 1986.
Revised January 23, 1987.
Accepted January 26, 1987.)