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Novel glycine$_B$ antagonists show neuroprotective activity *in vivo*

G. L. Wenk[1], **L. M. Baker**[1], **B. Hauss-Wegrzyniak**[1], **W. Danysz**[2], and **J. D. Stoehr**[3]

[1] Arizona Research Laboratories, Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, Arizona, U.S.A.
[2] Department of Pharmacology, MERZ + Co. GmbH & Co., Frankfurt/Main, Federal Republic of Germany
[3] Department of Physiology, Arizona College of Osteopathic Medicine, Midwestern University, Phoenix, Arizona, U.S.A.

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Summary. The degeneration or dysfunction of cholinergic neurons within the basal forebrain of patients with Alzheimer's disease (AD) 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. Two approaches to neuroprotection from endogenous glutamatergic function were investigated and found to be effective: blockade of voltage-dependent, NMDA-type glutamate receptor channels and antagonism of an NMDA-receptor related glycine$_B$ modulatory site.

Keywords: Glycine – Basal forebrain – Neuroprotection – Neurotoxicity – Acetylcholine

Introduction

The acidic amino acid glutamate may be the primary excitatory synaptic transmitter in the brain. Glutamate is thought to act via three different types of ionophore-coupled receptors that have been named for the selective pharmacological agonists that can stimulate them: N-Methyl-D-Aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (Watkins and Evans, 1981). Multiple interesting characteristics of the NMDA receptor are pertinent to both its excitotoxic and physiological actions. For example, glutamate is not effective at its receptor site unless a glycine modulatory site is also occupied (Kleckner and Dingledine, 1988; Lester et al., 1993). In addition, the NMDA ion channel is normally blocked in a voltage-dependent manner by Mg^{2+} (Nowak et al., 1984). Therefore, activation of the channel is partially dependent upon two factors, the binding of the agonist at the glycine and NMDA sites and the postsynaptic membrane potential. The depolarization of the membrane, the removal of the

Mg^{2+} block, and the ultimate opening of the NMDA channel, function in sequence to allow 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 to a specific site within the NMDA channel, including the dissociative anesthetic phencyclidine and dizocilpine. These drugs are non-competitive antagonists of NMDA receptors and produce a use-dependent blockade of the channel and the influx of Ca^{2+} (Huettner and Bean, 1988). Neuroprotection also can be provided against NMDA receptor stimulation by administration of non-competitive antagonists such as memantine (Wenk et al., 1996).

Antagonists at the glycine binding site, such as D-cycloserine (Lanthorn, 1994) and analogues of kynurenate (Kemp et al., 1988), including 7-chlorothiokynurenic acid (Chen et al., 1993) can also provide neuroprotection from NMDA receptor agonists. These antagonists have all been considered for possible use in patients with dementia and related degenerative disorders.

Acetylcholinergic neurons within the basal forebrain 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 basal forebrain cholinergic neurons (Wenk et al., 1995). The current study investigated the ability of four novel non-competitive antagonists at these two sites: three $glycine_B$ antagonists (MRZ 2/570, 2/571, 2/576) and one NMDA open channel antagonist (MRZ 2/579), administered either acutely and chronically, to provide neuroprotection from a NMDA receptor agonist within the nucleus basalis magnocellularis (NBM) of young rats.

Materials and methods

Male Long-Evans rats (250 gm) were anesthetized (pentobarbital 50 mg/kg, i.p.) and placed into a stereotaxic apparatus. Each rat received a single unilateral injection into the NBM at the following coordinates: 0.6 mm posterior to Bregma, 2.8 mm lateral to the midline, and 6.9 mm ventral to the skull. Rats were injected (1.0 ul total volume) with NMDA (0.015 M). The concentration of NMDA was chosen from preliminary studies (Wenk et al., 1996).

Acute Administration Studies: Thirty min prior to the NBM infusions of NMDA, MRZ 2/579 was administered (i.p. in PBS) at one of the following doses: 1, 3 or 10 mg/kg. Fifteen min prior to, and 15 min after, the NBM infusion of NMDA, MRZ 2/570 was administered (i.p.) at one of the following doses: 5, 10, 20, or 40 mg/kg. Fifteen min prior to, and 15 min after, the NBM 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.

Chronic Administration Studies: Immediately after injection of NMDA into the NBM an osmotic minipump, that had been prefilled with MRZ 2/570 (model 2ML2 pumps, 20 and 40 mg/kg/day), was implanted subcutaneously. All minipumps were pre-incubated for 12–24 hrs in warm (37°C) phosphate-buffered saline prior to implantation to ensure immediate drug administration.

Two weeks later, each rat was anesthetized with metophane gas and killed 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 (ChAT) activity

(Fonnum, 1969), as an indicator of NBM cholinergic neuronal integrity. The biochemistry data were analyzed by ANOVA.

Results

Acute administration studies

Injection of NMDA into the NBM significantly decreased cortical ChAT activity on the side ipsilateral to the lesion. Acute administration (i.p.) of MRZ 2/579 (channel blocker), 30 minutes prior to the NBM injection, provided significant neuroprotection from NMDA ($F_{3,28} = 6.26$, $p < 0.05$). Acute administration of MRZ 2/570 (glycine$_B$ antagonist), given 15 minutes prior to, and 15 minutes after, the NBM injection, provided significant ($F_{4,35} = 7.74$, $p < 0.001$) neuroprotection from NMDA, but only at the two intermediate doses, i.e. 10 and 20 mg/kg ($p < 0.05$). Acute administration of MRZ 2/571 (glycine$_B$ antagonist), given 15 minutes prior to, and 15 minutes after, the NBM injection, provided a 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 (glycine$_B$ antagonist), given 15 minutes prior to, and 15 minutes after, the NBM injection, also provided significant ($F_{4,35} = 7.74$, $p < 0.001$) neuroprotection from NMDA that was significant ($p < 0.05$) at all doses.

Chronic administration studies

Injection of NMDA into the NBM significantly decreased cortical ChAT activity on the side ipsilateral to the lesion. Chronic administration of MRZ 2/570 provided significant ($F_{2,21} = 4.46$, $p = 0.02$) neuroprotection against NMDA at 20 mg/kg/day ($p < 0.05$), but not at 40 mg/kg/day.

Discussion

The results are consistent with our previous study showing that NBM cholinergic neurons die under conditions of excess NMDA receptor activation and that antagonism by channel blockers, such as MK-801 and memantine, can provide neuroprotection (Wenk et al., 1996). In the present study, a similar non-competitive NMDA receptor channel antagonist MRZ 2/579 also provided neuroprotection from NMDA. The glycine$_B$ antagonist MRZ 2/570 provided neuroprotection from NMDA in a dose-dependent fashion, although the highest dose provided no neuroprotection in both the acute and chronic administrations studies. This may have been because the highest dose of MRZ 2/570 was prepared at the limits of its solubility in PBS. The glycine$_B$ antagonist MRZ 2/571 also provided a dose-dependent neuroprotection, although the lowest did not provide significant protection. In the present study, the glycine$_B$ antagonist MRZ 2/576 provided equivalent levels of neuroprotection at all doses. In summary, both the NMDA channel antagonist and the glycine$_B$ antagonists were able to provide neuroprotection from stimulation of NMDA receptors on the cholinergic cells within the basal forebrain of young rats.

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Authors' address: G. L. Wenk, Ph.D., Division of Neural Systems, University of Arizona, 384 Life Sciences North Building, Tucson, AZ 85724, U.S.A.

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