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Carbamazepine inhibits NMDA-induced depolarizations in cortical wedges prepared from DBA/2 mice

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Abstract. There is some doubt as to the mechanism of action of the widely-used anticonvulsant drug, carbamazepine. In cortical wedges prepared from genetically epilepsy-prone DBA/2 mice, carbamazepine at therapeutic concentrations (1-10 μM) markedly reduced the depolarization produced by N-methyl-D-aspartate (NMDA). The NMDA sub-type of glutamate receptor has been implicated in the pathogenesis of epilepsy and the inhibitory action of carbamazepine on this response suggests that the anticonvulsant action of the drug may be due to its blockade of NMDA receptor-mediated events.
Key words. Carbamazepine; NMDA; cortical wedges; DBA/2 mice.

Carbamazepine has been found to be one of the most effective antiepileptic drugs in clinical use for generalised tonic/clonic seizures. It has also been used for the treatment of trigeminal neuralgia and more recently for bipolar illness[1]. However, although it has been in use for more than twenty years, its mechanism of action has still not been proven. There is evidence that carbamazepine blocks sodium channels[2] and also that it facilitates potassium efflux[3], either of these mechanisms would reduce neuronal excitability and could account for its antiepileptic action. A further recent report[4] has shown that carbamazepine blocked NMDA-activated currents in cultured spinal cord neurones.

In this present study we have investigated the action of carbamazepine on NMDA-induced depolarizations in cortical wedges prepared from genetically epilepsy-prone DBA/2 mice.

Materials and methods

Male or female DBA/2 mice aged between 21 and 30 days, which corresponds to the time of peak audiogenic seizure susceptibility, were used throughout. Mice were killed by cervical dislocation and the brain rapidly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF). Coronal slices (500 μm) were cut using a McIlwain tissue chopper, and cortical wedges were prepared as described by Burton et al.[5]. The tissue was placed in a two-compartment bath with a grease seal isolating the grey cortical matter from the callosum. Each compartment was perfused independently with gassed (95% O_2/5% CO_2) aCSF at 2 ml/min at room temperature (20–22 °C). The composition of aCSF in mM was: NaCl 124; KCl 5; NaH_2PO_4 1.25; $MgSO_4$ 2; $CaCl_2$ 2; $NaHCO_3$ 26; glucose 10; pH 7.4. For Mg^{2+}-free aCSF a corresponding increase in NaCl concentration was made.

Following slicing, both compartments were perfused with normal aCSF for 45–60 min to allow the tissue to equilibrate; perfusion of the cortical side was then continued with Mg^{2+}-free aCSF to facilitate NMDA-receptor activation, while the callosal side was perfused with normal aCSF throughout the experiment. The NMDA-induced depolarizations were monitored continuously via Ag/AgCl electrodes and amplified (Flyde 2601A), filtered and displayed on a BBC Goertz-Metrawatt chart recorder. The depolarization of individual neurones by NMDA was recorded as a population response in this preparation. Drugs were perfused into the 'cortical' side of the bath. NMDA (20 μM) was perfused for 2 min at approximately 15-min intervals and once stable recordings were obtained carbamazepine at varying concentrations (0.5–200 μM) was perfused for 10 min prior to, and during, the perfusion with NMDA. In a further series of experiments concentration-response curves were constructed for NMDA alone and in the presence of either 2.5 or 100 μM carbamazepine. Carbamazepine was dissolved in dimethyl sulphoxide (DMSO) and dilut-

ed with Mg^{2+}-free aCSF to give a final concentration of 1.25% DMSO.
NMDA was obtained from Tocris and carbamazepine from Sigma.

Results

The concentration-response curve for NMDA was sigmoidal over the concentration range 2.5–80 μM (fig. 1) and for the subsequent experiments with carbamazepine an NMDA concentration of 20 μM was used. Carbamazepine at concentrations of 1.25–10 μM significantly reduced the NMDA-induced depolarization, whilst concentrations of 100 and 200 μM significantly potentiated the effect of NMDA (fig. 2). Neither DMSO (1.25%) nor 0.625 μM carbamazepine had any effect on the responses.
2.5 μM carbamazepine produced a decrease in the NMDA concentration-response curve with maximal effects on NMDA responses between 10–80 μM (fig. 3). 100 μM carbamazepine, on the other hand, produced a parallel shift to the left on the NMDA-induced concentration-response curve.
A large majority of the cortical wedges exhibited spontaneous depolarizing shifts when perfused with Mg^{2+}-free aCSF. Carbamazepine at all concentrations tested had no significant effect on the frequency of these depolarizations.

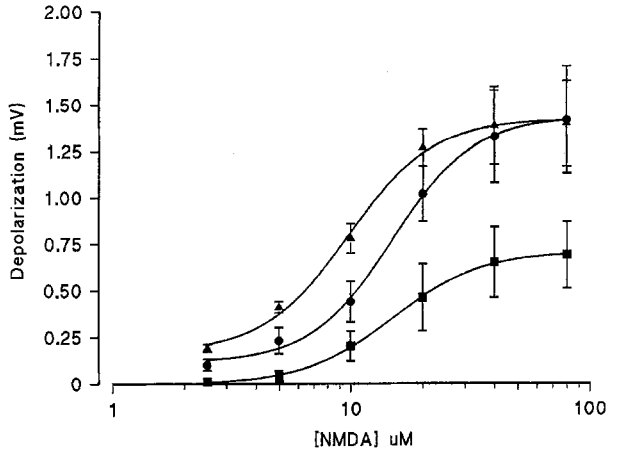

Figure 3. The effect of carbamazepine at 100 μM (▲) and 2.5 μM (■) on the concentration-response curve to NMDA. (●) control curve for NMDA. Mean ± SEM, for 2.5 μM carbamazepine n = 8–9 and for 100 μM n = 7, NMDA n = 12.

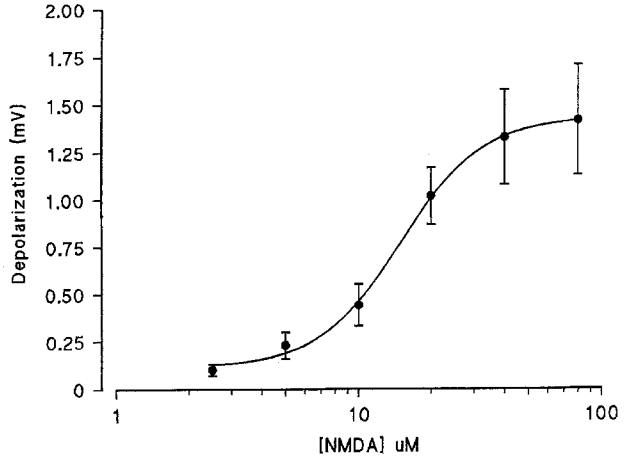

Figure 1. Log concentration-response curve for NMDA on cortical wedges prepared from DBA/2 mice. Each point represents the mean ± SEM from 12 slices.

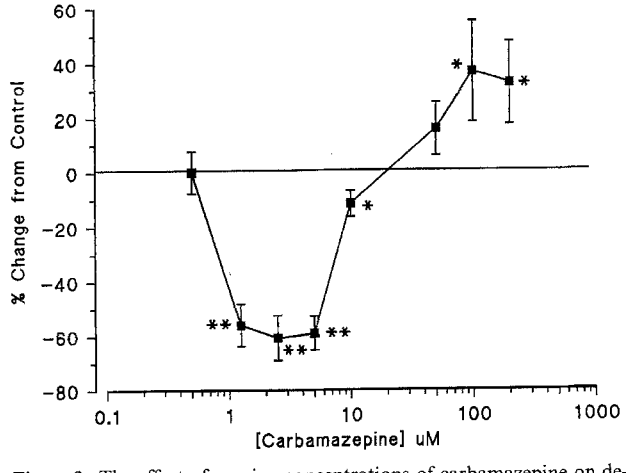

Figure 2. The effect of varying concentrations of carbamazepine on depolarization induced by 20 μM NMDA expressed as percentage change from control. Mean ± SEM, n = 6–10, *p < 0.05, **p < 0.01.

Discussion

The mechanism of action of carbamazepine as an antiepileptic drug is far from clear. Previous studies have shown that carbamazepine at therapeutically relevant concentrations blocks Na^+ channels in a use-, concentration- and voltage-dependent manner in cultured mouse neurones[2]. Another study[3] using penicillin-induced discharges in rat hippocampal slices suggested that the drug increased K^+ fluxes leading to hyperpolarization of the neurone and a subsequent decrease in firing. However, concentrations of 60–200 μM were used in this study. Finally, carbamazepine at concentrations of 20–50 μM has been shown to block NMDA-activated (100 μM) currents in cultured mouse spinal cord neurones[4].
This present study has shown that carbamazepine has a biphasic effect on NMDA-induced depolarizations in cortical wedges prepared from genetically epilepsy-prone DBA/2 mice. The use of these mice is considered to be a better reflection of the underlying mechanisms involved in epileptogenesis. Low concentrations of carbamazepine decreased NMDA-induced depolarizations, with maximal effects being obtained with 1.25–10 μM concentrations which correspond well with the quoted cerebrospinal fluid concentrations of 4–14 μM found in patients treated chronically with carbamazepine for the control of epilepsy[6]. There are also reports in the literature of exacerbation of epileptic episodes in patients with

high CSF levels of carbamazepine[7]. This equates with the potentiation of NMDA-induced depolarization reported in this paper with concentrations of carbamazepine exceeding 50 μM. We have preliminary evidence that perfusion of cortical slices with NMDA releases endogenous glutamate. Thus, carbamazepine, being a tricyclic structure, might inhibit the Na^+-dependent uptake of glutamate at these higher concentrations leading to a potentiated depolarization.

Carbamazepine at all concentrations used in our experiments had no effect on the spontaneous depolarizing shifts which suggests a lack of blocking effect at Na^+ channels especially since tetrodotoxin at 1 μM completely inhibits these spontaneous depolarizing shifts[5]. The site of action of carbamazepine on the NMDA receptor/channel complex cannot be defined from these present experiments. However, it does appear unlikely that it is within the NMDA receptor-operated channel, as compounds such as ketamine and dizocilipine, which block the channel, also inhibit the spontaneous depolarizations[8]. An inhibitory action at either the NMDA recognition site, or the glycine modulatory site, remain as possibilities. Antagonists at the NMDA recognition site have been shown to have potent anticonvulsant activity while antagonists at the glycine site are weakly anticonvulsant[9,10]. The results presented in this paper would favour an antagonistic action at the NMDA receptor site in view of the profound reduction in response seen with low concentrations of carbamazepine.

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Involvement of D-amino acid oxidase in elimination of D-serine in mouse brain

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Abstract. The physiological role of D-amino acid oxidase (EC 1. 4. 3. 3) in mouse brain is described. The presence of D-enantiomers of neutral common amino acids was surveyed in the brain. D-serine was shown to be present at high concentration only in regions where the enzyme activity was low. In normal mice whose D-amino acid oxidase activity was much higher in the cerebellum than in the cerebrum, free D-serine content was apparently lower in the cerebellum than in the cerebrum. In mice of a mutant strain lacking D-amino acid-oxidase activity, the free D-serine level was remarkably high both in the cerebrum and cerebellum. The results suggest that the enzyme is involved in the elimination of free D-serine in the cerebellum.
Key words. D-amino acid oxidase; D-serine; cerebellum; cerebrum.

D-Amino acid oxidase (DAAO) is a flavoenzyme widespread in many animal tissues that catalyzes the oxidative deamination of free neutral D-amino acids to the corresponding 2-oxo acids[1]. However, the physiological function of the enzyme has remained unknown. One reason is that the substrate D-amino acids have not been believed to be present in animal tissues to any significant extent. Hamilton et al.[2] suggested that the physiological substrates are adducts of amines with glyoxylate. However, substantial amounts of free neutral D-amino acids have recently been found in samples free from the action of DAAO, i.e., human plasma from patients with renal diseases[3], and DAAO-lacking mutant-mouse tissues such as kidney, liver, lung, heart, brain and serum[4]. The presence of D-alanine, D-proline and D-serine has been recently demonstrated by high-performance liquid chromatography (HPLC) analysis in mouse kidney and serum[5]. These studies suggest that DAAO is involved in the catabolism of free neutral D-amino acids.

In the present experiment, the relationship between amounts of D-amino acids and DAAO activity was investigated in order to confirm the above hypothesis for the brain, and it has been revealed that the content of free D-serine is high where DAAO activity is low.