Amino Acids (1998) 14: 217–221

Amino
Acids


# Effects of NMDA receptor antagonists on nociceptive responses *in vivo*: comparison of antagonists acting at the glycine site with uncompetitive antagonists

**M. McClean, B. A. Chizh,** and **P. M. Headley**

University of Bristol, Department of Physiology, School of Medical Sciences, Bristol, United Kingdom

Accepted September 26, 1997

**Summary.** We have shown that members of a new series of tricyclic pyrido-phthalazine diones, defined as glycine$_B$ site NMDA antagonists *in vitro*, are selective and systemically active NMDA antagonists *in vivo*. In electrophysiological tests in $\alpha$-chloralose anaesthetised rats, these compounds reduced nociceptive reflex responses. In conscious rats they displayed analgesic properties. These glycine$_B$ antagonists were compared electrophysiologically with several uncompetitive NMDA channel blockers. The degree of voltage dependence previously reported *in vitro* related to the effectiveness of the agents against different amplitude nociceptive responses of spinal cord neurones *in vivo*.

**Keywords:** Analgesia – NMDA – Electrophysiology – Behaviour – Voltage dependence

## Introduction

Within the vertebrate CNS, NMDA (*N*-methyl-D-aspartate) receptors contribute to synaptic transmission in numerous pathways including those mediating the transmission of nociceptive information. The uncompetitive NMDA channel blocker ketamine has been shown to have analgesic properties in experimental and clinical pain states in man (e.g. Stannard and Porter, 1993; Persson et al., 1995). However ketamine and similar compounds exhibit dysphoric side effects that limit their clinical use.

In view of extensive evidence that NMDA receptors may trigger and/or maintain hyperalgesic states (Dickenson, 1990; Meller and Gebhart, 1993), the effectiveness of other classes of NMDA antagonists is of interest for the treatment of pain states. We have tested several members of a new series of tricyclic pyrido-phthalazine diones that are selective for the glycine binding site of the NMDA receptor *in vitro* (Parsons et al., this meeting) for their ability to reduce NMDA responses and nociceptive activity following systemic administration *in vivo* (Chizh et al., 1996; McClean et al., 1996).

In isolated tissues, uncompetitive NMDA antagonists show voltage dependence (to different degrees) whereas NMDA receptor associated glycine$_B$ antagonists do not. The extent to which this property has functional significance *in vivo* is not yet established and could provide an explanation for the varied therapeutic profiles of NMDA antagonists. We have now compared the effects of several uncompetitive channel blockers and two glycine$_B$ site antagonists on simple spinal nociceptive reflexes (Jones et al., 1996).

## Material and methods

### *Electrophysiology*

Extracellular action potentials of spinal neurones were recorded in $\alpha$-chloralose anaesthetised spinalised male Wistar rats (280–350 g) using methods described in full elsewhere (Chizh et al., 1996; Herrero and Headley, 1996). Responses were elicited in regular cycles and were quantified as spikes evoked during the last 10 s of the stimulus. Test compounds were administered in a dose doubling regime and effects expressed as % of the last three pre-drug control responses (mean ± s.e.m.).

With dorsal horn neurones, action potentials were recorded using 7 barrel glass micropipettes. For selectivity studies, responses were elicited by alternating microiontophoretic ejections of NMDA and AMPA in 3–4 min cycles. In 'voltage dependence' studies, NMDA ejection currents were selected to elicit alternating 'high' and 'low' frequencies of spike discharge.

Nociceptive reflex responses of single motoneurones were recorded as action potentials from hindlimb muscle single motor units using a bipolar needle electrode. Responses were evoked by alternating 'high' and 'low' intensities of noxious pinch stimuli applied to the hindpaw receptive field. Linear regression analysis was used to obtain slope values for the relationship between the log of the doses reducing the responses to 50% control ($ID_{50}$) plotted against control response amplitude.

### *Behavioural tests*

Male Sprague-Dawley rats (250–350 g) underwent paw pressure, paw thermal (Hargreaves et al., 1988) and rotarod (accelerating) tests. All rats were acclimatised to handling and to the testing environment for at least 2 days prior to testing. Thermal and mechanical latencies of withdrawal were measured for both paws in tests involving normal animals. Thermal tests were performed on rats with acute inflammation of one hindpaw. Threshold values were measured 1 h before and 3 h following intra-plantar injection of carrageenan (1 mg in 0.1 ml). In all cases control values were obtained prior to i.p administration of test compound. Drug effects are presented as differences from these values. For each drug a time course analysis was performed and a dose response curve constructed.

At the completion of studies animals were killed humanely by an overdose of pentobarbitone.

## Results

### *Selectivity of tricyclic pyrido-phthalazine diones for responses to exogenous NMDA*

The most potent and selective NMDA antagonists were Mrz 2/502, Mrz 2/576 and Mrz 2/570 ($ID_{50}$ ± s.e.m 1.6 ± 0.3, 2.8 ± 0.7 and 4.5 ± 0.7 mg/kg i.v., n = 6–7, c.f. 1.3 ± 0.3 mg/kg i.v. for ketamine, n = 9). At the highest doses tested,

responses to NMDA were reduced by Mrz 2/502 (2–4, mean 3 mg/kg) to 17 ± 6% control (AMPA 76 ± 8%); by Mrz 2/576 (2–8, mean 4 mg/kg) to 30 ± 7% control (AMPA 88 ± 6%); by Mrz 2/570 (4–16, mean 9 mg/kg) to 29 ± 4% control (AMPA 100 ± 9%). On the same cells, ketamine (1–4, mean 3 mg/kg, n = 17) reduced NMDA to 15 ± 3% control (AMPA 91 ± 4%).

### *Glycine$_B$ antagonists: effects on spinal nociceptive reflexes*

The potency rank order and $ID_{50}$ values were similar in electrophysiological tests to those obtained with NMDA responses of dorsal horn neurones: Mrz 2/502 1.0 ± 0.3 mg/kg (n = 10) and Mrz 2/576 3.3 ± 0.4 mg/kg (n = 5).

### *Glycine$_B$ antagonists: effects on reflex withdrawal in conscious rats*

Time course analysis showed that following i.p. administration the effects of Mrz 2/502 and Mrz 2/576 peaked at 5 min while morphine effects peaked at 30 min. In normal rats Mrz 2/576 and morphine produced a dose dependent increase in both thermal and mechanical nociceptive thresholds. The lowest dose of Mrz 2/576 increasing withdrawal latency in both mechanical and thermal tests was 14 mg/kg. At this dose Mrz 2/576 impaired rotarod performance.

Intraplantar injection of carrageenan produced a marked inflammation of the hindpaw and a significant reduction in both mechanical and thermal withdrawal latencies of the inflamed, but not of the contralateral, limb. Treatment with either Mrz 2/576 or morphine produced a statistically significant and dose dependent reversal of thermal hyperalgesia in the carrageenan-injected paws. The minimum effective dose that significantly enhanced the mechanical latency of withdrawal of the inflamed limbs was 20 mg/kg of Mrz 2/576 ($p < 0.05$, n = 8).

### *Relative efficacy of different NMDA antagonists on spinal nociceptive responses*

Slope values (log mg/kg per spikes/s) were calculated for three uncompetitive NMDA antagonists and for one glycine$_B$ antagonist. The rank order of slope values for memantine, ketamine, MK-801 and Mrz 2/576 matched the rank order of voltage dependence found *in vitro* (Parsons et al., 1993 and unpublished data). Values were memantine ($8.5 \times 10^{-2}$, n = 28) > ketamine ($5.4 \times 10^{-2}$, n = 49) > MK-801 ($4.7 \times 10^{-2}$, n = 21) with Mrz 2/576 ($2 \times 10^{-2}$, n = 12) being the least dependent on control firing rate.

## Discussion

The tricyclic pyrido-phthalazine diones tested here are systemically active and have a short time course of action. Our results are consistent with previous *in vitro* experiments and show that compounds of this series are selective antagonists of responses to exogenous NMDA *in vivo*.

Mrz 2/576 and Mrz 2/502 also reduced spinal nociceptive reflex responses that have an NMDA receptor mediated component as defined by the effectiveness of the established NMDA antagonist ketamine. Mrz 2/576 also produced antinociception in conscious rats at doses comparable to those abolishing nociceptive responses of single motor units in anaesthetised rats.

Because NMDA receptors have been suggested to have a role in mediating persistent pain and hyperalgesia (Dickenson, 1990; Meller and Gebhart, 1993) we studied animals with normal paws and others with a carageenan-inflamed paw to evaluate the compounds under conditions of normal vs. increased excitability within nociceptive pathways. A significant decrease in paw thermal thresholds was observed in inflamed paws while contralateral paws remained unaffected. Consistent with studies suggesting that excessive activation of NMDA receptors may be involved in the development of behavioural hyperalgesia (Meller and Gebhart, 1993), Mrz 2/576 reversed hyperalgesia and produced analgesia. As NMDA receptor antagonists are known to disrupt motor function (Headley et al., 1987; Kemp and Leeson, 1993) motor function assessments were made for comparison with the behavioural nociception tests. Our results suggest that in conscious animals antinociception is produced by the glycine$_B$ site antagonist Mrz 2/576 but only at doses that cause some impairment of the motor system. However, under the conditions tested morphine also caused some motor impairment at doses causing antinociception.

Existing uncompetitive channel blocking agents such as memantine, ketamine and MK-801, when tested on isolated tissues, each display varying degrees both of voltage dependence and of channel binding kinetics. Both properties may affect their clinical profile. The present results show that these compounds reduce receptor mediated responses but their efficacy is influenced by the spike discharge rate of the unit. Memantine had the greatest slope value and was therefore more greatly affected by spike firing rate than were the other channel blockers and Mrz 2/576. This rank order of slope values matches the rank order of voltage dependence found with isolated neurones *in vitro* (Parsons et al., 1993 and unpublished data). We consequently suggest that the varied voltage dependence of these uncompetitive NMDA antagonists demonstrated *in vitro* does have correlates *in vivo*. This property may therefore contribute to the markedly different clinical profiles of memantine compared with other uncompetitive NMDA antagonists such as ketamine.

## References

Chizh BA, McClean M, Parsons CG, Headley PM (1996) An *in vivo* study of the potency and selectivity of novel systemically active antagonists at the glycine site of the NMDA receptor. Soc Neurosci 22: 604.18

Chizh BA, Headley PM (1996) Thyrotropin-releasing hormone facilitates spinal nociceptive responses by potentiating NMDA receptor mediated transmission. Eur J Pharmacol 300: 183–189

Dickenson AH (1990) A cure for wind-up: NMDA receptor antagonists as potential analgesics. Trends Pharmacol Sci 11: 307–309

Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32: 77–88

Headley PM, Parsons CG, West DC (1987) The role of N-methylaspartate receptors in mediating responses of rat and cat spinal neurones to defined sensory stimuli. J Physiol 385: 169–188

Herrero JF, Headley PM (1996) Reversal by naloxone of the spinal antinociceptive actions of systemically administered NSAID. Br J Pharmacol 118: 968–972

Jones MW, McClean M, Headley PM (1996) Do differing levels of voltage dependence of NMDA open channel-blockers affect their *in vivo* actions on spinal neurones in anaesthetised rats? Br J Pharmacol: 119: 34P

Kemp JA, Leeson PD (1993) The glycine site of the NMDA receptor-five years on. Trends Pharmacol Sci 14: 20–25

McClean M, Chizh BA, Jones MW, Parsons CG, Headley PM (1996) *In vivo* significance of the different voltage dependence of uncompetitive and glycine$_B$ NMDA antagoniststst. Soc Neurosci 22: 604.19

Meller ST, Gebhart GF (1993) Nitric oxide (NO) and nociceptive processing in the spinal cord. Pain 52: 127–136

Parsons CG, Gruner R, Rozenthal J, Millar J, Lodge D (1993) Patch clamp studies on the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). Neuropharmacology 32: 1337–1350

Persson J, Axelddon G, Hallin G, Gustafsson LL (1995) Beneficial effects of ketamine in a chronic pain state with allodynia, possibly due to central sensitization. Pain 60: 217–222

Stannard CF, Porter GE (1993) Ketamine hydrochloride in the treatment of phantom limb pain. Pain 54: 227–230

**Authors' address:** M. McClean, University of Bristol, Department of Physiology, School of Medical Sciences, Bristol BS8-1TD, U.K.

Received August 25, 1997