

Protection against post-ischaemic neuronal loss in gerbil hippocampal CA1 by glycine_B and AMPA antagonists

Short Communication

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Summary. Novel antagonists of the glycine_B site of the NMDA receptor (MRZ 2/570, MRZ 2/576), and an AMPA receptor antagonist, NBQX were tested in 3-min. global ischaemia in gerbils. Untreated animals showed after 14 days a loss of almost 90% of pyramidal neurones in the CA1 region, which was prevented by NBQX, and reduced to 50% by both glycine_B antagonists. NBQX produced a delayed, long lasting (up to 24hr) hypothermia while hypothermia with both glycine_B antagonists was transient.

Keywords: Forebrain ischaemia, gerbil, glycine_B antagonists, MRZ 2/570, MRZ 2/576, NBQX, hippocampus.

Introduction

NMDA receptor antagonists provide clear neuroprotection in focal models of brain ischaemia (Park et al., 1988). However, in models of global/forebrain ischaemia. e.g. in Mongolian gerbils, the protective effect of the uncompetitive NMDA receptor antagonist MK-801 (Gill et al., 1988) appeared to be dependent on postischemic hypothermia (Buchan and Pulsinelli, 1990; Lazarewicz et al., 1994).

Apart from glutamate, a more prolonged, but relatively modest increase in the concentration of extracellular glycine has been observed under ischemic conditions (Globus et al., 1991). Therefore, antagonists of the glycine site of the NMDA receptor (glycine_B) may offer an alternative neuroprotective approach. Unfortunately, little is known about the therapeutic potential of such compounds due to poor bioavailability of most glycine_B antagonists (Wood et al., 1992). Recently, Pellegrini-Giampietro and colleagues (1994) reported that the glycine_B antagonist, 7-chlorothiokynurenic acid is neuro-

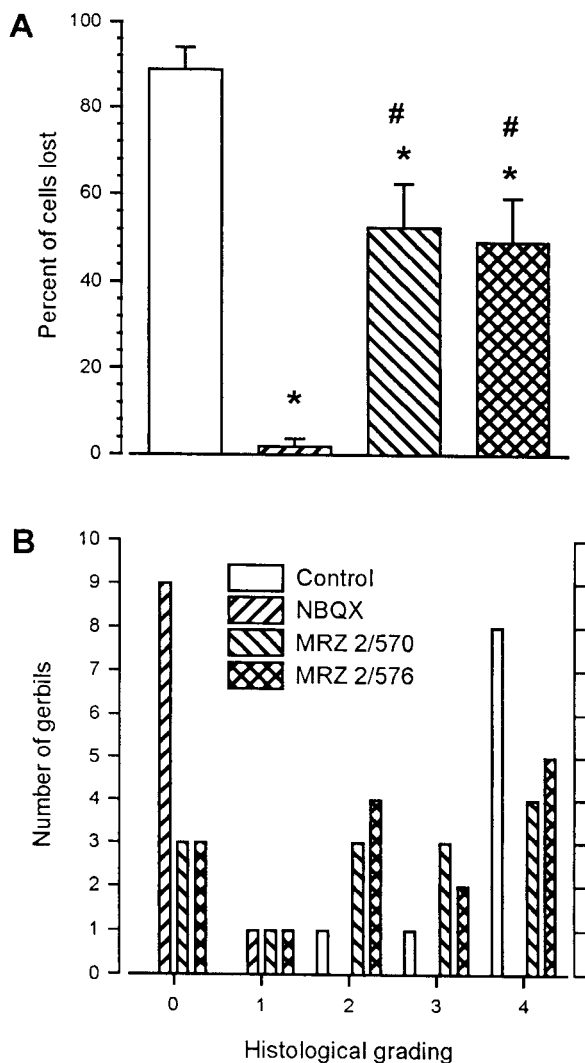


Fig. 1. Effect of post-treatment with NBQX, MRZ 2/570 and MRZ 2/576 on histological damage in the CA1 area of the gerbil hippocampus 14 days after 3-min forebrain ischaemia. Results are expressed as per cent cell loss (**A**) or as the number of rats showing respective grading (**B**). * $p < 0.05$ vs. control. # $p < 0.05$ vs. NBQX (Dunnett's test). $N = 10, 14, 14$ and 10 respectively

protective in global ischaemia in gerbils. Recently a series of novel glycine_B antagonists, tricyclic pyridino-phthalazindiones, which are moderately potent *in vitro*, but potent *in vivo*, has been characterised (Parsons et al., 1997).

The aim of this study was to assess the neuroprotective potential of two of these tricyclic pyrido-phthalazindione glycine_B antagonists, MRZ 2/570 and MRZ 2/576 using an AMPA receptor antagonist, NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)qui-noxaline), as a reference agent (Buchan et al., 1991; Frank et al., 1993; Sheardown et al., 1990).

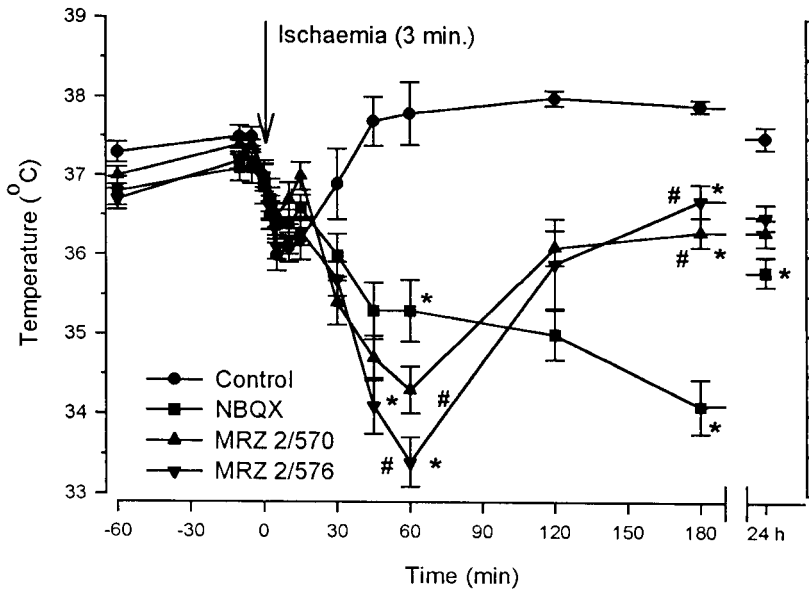


Fig. 2. Effect of NBQX, MRZ 2/570 and MRZ 2/576 on rectal body temperature of gerbils after 3-min forebrain ischaemia. Results are expressed as means \pm S.E. For clarity indications of statistical differences are given only for 60 min, 180 min and 24 hr. * $p < 0.05$ vs. control. # $p < 0.05$ vs. NBQX (Dunnett's test). $N = 10, 14, 15$ and 10 respectively

Materials and methods

Male Mongolian gerbils (*Meriones unguiculatus*, $n = 50$, 60–80 g), obtained from Centre d'élevage R. Janvier, Le Genest-St-Isle, France) were anaesthetised with 2% halothane in O_2 and N_2O (3:7), and bilateral carotid occlusion was induced for 3 min. as described previously (Łazarewicz et al., 1994). Animals were warmed during the whole surgical procedure and rectal temperature was measured before, during and for up to 3 h after carotid occlusion, and additionally 24 h later, with a thermistor (ELLAB A/S, Denmark). After ischaemia, animals were kept for 3 h at room temperature (20–23°C). The experimental groups were treated i.p 15, 30 and 45 min after ischaemia with 30 mg/kg of MRZ 2/570 ($n = 15$), MRZ 2/576 ($n = 14$) NBQX ($n = 10$), or vehicle ($n = 10$), (Merz + Co, Frankfurt/M, Germany). 14 days after ischaemia, histopathological scoring was performed as described previously (Łazarewicz et al., 1994). Thereafter grades of damage for each animal were determined, assuming 0 = no damage, 1 = up to 30% of necrotic cells, 2 = 31% to 50% of necrotic cells, 3 = 51% to 70% of necrotic cells, 4 = 71% to 100% of necrotic cells.

Temperature, and per cent of cell loss data, expressed as mean \pm S.E., were analysed by Two Way ANOVA followed by the Dunnett test. Histological grading was analysed by Kruskal-Wallis ANOVA on ranks followed by Dunn's test.

Results

Treatment with NBQX almost completely prevented neuronal damage, whereas the glycine_B antagonists: MRZ 2/570 and MRZ 2/576 reduced damage to approximately 50% (Fig. 1A). Also histological grading (Fig. 1B)

Sheardown MJ, Nielsen EO, Hansen AJ, Jacobsen P, Honore T (1990) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline – a neuroprotectant for cerebral ischemia. *Science* 247: 571–574

Wood ER, Bussey TJ, Phillips AG (1992) A glycine antagonist reduces ischemia-induced CA1 cell loss in vivo. *Neurosci Lett* 145: 10–14

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