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Effect of low-affinity NMDA receptor antagonists on electrical activity in mouse cortical slices

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Abstract

The objective of this study was to investigate the effects of three low-affinity NMDA receptor antagonists, MRZ 2/279 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane HCl), AR-R 15896AR ([+]-α-phenyl-2-pyridine-ethanamine diHCl) and dextromethorphan on epileptiform activity in vitro. Epileptiform discharges were elicited in DBA/2 mouse cortical slices by perfusion with Mg^{2+}-free artificial cerebrospinal fluid. MRZ 2/279, AR-R 15896AR and dextromethorphan all reversibly decreased the frequency of the discharges in a concentration-dependent manner. The IC_{50}'s for MRZ 2/279, AR-R 15896AR and dextromethorphan were 5.2, 10.8 and 55.9 μM, respectively. These low-affinity NMDA receptor antagonists may be proved to be clinically effective with fewer adverse effects than drugs with high-affinity for the NMDA receptor-operated channel.

Keywords: Cortical slice; Epileptiform activity; NMDA receptor antagonist, low-affinity; Mg^{2+}, low

1. Introduction

The NMDA sub-type of glutamate receptor has a number of binding sites at which potential therapeutic agents may interact (Wong and Kemp, 1991). One of these sites is deep within the channel of the receptor complex and has been designated the phencyclidine (PCP) site following the observation that the psychotomimetic binds avidly in this location. Besides PCP, a number of other compounds bind uncompetitively, with high-affinity, at this site to block the channel, notably dizocilpine and ketamine (Iversen and Kemp, 1994). This blockade is both use- and voltage-dependent (Huettner and Bean, 1988). The high-affinity binding is correlated with serious behavioural side effects which limit the therapeutic use of such compounds (Kornhuber and Weller, 1997).

Low-affinity, uncompetitive open-channel blocking agents exhibit rapid blocking kinetics as well as use-dependent block with strong voltage dependency (Parsons et al., 1999). The drugs under investigation addressed in this paper are three, low-affinity uncompetitive antagonists, 1-amino-1,3,3,5,5-pentamethyl-cyclohexane HCl (MRZ 2/279; Neramexane), [+]-α-phenyl-2-pyridine-ethanamine diHCl (AR-R 15896AR) and dextromethorphan. MRZ 2/279 has been shown to possess anticonvulsant activity in the mouse maximal electroshock test and to be neuroprotective in glutamate-induced excitotoxicity in cultured neurones (Parsons et al., 1999). Similarly, AR-R 15896AR has been shown to be neuroprotective in animal models of cerebral ischaemia (Palmer et al., 1997), to be effective against NMDA-induced toxicity in cortical neurones and to decrease Ca^{2+} influx (Black et al., 1995). This latter effect was seen to occur more rapidly than with the high-affinity antagonists PCP and dizocilpine (Black et al., 1995). Dextromethorphan has similar effects to the other two compounds in that it protects against focal cerebral ischaemia (Steinberg et al., 1988), blocks NMDA-induced epileptiform activity in cortical slices (Aram et al., 1989; Apland and Braitman, 1990) and inhibits NMDA-induced convulsions (Ferkany et al., 1988).

The purpose of this present study was to investigate the effects of MRZ 2/279, AR-R 15896AR and dextromethorphan on epileptiform activity elicited in mouse cortical slices on perfusion with Mg^{2+}-free artificial cerebrospinal fluid.

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2. Materials and methods

2.1. Preparation of cortical wedges

Male or female DBA/2 mice aged 21–45 days, bred from the colony at the University of Wales College of Medicine, were killed by cervical dislocation followed by decapitation. The brain was quickly removed and placed in ice-cold gassed (95% O_2/5% CO_2) normal artificial cerebrospinal fluid (aCSF). Coronal slices (500 μm) were cut using a MacIlwain tissue chopper and the slices transferred to aCSF in a Petri dish. Wedges, approximately 3 mm wide at the pial surface and 2 mm at the striatum were prepared containing cortex, callosum and striatum. Two-compartment tissue baths were used with the static pool compartment, containing a portion of striatum and the callosum, filled with aCSF and the second compartment, containing the cortex was perfused at 2 ml/min with gassed aCSF at room temperature. The tissue was left to equilibrate for 60–90 min in normal aCSF and then the cortex was perfused with magnesium-free aCSF (Mg^{2+}-free aCSF) to elicit spontaneous depolarizations. The tissue was again allowed 90 min for the spontaneous depolarisations to reach a stable frequency before perfusing the drugs under investigation.

Composition of aCSF (mM): NaCl 124, KCl 5, NaH_2PO 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 2, Glucose 10. For Mg^{2+}-free aCSF a corresponding increase in NaCl concentration was made to maintain osmolarity.

2.2. Electrical recording

Sliver/silver chloride electrodes were used to measure the extracellular field potential difference between the compartments of the bath. The output was filtered, amplified and displayed on a chart recorder and also stored on a Maclab computer system. Spontaneous activity was measured by counting the number of depolarisations in 5-min epochs throughout the course of the experiment and then calculated as a percentage of the frequency in five-minute period before drug application in order to normalise the data. In the majority of the experiments, recording continued until the frequency of the depolarizations returned to control values.

2.3. Drugs

The drugs were dissolved in Mg^{2+}-free aCSF at their final concentrations and applied only to the cortical side of the tissue at 2 ml/min. Dextromethorphan (10, 20, 50, 100 and 200 μM) and AR-R 15896AR (1, 5, 10 and 20 μM) were applied for 15 min whilst MRZ 2/579 (1, 5, 10 and 20 μM) was applied for 1 h. Dextromethorphan was obtained from Sigma, AR-R 15896AR was a gift from AstraZeneca and MRZ 2/579 a gift from Merz+Co.

2.4. Statistical analysis

The maximum percentage inhibition for each drug concentration was taken and used to calculate the IC_{50}'s for the drugs. One-way analysis of the variance (ANOVA) followed by Dunnett's post hoc test was used to assess the significance of the drug effects from control. The IC_{50}'s in this paper are given with 95% confidence intervals.

3. Results

Perfusion of Mg^{2+}-free aCSF resulted in the onset of epileptiform activity within 5–10 min and the frequency increased gradually until stability was reached after 30–45 min. The frequency of the depolarizations in the 5-min epoch prior to perfusion of the drug solutions was 32 ± 6.3 ($n = 47$). In control experiments, the frequency of the depolarizations did not change significantly over 4 h ($n = 5$). The depolarizations had a rise time of approximately

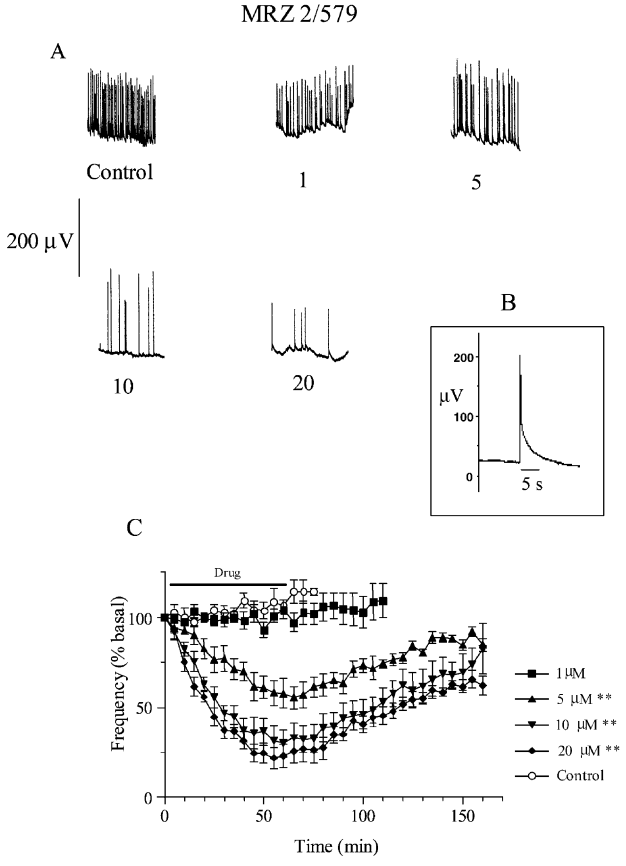

Fig. 1. (A) The effect of micromolar concentrations of MRZ 2/579 on spontaneous depolarizations elicited from mouse cortical wedges on perfusion with Mg^{2+}-free aCSF, each panel represents 5 min of recording after 60 min of drug perfusion. (B) A single depolarization. (C) Time course effects of varying concentrations of MRZ 2/579 perfused for 60 min. Mean ± S.E.M., one-way ANOVA followed by Dunnett's post hoc test, ** $P < 0.01$, $n = 6$.

90–120 ms followed by a slower repolarization phase; the total duration of each event was between 1 and 4 s (Fig. 1B).

The effect of MRZ 2/279 on the frequency of the spontaneous depolarizations was relatively slow in onset which accounted for the perfusion time of 1 h. This compound had a concentration-dependent inhibitory effect on the frequency of the depolarizations with 5, 10 and 20 μM significantly decreasing the frequency ($P<0.01$ for all concentrations, $n=6$, Fig. 1A and C). The IC_{50} for MRZ 2/279 was 5.2 μM (4.0–6.7; Fig. 4). The amplitude of the depolarizations was not reduced except by the highest concentration (20 μM) of MRZ 2/279 (Fig. 1A) and the frequency of the responses did not recover to control levels.

AR-R 15896AR also decreased the frequency of the depolarizations in a concentration-dependent manner with

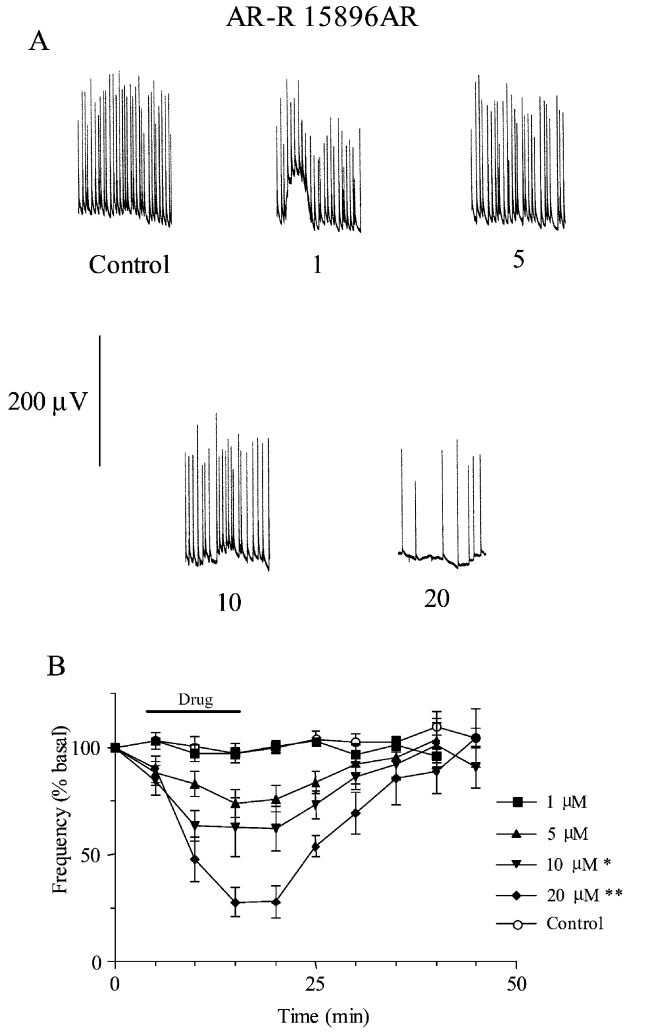

Fig. 2. (A) The effect of micromolar concentrations of AR-R 15896AR on spontaneous depolarizations elicited from mouse cortical wedges on perfusion with Mg^{2+}-free aCSF, each panel represents 5 min of recording after 15 min of drug perfusion. (B) Time course effects of varying concentrations of AR-R 15896AR perfused for 15 min. Mean ± S.E.M., one-way ANOVA followed by Dunnett's post hoc test, * $P<0.05$, ** $P<0.01$, $n=5–6$.

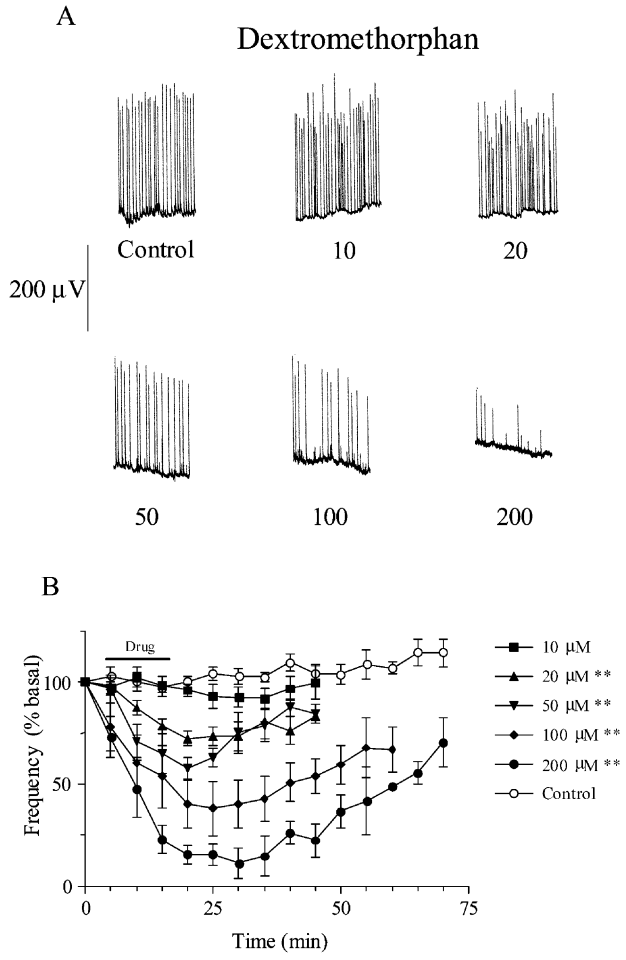

Fig. 3. (A) The effect of micromolar concentrations of dextromethorphan on spontaneous depolarizations elicited from mouse cortical wedges on perfusion with Mg^{2+}-free aCSF, each panel represents 5 min of recording after 15 min of drug perfusion. (B) Time course effects of varying concentrations of dextromethorphan perfused for 15 min. Mean ± S.E.M., one-way ANOVA followed by Dunnett's post hoc test, ** $P<0.01$, $n=5–7$.

10 and 20 μM being significantly different from control ($P<0.05$ and 0.01, respectively, $n=5$, Fig. 2A and B). However, maximum effects were seen within 15 min at all

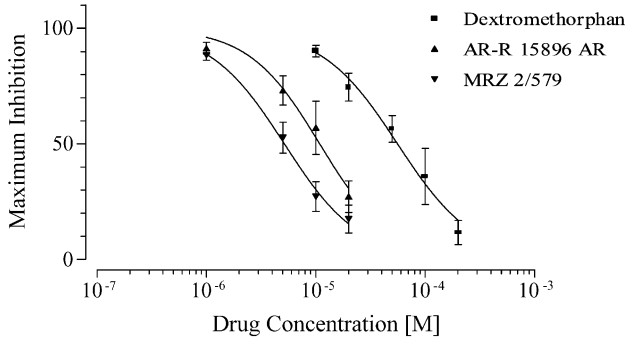

Fig. 4. Inhibitory concentration curves for MRZ 2/579, AR-R 15896AR and dextromethorphan on maximum inhibition of spontaneous depolarizations. Mean ± S.E.M., $n=5–7$.

concentrations and recovery was complete after 45 min (Fig. 2B). The IC_{50} for AR-R 15896AR was 10.8 μM (8.0–14.7; Fig. 4).

Dextromethorphan at 20, 50 100 and 200 μM significantly decreased the frequency of the depolarizations ($P < 0.01$ for all concentrations, $n = 5–7$, Fig. 3A and B). Recovery was attained after 45 min with 20 and 50 μM but not with the higher concentrations. The amplitude of the responses was also decreased with 200 μM. The IC_{50} for dextromethorphan was 55.9 μM (42.7–73.0; Fig. 4).

4. Discussion

In this study, we have compared the effects of three low-affinity NMDA receptor antagonists on epileptiform activity in cortical wedges. All three compounds reversibly decreased the frequency of the depolarizations with the potency being in the order MRZ 2/279 < AR-R 15896AR < dextromethorphan.

Removal of Mg^{2+} from the perfusion medium elicits spontaneous events from a variety of in vitro slice preparations, for example rat hippocampal slices (Armand et al., 2000), rat cortical slices (Apland and Braitman, 1990) and DBA/2 mouse cortical wedges (Hu and Davies, 1997). These spontaneous depolarizations are considered to be a valid model for use in the evaluation of compounds interacting with the NMDA receptor-operated complex with a view to their being used either as anticonvulsant drugs or as neuroprotectants (Aram and Lodge, 1988). These spontaneous depolarizations have been shown to be susceptible to the anticonvulsants, *S*(+)-losigamone (Jones and Davies, 1999) and retiagabine (Armand et al., 2000) and the anaesthetic/neuroprotectant, ketamine (Hu and Davies, 1997). Whilst previous studies with dextromethorphan on guinea pig cortical or hippocampal slices showed that this compound also decreased spontaneous epileptiform activity (Wong et al., 1988; Apland and Braitman, 1990).

The onset and duration of action of the three compounds under investigation in this paper showed considerable differences. MRZ 2/279 was perfused for 1 h as it is a highly lipophilic compound and previous studies using cortical slices have required long incubation periods and consequently delayed recovery following washout (C.G. Parsons, personal communication). In our experiments, 5, 10 and 20 μM MRZ 2/579 significantly decreased the frequency of the depolarizations within 10–20 min, however, at these concentrations recovery to baseline frequency did not occur within 150 min. The onset of inhibition with AR-R 15896AR and dextromethorphan was more rapid than with MRZ 2/579 and, especially with the lower concentrations, recovery was complete within 1 h. Whilst one cannot equate the three compounds, owing to the varying perfusion times, there is evidence that the degree of trapping of these compounds, within the NMDA channel varies markedly. AR-R 15896AR block was reduced to 54% 120 s after washout (Mealing et al., 1997) whereas there was no reduction in the degree of blocking 120 s after washout of MRZ 2/579 (Parsons et al., 1999), both these studies being on cultured cortical neurones. Recovery of spontaneous bursting after dextromethorphan occurred after 75 min in rat hippocampal slices (Apland and Braitman, 1990).

The IC_{50}'s for the three compounds in this present investigation are in broad agreement with published data in that MRZ 2/579 < AR-R 15896AR < dextromethorphan. The IC_{50} for MRZ 2/579 on NMDA-induced currents in cultured neurones was 1.29 μM and against glutamate-induced toxicity was 2.16 μM (Parsons et al., 1999) and in a similar experiment AR-R 15896AR gave an IC_{50} of 9.8 μM (Mealing et al., 1997); whilst the EC_{50} for dextromethorphan was 18 μM on spontaneous epileptiform activity in hippocampal slices (Apland and Braitman, 1990).

The action of these three compounds in reducing the frequency of the spontaneous depolarizations can be attributed to blockade of Ca^{2+} entry through the NMDA-receptor-operated channel. A similar, more persistent, action could account for the reduction in amplitude with the highest concentrations of MRZ 2/579 and dextromethorphan. Low affinity, uncompetitive open-channel blocking agents exhibit rapid kinetics as well as use-dependent block with strong voltage dependency. This rapid voltage-dependent block allows the drug to leave the channel once glutamate occupies its recognition site on the synaptic aspect of the receptor complex and would result in rapid, preferential blockade at high levels of sustained receptor excitation (Rogawski, 1993). The corollary to this being that at normal, physiological levels of synaptic activity there would be relatively little block as the compounds would rapidly leave the channel due to their low-affinity for the binding site. These compounds have a potential therapeutic role in conditions such as epilepsy and cerebral ischaemia where there is excessive synaptic glutamate.

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