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Neuroprotection by novel antagonists at the NMDA receptor channel and glycine$_B$ sites

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Abstract

Glutamate may act via an *N*-methyl-D-Aspartate (NMDA)-sensitive receptor site to destroy cholinergic neurons within the nucleus basalis magnocellularis in age-associated neurodegenerative diseases. Multiple interesting properties of the NMDA receptor are relevant to its excitotoxic actions, e.g., glutamate is ineffective unless a glycine (gly) modulatory site is also occupied. Thus, the antagonism of glutamate receptor-related toxicity by blockade of either the NMDA-sensitive recognition site or the gly binding site may therefore have therapeutic applications. The current study investigated the ability of four novel noncompetitive antagonists at these two sites: one NMDA open channel antagonist (MRZ 2/579: 1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride), and three gly$_B$ receptor antagonists (MRZ 2/570: 8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridaziono [4,5-β] quinoline-5-oxide choline salt; MRZ 2/57: 8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridaziono [4,5-β] quinoline-5-oxide choline; MRZ 2/576: 8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridaziono [4,5-β] quinoline-5-oxide choline) administered acutely, to provide neuroprotection from a NMDA receptor agonist within the nucleus basalis magnocellularis of young rats. Injection of NMDA into the nucleus basalis magnocellularis significantly decreased cortical choline acetyltransferase activity. Acute administration (i.p.) of MRZ 2/579, 2/570, 2/571 and 2/576 provided significant neuroprotection from NMDA. © 1998 Elsevier Science B.V.

Keywords: Nucleus basalis magnocellularis; Acetylcholine; Neuroprotection; Glycine$_B$ receptor site

1. Introduction

The acidic amino acid glutamate may be the primary excitatory synaptic transmitter in the brain. Glutamate may act via three different types of ionophore-coupled receptors that have been named for the agonists that can stimulate them: *N*-methyl-D-Aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate (Watkins and Evans, 1981). Multiple interesting properties of the NMDA receptor are relevant to its excitotoxic and physiological actions. For example, glutamate is ineffective at its receptor site unless a glycine$_B$ (gly$_B$) modulatory site is also occupied (Kleckner and Dingledine, 1988). In addition, the ion channel is blocked in a voltage-dependent manner by Mg^{2+} (Nowak et al., 1984). Therefore, activation of the channel is dependent upon binding of the appropriate agonists at the gly$_B$ and NMDA receptor sites and on the postsynaptic membrane potential. Opening of the NMDA channel allows an influx of Ca^{2+} that is believed to be an important intracellular signal that dose-dependently initiates either neuroplasticity or cytotoxicity (Rothman, 1992).

A variety of chemicals have been investigated that can bind pharmacologically to a specific site within the NMDA channel, including the dissociative anesthetic phencyclidine and dizocilpine. These drugs are noncompetitive antagonists of NMDA receptor function and produce a use-dependent blockade of the channel and thereby inhibit the influx of Ca^{2+} (Huettner and Bean, 1988). Neuroprotection can be provided against NMDA receptor stimulation by administration of noncompetitive antagonists such as memantine (Wenk et al., 1996). Similarly, antagonists at the gly$_B$ receptor binding site, such as analogues of

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kynurenate (Kemp et al., 1988), can also provide neuroprotection from NMDA receptor agonists. Acetylcholinergic neurons within the nucleus basalis magnocellularis are vulnerable to excess stimulation of glutamatergic NMDA receptors, possibly due to the fact that they receive a dense glutamatergic projection from pedunculopontine tegmentum (Rasmusson et al., 1994). Injection of NMDA receptor agonists produce a significant decline in the number of nucleus basalis magnocellularis cholinergic neurons (Wenk et al., 1995). The current study investigated the ability of four novel noncompetitive antagonists at these two sites: three gly_B receptor antagonists (MRZ 2/570, 2/571, 2/576; Parsons et al., 1997a,b) and one NMDA open channel antagonist (MRZ 2/579; Parsons et al., 1997a,b), administered acutely, to provide neuroprotection from a NMDA receptor agonist within the nucleus basalis magnocellularis of young rats.

A defect in energy production may make neurons that express NMDA receptors more vulnerable to elevated or normal levels of endogenous glutamate (Albin and Greenamyre, 1992). Decreased levels of intracellular ATP would lead to a partial, and chronic, membrane depolarization, the relief of the voltage-dependent Mg^{2+} blockade at NMDA receptors, and a persistent increase in the influx of Ca^{2+} ions into the cells (Zeevalk and Nicklas, 1990). The accumulation of intracellular Ca^{2+} following the activation of NMDA receptors by glutamate would lead to neuronal death (Frandsen and Schousboe, 1992). Neuroprotection from mitochondrial metabolic toxins can be provided by NMDA receptor ion channel antagonists such as ARL-15896 (Greene et al., 1996) and memantine (Wenk et al., 1996). The present study, therefore, also investigated whether the novel NMDA receptor channel antagonist, MRZ 2/579, could attenuate the effects of mitochondrial failure, produced by an injection of 3-nitropropionic acid, upon cholinergic neuronal integrity within the nucleus basalis magnocellularis. 3-Nitropropionic acid is a naturally occurring toxin that is an irreversible inhibitor of succinate dehydrogenase (Coles et al., 1979).

2. Materials and methods

2.1. Animals

Male Long–Evans hooded rats (250–300 g, Charles River, USA) were housed two per cage in a 12 h light–dark cycle (lights on at 06:00) in a controlled temperature (21°C) room. Water and food were available ad libitum.

2.2. Surgery

The rats were anesthetized sodium pentobarbital (50 mg/kg, i.p.) and placed into a stereotaxic apparatus. Each rat received a single unilateral injection into the left nucleus basalis magnocellularis at the following coordinates: 0.6 mm posterior to Bregma, 2.8 mm lateral to the midline, and 7.9 mm ventral to the skull. Rats were injected (1.0 μl total volume) with either NMDA (0.015 M) or 3-nitropropionic acid (0.25 M). The concentrations of NMDA and 3-nitropropionic acid were chosen from preliminary studies (Wenk et al., 1996).

At the completion of surgery, chloramphenicol (1% solution) was applied to the exposed skull and scalp prior to closure to limit local infection, lidocaine was applied locally to the scalp to lessen pain and 5 ml of sterile isotonic saline were injected subcutaneously to prevent dehydration during recovery. Each rat was also given diluted Tylenol (cherry-flavored) to drink for 24 h after surgery to lessen postoperative pain.

2.2.1. Treatment

Thirty minutes prior to the nucleus basalis magnocellularis infusions of either toxin, MRZ 2/579 was administered (i.p., in phosphate-buffered saline) at one of the following doses: 1, 3 or 10 mg/kg vs. NMDA and 3, 10, or 30 mg/kg vs. 3-nitropropionic acid. Fifteen minutes prior to, and 15 min after, the nucleus basalis magnocellularis infusion of NMDA, MRZ 2/570 was administered (i.p.) at one of the following doses: 5, 10, or 20 mg/kg. Fifteen minutes prior to, and 15 min after, the nucleus basalis magnocellularis infusion of NMDA, either MRZ 2/571 or MRZ 2/576, was administered (i.p.) at one of the following doses: 5, 10, or 20 mg/kg.

2.3. Preparation of tissues

Two weeks later, each rat was sacrificed by decapitation and the brain was removed and dissected on ice (4°C). The right and left anterior sensorimotor cortex were analyzed for choline acetyltransferase activity (Fonnum, 1969), as an indicator of cholinergic neuronal integrity within the nucleus basalis magnocellularis region of the brain. The biochemistry data were analyzed by an analysis of variance.

Plasma and brain levels of MRZ 2/579 were determined using a separate group of rats. Samples of blood and brain (approximately 30 mg of frontal cortex) were taken 30, 90 and 150 min after an injection of MRZ 2/579 (10 mg/kg, i.p.). The blood was centrifuged ($4000 \times g$) and the serum was stored with the brain samples (-20°C) until analyzed.

2.4. Drugs

All agents were dissolved in phosphate-buffer saline (pH 7.4). NMDA and 3-nitropropionic acid were obtained from Sigma (USA) and injected directly into the nucleus basalis magnocellularis brain region. All other drugs were obtained from Merz (Frankfurt) and their structures have been published (Parsons et al., 1997a); MRZ 2/570: 8-

bromo-4-hydroxy-1-oxo-1,2-dihydropyridaziono [4,5-β] quinoline-5-oxide choline salt; MRZ 2/57: 8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridaziono [4,5-β] quinoline-5-oxide choline salt; MRZ 2/576: 8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridaziono [4,5-β] quinoline-5-oxide choline salt; MRZ 2/579: 1-amino-1,3,3,5,5-pentamethylcyclohexane hydrochloride.

3. Results

Injection of NMDA or 3-nitropropionic acid into the nucleus basalis magnocellularis significantly decreased cortical choline acetyltransferase activity on the side ipsilateral to the lesion (Tables 1 and 2). Acute administration (i.p.) of MRZ 2/579 (NMDA channel blocker), 30 min prior to the nucleus basalis magnocellularis injection, provided significant neuroprotection from NMDA ($F(3,28) = 6.26$, $P < 0.05$), but not from 3-nitropropionic acid ($F(3,27) = 3.44$, $P > 0.05$). Acute administration of MRZ 2/570 (gly_B receptor antagonist), given 15 min prior to, and 15 min after, the nucleus basalis magnocellularis injection, provided significant ($F(4,35) = 7.74$, $P < 0.001$) neuroprotection from NMDA, but only at the two higher doses, i.e., 10 and 20 mg/kg ($P < 0.05$).

Acute administration of MRZ 2/571 (gly_B receptor antagonist), given 15 min prior to, and 15 min after, the nucleus basalis magnocellularis injection, provided significant ($F(6,49) = 7.31$, $P < 0.001$) dose-dependent neuroprotection from NMDA, that was only significant ($P < 0.05$) at the two higher doses, i.e., 10 and 20 mg/kg. Acute administration of MRZ 2/576 (gly_B receptor antagonist), given 15 min prior to, and 15 min after, the nucleus basalis magnocellularis injection, also provided significant ($F(4,35) = 7.74$, $P < 0.001$) neuroprotection from NMDA, that was significant ($P < 0.05$) at all doses. Serum and brain levels of MRZ 2/579 after a single intraperitoneal injection are shown in Fig. 1. Brain and serum levels reach maximal levels within 30 min after an i.p. injection and

Table 1
Endogenous levels of choline acetyltransferase activity (nmol h^{-1} mg^{-1} protein) in left and right frontal cortex following injection of NMDA into the left nucleus basalis magnocellularis

	Left	Right	% Decline
Studies of neuroprotection by MRZ 2/579			
NMDA vs. saline	27.7±1.4	32.7±1.4	−15.7±1.1
vs. 1 mg/kg 2/579	24.2±1.5	26.4±1.3	−8.5±0.7[a]
vs. 3 mg/kg 2/579	26.3±1.1	28.3±1.1	−6.9±0.7[a]
vs. 10 mg/kg 2/579	31.3±1.2	31.2±0.9	−0.1±0.6[a]
Studies of neuroprotection by MRZ 2/570			
NMDA vs. saline	25.4±2.4	28.9±2.7	−12.3±0.4
vs. 5 mg/kg 2/570	33.1±1.5	37.7±1.3	−13.2±0.7
vs. 10 mg/kg 2/570	30.4±1.4	31.6±1.8	−3.3±0.9[a]
vs. 20 mg/kg 2/570	37.1±0.8	36.3±0.7	−2.4±0.7[a]
Studies of neuroprotection by MRZ 2/571			
NMDA vs. saline	32.3+1.1	25.2±1.2	−22.1±1.0
vs. 5 mg/kg 2/571	28.7±1.2	24.0±1.6	−16.6±0.7
vs. 10 mg/kg 2/571	28.2±1.7	24.7±1.4	−11.7±0.5[a]
vs. 20 mg/kg 2/571	34.1±1.2	31.3±1.1	−8.2±0.6[a]
Studies of neuroprotection by MRZ 2/576			
NMDA vs. saline	32.3±1.1	25.2±1.2	−22.1±1.2
vs. 5 mg/kg 2/576	33.1±2.4	30.7±2.0	−6.6±0.4[a]
vs. 10 mg/kg 2/576	32.7±1.1	30.1±1.1	−7.7±0.5[a]
vs. 20 mg/kg 2/576	31.2±0.7	29.9±0.7	−4.3±0.7[a]

Each section of this table compares the ability of either an NMDA (2/579) or gly_B receptor antagonists (administered i.p. at different doses) to attenuate the effects of an injection of the neurotoxin NMDA into the left nucleus basalis magnocellularis. In each case, the neuroprotective effect of the drug is compared against the effects of saline treatment.
[a] $P < 0.05$ vs. saline. Each section shows the results from a single study and each data point represents the results, expressed as the mean±S.E.M., obtained from eight rats.

Table 2
Endogenous levels of choline acetyltransferase activity (nmol h^{-1} mg^{-1} protein) in left and right frontal cortex of rats following injection of 3-nitropropionic acid into the left nucleus basalis magnocellularis

	Left	Right	% Decline
Studies of neuroprotection by MRZ 2/579			
3-Nitropropionic acid vs. saline	20.6±1.6	30.2±0.6	−31.6±1.3
vs. 3 mg/kg 2/579	22.4±1.5	29.4±1.8	−23.5±1.8
vs. 10 mg/kg 2/579	18.1±1.2	28.4±1.1	−36.2±1.0
vs. 30 mg/kg 2/579	15.7±1.0	26.5±1.8	−40.6±0.9

This table compares the ability of the NMDA receptor antagonist (administered i.p. at three different doses) to attenuate the effects of an injection of the neurotoxin 3-nitropropionic acid into the left nucleus basalis magnocellularis. Each data point represents the results, expressed as the mean±S.E.M., obtained from eight rats.

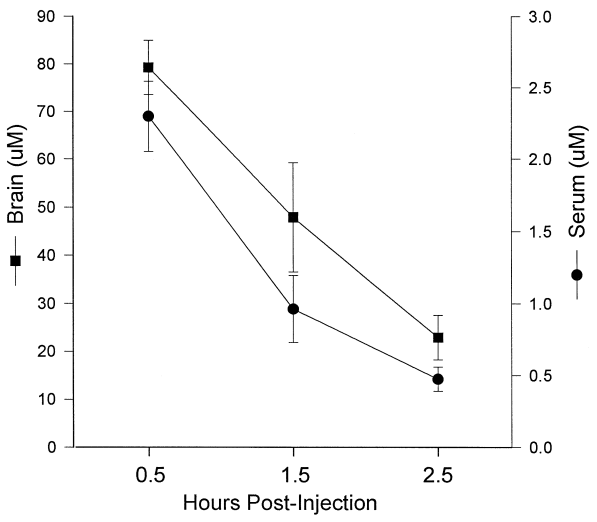

Fig. 1. Brain (– ■ –) and serum (– • –) levels of MRZ 2/579 at different time points (hours) following a single intraperitoneal injection at the dose of 10 mg/kg. Each data point represents the mean±S.D. obtained from four treated rats.

reach maximal levels of 2.3 μM in the plasma and 0.79 μM in the brain.

4. Discussion

The results are consistent with our previous study showing that nucleus basalis magnocellularis cholinergic neurons die under conditions that lead to a mitochondrial energy deficit, such as that produced by 3-nitropropionic acid, or following excess NMDA receptor activation (Wenk et al., 1996). The activation of NMDA receptors may be a critical event underlying the cytotoxicity of 3-nitropropionic acid in the nucleus basalis magnocellularis, inasmuch as another NMDA open-channel antagonist, i.e., memantine, significantly attenuated its cytotoxicity (Wenk et al., 1996). In the present study, the noncompetitive NMDA receptor channel antagonist MRZ 2/579 was able to provide neuroprotection from the effects of NMDA, but failed to provide neuroprotection from 3-nitropropionic acid. It is unknown why two different open channel antagonists, i.e., memantine and MRZ 2/579, were able to provide a similar degree of neuroprotection from NMDA, but that only memantine was able to provide neuroprotection from the mitochondrial poison 3-nitropropionic acid. The difference in their actions may be due to the fact that MRZ 2/579 has a much shorter half-life than memantine (Hesselink et al., 1997); the relatively long duration of increased vulnerability due to 3-nitropropionic acid exposure may require the prolonged presence of a neuroprotective agent at the NMDA receptor site. Acute administration of MRZ 2/579 (10 mg/kg) produced a concentration in brain homogenates that was almost 80 μM. However, free in vivo brain levels (measured in extracellular fluid) are usually closer to plasma levels (2.3 μM at peak) than to brain homogenate levels (Hesselink et al., 1997; see Danysz et al., 1997 for discussion). Although the higher concentration found in the present study may be due to the presence of the drug within the blood contained within the tissue sample, this is unlikely because MRZ 2/579 is accumulated and concentrated by the brain. A lower dose of MRZ 2/579 (5 mg/kg) produced a free brain level of 0.8 μM (based on in vitro recovery of 18%, Hesselink et al., 1997).

The gly_B receptor antagonist MRZ 2/570 provided neuroprotection from NMDA in a dose-dependent fashion. Another gly_B receptor antagonist, MRZ 2/571, also provided a dose-dependent neuroprotection starting at 10 mg/kg while MRZ 2/576 provided equivalent levels of neuroprotection at all doses. In summary, both the NMDA channel antagonist and the gly_B receptor antagonist were able to provide neuroprotection from stimulation of NMDA receptors on the cholinergic cells within the basal forebrain of young rats. It should be stressed that the neuroprotective doses of MRZ 2/570 and 2/576 used in the present study will lead to brain concentrations (as studied with microdialysis) that are close to their potency as NMDA receptor antagonists (approximately 0.5–1 μM, based on in vitro recovery of 13%, Hesselink et al., 1997).

The degeneration or dysfunction of cholinergic neurons within the basal forebrain of patients with Alzheimer's disease may be related to the vulnerability of these cells to endogenous glutamate (Beal, 1995; Greenamyre and Young, 1989). The administration of drugs that attenuate the toxic actions of glutamate in the early stages of the disease might significantly delay its rate of progression.

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