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The effect of the desglycinyl metabolite of remacemide on cortical wedges prepared from DBA/2 mice

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Abstract

Remacemide hydrochloride is currently undergoing clinical trials for use as an anticonvulsant agent in the treatment of epilepsy. It is considered that the desglycinyl metabolite (FPL 12495AA) of the parent compound accounts for the majority of the anticonvulsant activity. In this study we have investigated the effects of FPL 12495AA on electrical activity in the cortical wedges prepared from audiogenic seizure-prone DBA/2 mice. FPL 12495AA at varying concentrations (50–200 μM) significantly reduced both the spontaneous depolarizations (IC_{50} 102 μM) and the associated afterpotentials (IC_{50} 50 μM) which are characteristic in this preparation under magnesium-free conditions. The compound also concentration-dependently reduced *N*-methyl-D-aspartate (NMDA)-induced depolarizations of the tissue (IC_{50} 43 μM) and the antagonism by FPL 12494AA was not overcome by increasing NMDA concentrations. FPL 12495AA had no effect on (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)-induced depolarizations. The results suggest that FPL 12495AA has a specific antagonistic effect on the NMDA receptor complex possibly through non-competitive inhibition at the phencyclidine site in the ion channel. Such an action could contribute to its anticonvulsant properties.

Keywords: Remacemide hydrochloride; FPL 12495AA; Cortical wedge; NMDA receptor

1. Introduction

The two amino acid neurotransmitters, γ-amino-butyric acid (GABA) and glutamate, are implicated in the neurochemistry underlying epilepsy. The majority of the drugs currently used in the treatment of epilepsy facilitate, by various mechanisms, the inhibitory actions of GABA or inhibit ion channel activation. However, in the last 5 years a great deal of research effort has been invested into the elucidation of the excitatory role of glutamate in epilepsy. As a result of these studies it has been shown that compounds acting as antagonists at the different sub-types of glutamate receptor are potent anticonvulsants in animal models of epilepsy (Meldrum, 1992). The *N*-methyl-D-aspartate (NMDA) sub-type of glutamate receptor has been extensively investigated and this complex has been shown to contain a number of binding sites. Antagonists at the NMDA recognition site (Schmutz et al., 1990), the glycine modulatory site (Koek and Colpaert, 1990), and the cationic channel site (Aram et al., 1989) have all been shown to possess anticonvulsant properties.

Remacemide hydrochloride is a novel anticonvulsant undergoing clinical trials for patients with generalized tonic-clonic and complex partial epilepsy (Palmer et al., 1993). However, the mechanism of action of the compound remains unclear. It is thought that the active moiety of remacemide hydrochloride is the desglycinyl metabolite (Palmer et al., 1992a), and this compound has been shown to exhibit efficacy against NMDA-induced convulsions/mortality in mice and maximal electroshock seizure in mice and rats (Garske et al., 1991; Palmer et al., 1992a).

The present study was designed to investigate the pharmacological action of the desglycinyl metabolite of remacemide (FPL 12495AA) on spontaneous depolarizations and accompanying afterpotentials in cortical wedges prepared from genetically epilepsy-prone DBA/2 mice. The effect of this compound was also assessed on its ability to inhibit NMDA-and (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced depolarizations.

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2. Materials and methods

Male or female DBA/2 mice aged between 21–30 days from our own colony bred at the University of Wales College of Medicine were used. The animals were decapitated and the brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF). Coronal slices (500 μm) were cut using a McIlwain tissue chopper and wedges were prepared as reported by Burton and colleagues (Burton et al., 1987). The cortical wedges were placed in a two-compartment bath with a grease seal isolating the grey cortical matter from the callosum. The bath was a modification of that described by Harrison and Simmonds (1985), in that the callosal side of the preparation was maintained in a static pool of normal aCSF. The cortical side was perfused independently with gassed (95% O_2/5% CO_2) aCSF at 2 ml/min at room temperature (20–22° C) for 60–90 min to allow the slices to equilibrate. Perfusion of the cortical side was then continued with Mg^{2+}-free aCSF to facilitate NMDA receptor activation. Drugs were applied only to the cortical side of the preparation.

The difference in membrane potential between the two compartments was continuously monitored via Ag/AgCl electrodes, amplified (Flyde 2601A), filtered, and displayed on a BBC Goerz-Metrawatt chart recorder. The spontaneous activity was also recorded on a MacLab computer system (AD Instruments, Hastings, UK) and the quantification of these epileptiform events was carried out by counting both the number of spontaneous depolarizations and the number of afterpotentials per burst.

FPL 12495AA was routinely perfused for 15 min followed by reperfusion with Mg^{2+}-free aCSF. In order to examine the effect of FPL 12495AA on NMDA-or AMPA-induced depolarizations, perfusion at varying concentrations of FPL 12495AA was carried out 10 min prior to and during NMDA or AMPA administration. NMDA was perfused for 2 min and all responses were normalised to the mean of 3–4 reproducible applications of NMDA (10 μM) at the beginning of each experiment. Owing to the prolonged duration of action of FPL 12495AA the concentration-response curves for NMDA (2 min application) were constructed in the presence of varying concentrations of FPL 12495AA throughout.

Normal aCSF had the following composition (in

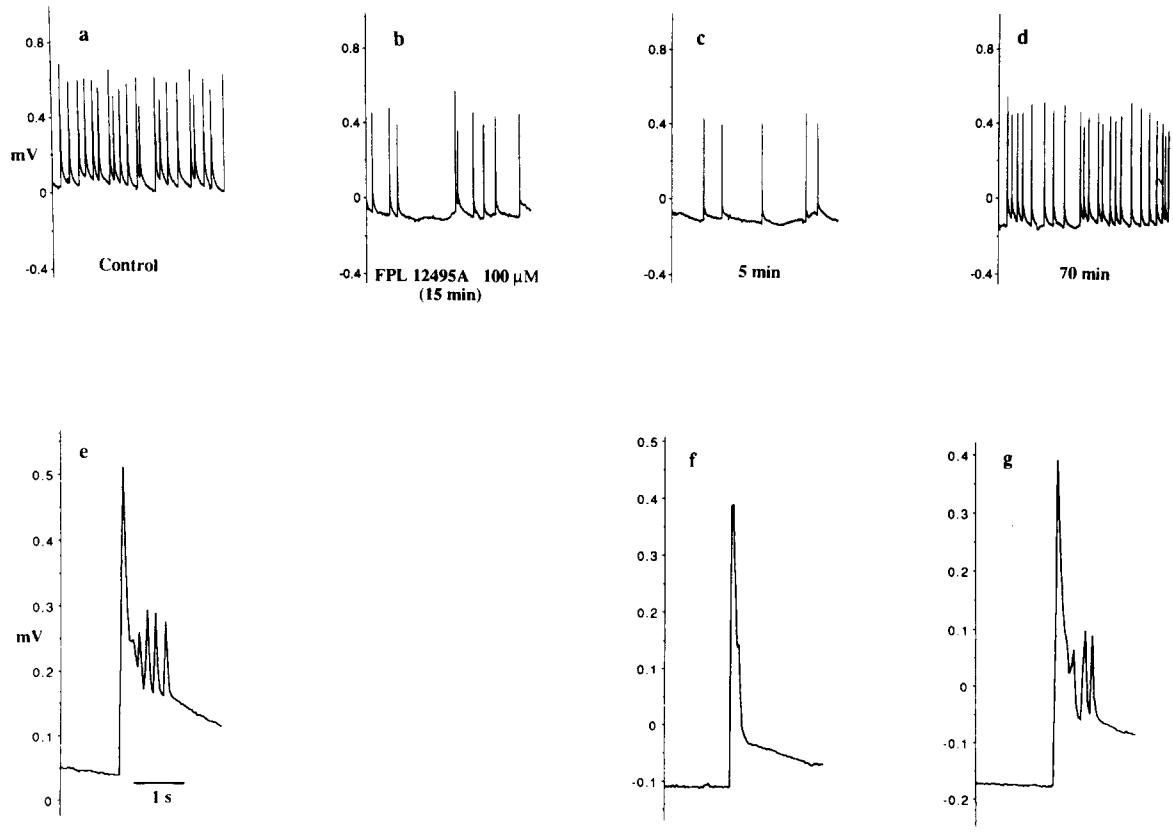

Fig. 1. The effect of FPL 12495AA on spontaneous depolarizations in a cortical wedge prepared from an audiogenic seizure-prone DBA/2 mouse (a,b,c,d). The top records are 5 min recordings before, during perfusion of FPL 12495AA (100 μM), and at 5 and 70 min after perfusion. The lower records (e,f,g) are of individual spontaneous depolarizations (SD) showing the afterpotentials associated with the SD for control, 5 and 70 min following FPL 12495AA.

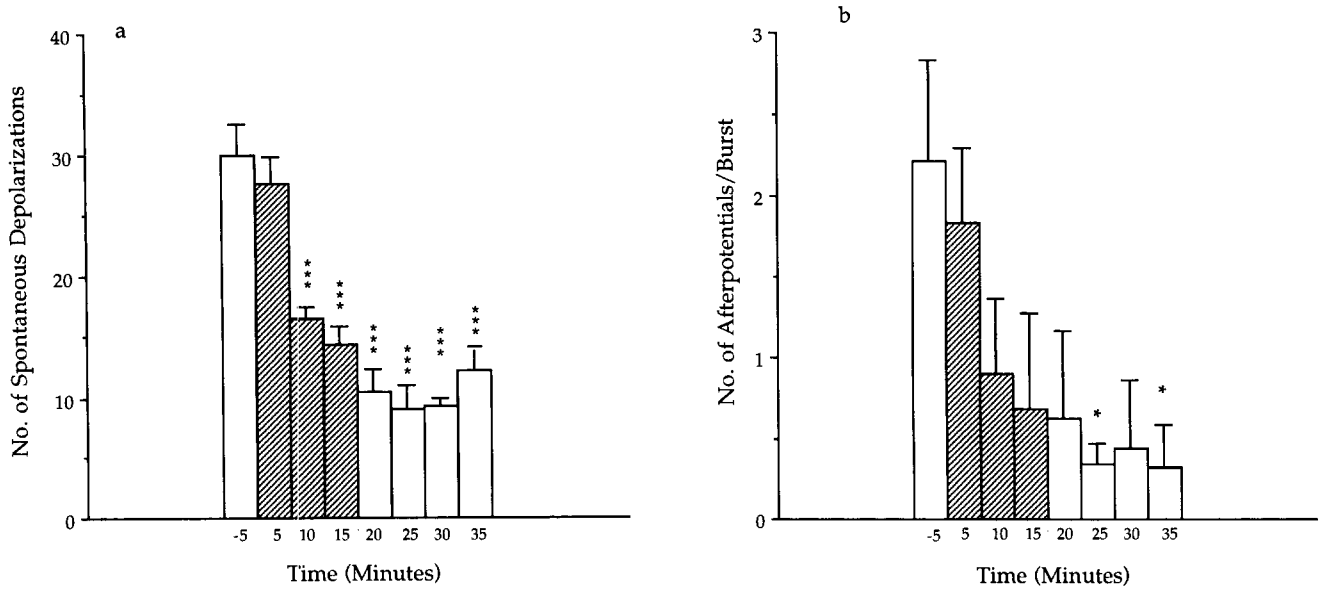

Fig. 2. The effect of FPL 12495AA (200 μM) perfused for 15 min (5–15; hatched columns) on (a) the mean number of spontaneous depolarizations in 5 min epochs recorded from DBA/2 cortical wedges and (b) on the mean number of afterpotentials per burst. Mean ± S.E.M.; * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, $n = 5–16$.

mM): NaCl 124; KCl 5; NaH_2PO 1.25; $MgSO_4$ 2; $CaCl_2$ 2; $NaHCO_3$ 26; glucose 10; pH 7.3–7.4. For Mg^{2+}-free aCSF a corresponding increase in NaCl concentration was made.

2.1. Statistics

The data were analyzed by one-way analysis of variance (ANOVA) followed by Student's-Newman-Keuls multiple comparison test.

3. Results

Ninety percent of the slices (81 out of 90) exhibited spontaneous depolarizations when perfused with Mg^{2+}-free aCSF, and the majority of them showed afterpotentials (Fig. 1). Spontaneous depolarizations were also seen during perfusion with normal aCSF (2 mM Mg^{2+}) in 28% of these slices. The frequency of the spontaneous depolarizations in Mg^{2+}-free aCSF varied in the range 3–12/min and were relatively fast in character with rise times of 90–120 ms and a duration of 1–5 s (Fig. 1). These depolarizations increased in frequency over the first 1–2 h following perfusion with Mg^{2+}-free aCSF but were then stable for 5–6 h with no significant change in amplitude. The afterpotentials, when present, varied between 1–8 on each depolarization (Fig. 1e).

FPL 12495AA at 100 (Fig. 1) and 200 μM (Fig. 2a) significantly decreased the frequency of spontaneous depolarizations with an IC_{50} value of 102 μM; lower concentrations (12.5, 25 and 50 μM; Table 1) had no significant effect. The reduction was seen within 10 min with 100 μM and 200 μM and responses returned to control levels within 30–60 min at 100 μM (Fig. 1),

Table 1
The effect of FPL 12494AA on spontaneous depolarizations and afterpotentials/burst as a percentage of baseline responses

	Spontaneous depolarizations				Afterpotentials/burst			
	n	Mean	S.E.M.	*P*	*n*	Mean	S.E.M.	*P*
Control	13	114.4	4.4		10	112.1	21.2	
FPL 12495								
12.5 μM	13	101.1	7.9	ns	10	126.0	22.4	ns
25 μM	11	93.3	5.3	ns	8	108.4	24.9	ns
50 μM	17	98.0	6.3	ns	16	65.2	10.2	ns
100 μM	10	56.5	7.5	< 0.001	7	9.3	6.0	< 0.01
200 μM	6	32.0	7.9	< 0.001	5	9.4	7.2	< 0.01

The effect of FPL 12495AA was calculated on the number of spontaneous depolarizations occurring between 15 and 20 min following perfusion compared to the number occurring in 5 min immediately before perfusion. ANOVA followed Student's-Newman-Keuls multiple comparison test. ns = not significant.

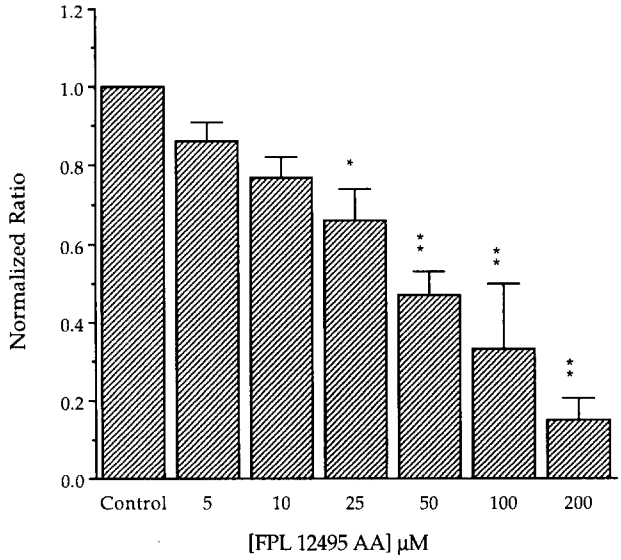

Fig. 3. The effect of varying concentrations of FPL 12495AA on NMDA-induced (10 μM) depolarizations. Responses were normalised to reproducible applications (3–4) of NMDA (10 μM) for standardization. Mean ± S.E.M.; $^{*}P < 0.05$, $^{**}P < 0.01$; $n = 5$–7.

but recovery from 200 μM took 2–3 h. The number of afterpotentials per depolarization (Figs. 1 and 2b) was greatly reduced by FPL 12495AA at 50, 100 and 200 μM with IC_{50} values of 50 and 59 μM respectively (Table 1). Recovery of the afterpotentials was delayed when compared with the recovery of the spontaneous depolarizations.

FPL 12495AA (25, 50, 100 and 200 μM) significantly reduced depolarizations induced by 10 μM NMDA in a concentration-dependent manner with an IC_{50} value of 43 μM (Fig. 3). Recovery of the response to NMDA (10 μM) following perfusion for 15 min with 100 μM FPL 12495AA took approximately 60 min (Fig. 4a). NMDA concentration-response curves (2.5–100 μM) in the presence of varying concentrations (5–100 μM) of FPL 12495AA were shifted to the right (Fig. 5). FPL 12495AA at concentrations up to 100 μM had no effect on AMPA-induced (5 μM; $n = 7$) depolarizations (Fig. 4b).

4. Discussion

The results presented in this paper show that FPL 12495AA significantly reduced the frequency of spontaneous depolarizations and the number of afterpotentials associated with these depolarizations. The compound also reduced NMDA-induced depolarizations but had no effect on AMPA-induced responses.

Drugs with an antagonistic action on the various binding sites associated with the NMDA-receptor complex exhibit significant anti-convulsant properties in a number of models of epilepsy. Antagonists at the endogenous ligand recognition site of the complex, such as 3-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (CPP), have been shown to block epileptiform activity in rat cortical slices (Aram et al., 1989) and also to protect against photically induced myoclonus in baboons (Meldrum, 1992). Antagonists at the other sites on the NMDA receptor complex such as 7-chlorokynurenic acid at the strychnine-insensitive glycine site (Croucher and Bradford, 1990), and the open channel blockers dizocilpine and phencyclidine, also exhibit anticonvulsant properties (Kemp et al., 1986). Furthermore, the selective non-NMDA receptor antagonists, such as the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo[*F*]quinoxaline (NBQX), has been reported to exert anticonvulsant activity in animal models of generalised epilepsy (Chapman and Meldrum, 1993).

The data presented in this paper support a specific antagonistic action for FPL 12495AA on the NMDA receptor complex as opposed to an action at AMPA receptors. At a concentration of 100 μM FPL 12495AA significantly reduced NMDA-induced depolarization, whereas there was no effect on AMPA-induced depolarization, although the frequency of spontaneous depolarizations in these preparations was decreased by FPL 12495AA.

DBA/2 mice are genetically epilepsy-prone and the behaviour is age-related in that mice of 20–30 days of age are far more susceptible than younger or older animals. In our colony, 96% of the mice at this age respond to a 110 dB sound with characteristic wild-running and tonic/clonic convulsions. As can be seen from the present paper, 90% of the slices prepared from animals aged between 20–30 days exhibit spontaneous depolarizations in magnesium-free aCSF. There is accumulating evidence that NMDA receptors participate in the initiation and/or propagation of epileptiform discharges (Dingledine et al., 1990; Horne et al., 1986). A wide range of compounds, with potential anticonvulsant activity, acting at the NMDA recognition site or the phencyclidine site on the receptor complex, as well as sigma ligands and barbiturates, have been shown to reduce burst frequency, and particularly the number of afterpotentials, when tested on rat cortical wedges (Aram et al., 1989; Horne et al., 1986; Palmer et al., 1992c). The reduction in the frequency of spontaneous depolarizations, and in the number of afterpotentials, suggests that the NMDA receptor is involved in these epileptiform events in this in vitro preparation. The decrease in the number of spontaneous depolarizations and afterpotentials seen with FPL 12495AA would thus suggest that its anticonvulsant activity is probably due to an effect on NMDA-mediated transmission. However, the IC_{50} results obtained in these present experiments, 102 μM and 50 μM for spontaneous depolarizations and after-

potentials respectively, are higher when compared with plasma levels of 750 nM obtained in human volunteers and thus another mechanism may be involved in the anticonvulsant action of the drug.

It is not possible from the results of the present experiments to ascertain the site of action on the NMDA-receptor complex of FPL 12495AA, as drugs acting on a number of different sites would be capable of decreasing both spontaneous and NMDA-induced depolarizations. However, inspection of the NMDA concentration-response curves in the presence of FPL 12495AA would imply that the antagonism was non-competitive, as increasing concentrations of NMDA did not surmount the antagonism with the response to the highest concentration of NMDA being less than the preceding response in the presence of 5, 25 and 50 μM FPL 12495AA. There is evidence that compounds acting within the channel exhibit non-competitive inhibition (Kemp et al., 1986) and a similar action may account for the effects of FPL 12495AA. Binding studies have shown that FPL 12495AA has a high affinity for NMDA receptors and that it displaces dizocilpine binding at sub-micromolar concentrations (Ray et al., 1991). It has lower affinity for the endogenous ligand recognition site, the glycine site, and the polyamine site on the NMDA complex, which suggests that FPL 12495AA interacts within the channel at the dizocilpine/phencyclidine site (Palmer et al., 1992b).

In summary, FPL 12495AA specifically reduced NMDA-mediated effects on the cortical wedge preparation at μM concentrations and this inhibition was relatively long-lasting. A non-competitive antagonistic action at the phencyclidine site of the NMDA receptor-operated cationic channel could account both for

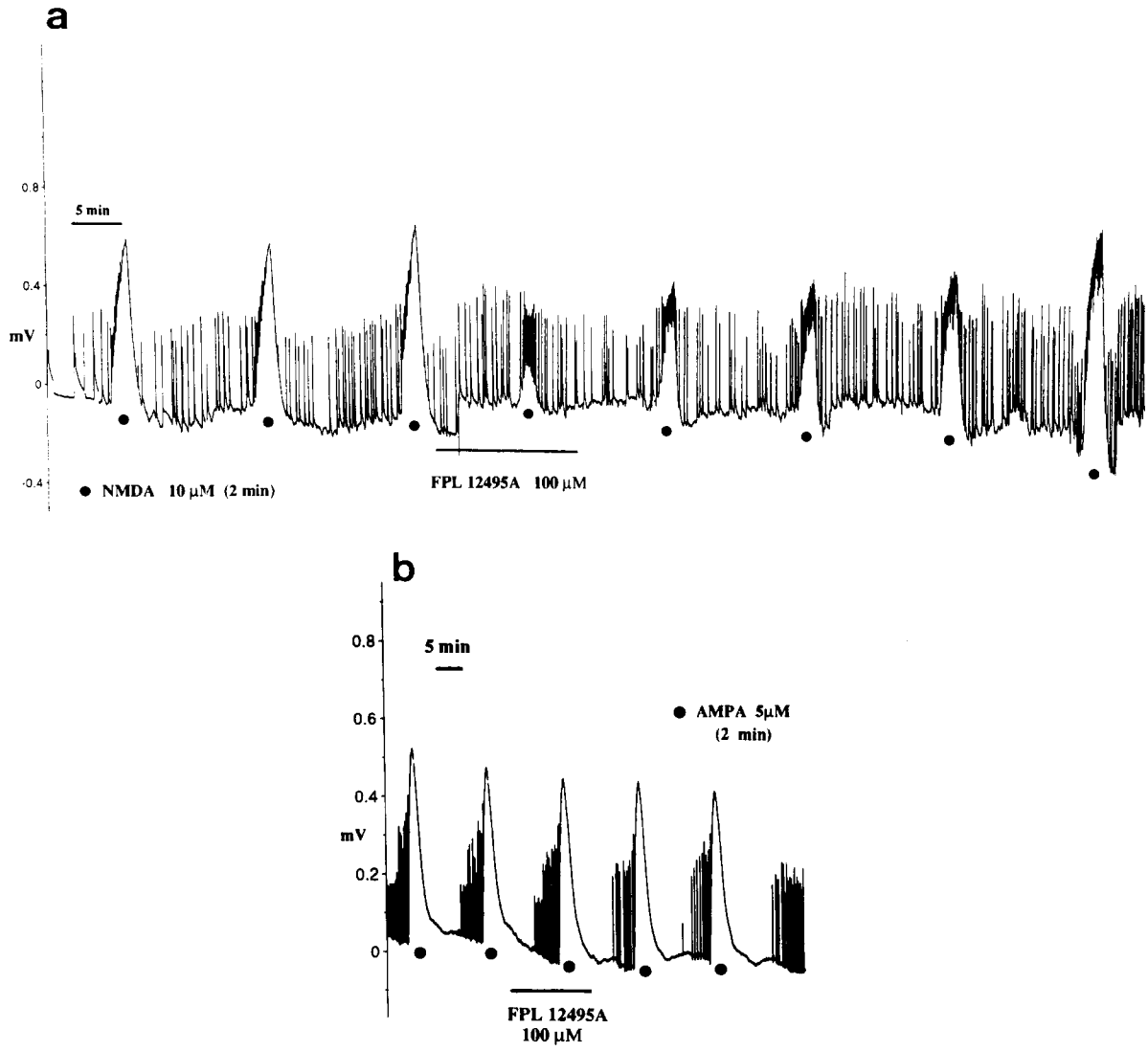

Fig. 4. Continuous records showing the effect of 100 μM FPL 12495AA perfused for 15 min on (a) NMDA-induced (10 μM) depolarizations and (b) on AMPA-induced (5 μM) depolarizations. Note the decrease in frequency of the spontaneous depolarizations following FPL 12495AA in (b).

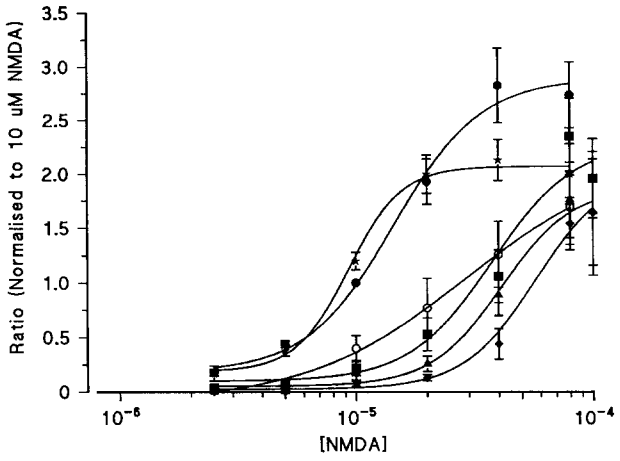

Fig. 5. Concentration-response curves for FPL 12495AA in the presence of varying concentrations of NMDA perfused for 2 min. FPL 12495AA was perfused throughout. The NMDA-induced depolarizations were normalised to repetitive applications of 10 μM NMDA. (●) Control; (⋆) 5 μM; (○) 10 μM; (■) 25 μM; (▲) 50 μM; (◆) 100 μM FPL 12495AA. Mean ± S.E.M.; $n = 6$–8.

the effects reported in this paper and also the anticonvulsant activity shown by this compound.

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