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Effects of memantine and MK-801 on NMDA-induced currents in cultured neurones and on synaptic transmission and LTP in area CA1 of rat hippocampal slices

Tadeusz Frankiewicz, *,[1]Brigitte Potier, *,[2]Zafar I. Bashir, *,[2]Graham L. Collingridge & [3]Chris G. Parsons

Dept. of Pharmacology, Merz + Co., Eckenheimer Landstraße 100-104, D-60318 Frankfurt am Main 1, Germany and *Dept of Pharmacology, The Medical School, University of Birmingham, Birmingham B15 2TT, England

1 The effects of the uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists, memantine (1-amino-3,5-dimethyladamantane) and MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzocyclo-hepten-5,10-imine maleate) were compared on synaptic transmission and long-term potentiation (LTP) in hippocampal slices and on NMDA-induced currents in cultured superior collicular neurones.

2 Memantine (10-100 μM) reversibly reduced, but did not abolish, NMDA receptor-mediated secondary population spikes recorded in area CA1 of hippocampal slices bathed in Mg^{2+}-free artificial cerebrospinal fluid.

3 Memantine (100 μM) antagonized NMDA receptor-mediated excitatory postsynaptic currents recorded in area CA1 in a strongly voltage-dependent manner i.e. depressed to $11 \pm 4\%$ of control at -35 mV and $95 \pm 5\%$ of control at $+40$ mV ($n=9$), with no apparent effect on response kinetics.

4 The effects of MK-801 and memantine on the induction of LTP were assessed after prolonged pre-incubations with these antagonists. When present for 6.6 ± 0.4 h prior to tetanic stimulation, memantine blocked the induction of LTP with an IC_{50} of 11.6 ± 0.53 μM. By comparison, similar long pre-incubations with MK-801 (6.4 ± 0.4 h) blocked the induction of LTP with an IC_{50} of 0.13 ± 0.02 μM.

5 Memantine and MK-801 reduced NMDA-induced currents in cultured superior colliculus neurones recorded at -70 mV with IC_{50}s of $2.2 \pm 0.2$$\mu$M and 0.14 ± 0.04 μM respectively. The effects of memantine were highly voltage-dependent and behaved as though the affinity decreased ε fold per 50 mV of depolarization (apparent $\delta = 0.71$). In contrast, under the conditions used, MK-801 appeared to be much less voltage-dependent i.e. affinity decreased ε fold per 329 mV of depolarization (apparent $\delta = 0.15$).

6 Depolarizing steps from -70 mV to $+50$ mV in the continuous presence of memantine (10 μM) caused a rapid relief of blockade of NMDA-induced currents from $83.7 \pm 1.9\%$ to $21.8 \pm 1.8\%$ ($n=5$). This relief was best fitted by a double exponential function (17.2 ± 11.7 and 698 ± 204 ms), the faster component of which was most pronounced.

7 In conclusion, whereas MK-801 is equipotent in blocking NMDA-induced currents (at -70 mV) and the induction of LTP, memantine is relatively less potent in blocking the induction of LTP. This is due to its rapid relief of blockade upon depolarization; a property which might explain its promising clinical profile in the treatment of chronic neurodegenerative diseases.

Keywords: Memantine (1-amino-3,5-dimethyladmantane); MK-801 (dizocilpine); LTP (long-term potentiation); NMDA (N-methyl-D-aspartate); hippocampal slice; area CA1; patch clamp; kinetics; voltage-dependency; superior colliculus culture

Introduction

Memantine (1-amino-3,5-dimethyladmantane) is an uncompetitive NMDA (N-methyl-D-aspartate) receptor antagonist (Kornhuber *et al.*, 1989; 1991; 1994; Bormann, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993; 1994; 1995a, b) that has been used clinically for many years in the treatment of spasticity and to a lesser exent Parkinson's disease (Grossmann & Schutz, 1982; Wesemann *et al.*, 1983; Schneider *et al.*, 1984; Kornhuber *et al.*, 1994; Danysz *et al.*, 1994a; 1995a). More recently, doses of this compound predicted to act selectively at NMDA receptors *in vivo* have been found to produce symptomatological improvements in the therapy of dementia (Ditzler, 1991; Görtelmeyer & Erbler, 1992; Pantev *et al.*, 1993).

Oversimplified interpretation of these observations might lead to the conclusion that they are incompatible with the accepted role of NMDA receptors in mediating synaptic plasticity and cognitive processes (Collingridge *et al.*, 1983; Morris *et al.*, 1986; see Bliss & Collingridge, 1993; Danysz & Archer, 1994; Danysz *et al.*, 1995b for reviews). However, therapeutically-relevant doses of memantine block NMDA receptor-mediated pathology in animal models of both acute excitotoxicity and chronic neurodegenerative diseases but exert none of the overt side effects classically associated with NMDA receptor antagonists (Danysz *et al.*, 1994b; Misztal *et al.*, 1994; Wenk *et al.*, 1994; 1995). Indeed, a symptomatological improvement in cognition and memory has been reported following chronic infusion of memantine in rats with lesions of the entorhinal cortex, a preclinical model for the cognitive deficits seen in Alzheimer's dementia (Danysz *et al.*, 1994b; Zajaczkowski *et al.*, 1995).

This promising profile of memantine has been attributed to its strong voltage-dependency and rapid, open-channel unblocking kinetics which may allow it to block the patho-

[1] Present address: INSERM Unite 161, 2 rue d'Alesia, 75104 Paris, France.
[2] Present address: Dept. of Anatomy, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, England.
[3] Author for correspondence.

logical activation of NMDA receptors whilst leaving their physiological activation relatively intact (Chen *et al.*, 1992; Parsons *et al.*, 1993; 1995b). The aim of the present study was to test whether these biophysical properties of memantine do indeed lessen its effects on the physiological activation of NMDA receptors, by assessing the potency of memantine and MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate) in blocking the induction of LTP in area CA1 of hippocampal slices and comparing these effects to the kinetics and voltage-dependency of NMDA receptor blockade in cultured neurones.

Methods

Hippocampal slice experiments

Initial experiments on synaptic field potentials and excitatory postsynaptic currents were performed on 400 μm hippocampal slices from young female rats as previously described in detail by Bashir & Collingridge (1992). Further experiments with prolonged incubations of memantine and MK-801 were performed as follows. Male rats (120–180 g, aged 4–7 weeks) were anaesthetized with chloroform before decapitation. The brains were removed rapidly and immediately cooled at 2–4°C in an ice bath. Transverse hippocampal slices (400 μm thick) were cut (Vibracut, FTB) and stored in artificial cerebrospinal fluid (aCSF) containing (mM): NaCl 124, KCl 3, $NaHCO_3$ 26, $NaHPO_4$ 1.25, $CaCl_2$ 2, $MgSO_4$ 1, D-glucose 10, bubbled with 95% O_2/5% CO_2 (pH 7.3) at room temperature, for at least 4 h before transfer to the recording chamber.

The CA3 region was dissected and discarded. Slices were then placed on a nylon mesh in an interface chamber (BSC-HT, Medical Systems) and perfused at a rate of 0.8 ml min^{-1} with normal aCSF at 33°C in an oxygen-enriched (95% O_2/5% CO_2) humidified atmosphere. After at least 30 min of incubation in the recording chamber, a glass recording electrode (2–3 MΩ, filled with aCSF) was positioned in the dendritic layer of area CA1 to record extracellular field excitatory postsynaptic potentials (f.e.p.s.p.). A concentric, bipolar tungesten stimulating electrode (WPI) was placed 500–700 μm away from the recording electrode but at the same dendritic level to activate the Schaffer collateral commissural fibres. Extracellular recordings were made in response to constant voltage (10–20 V, 20 μs) single shock stimulation once every 15 s (DS2 isolated stimulator, Digitimer). Stimulus intensities were adjusted to evoke f.e.p.s.ps of half maximal amplitude. Responses were recorded with an Axoclamp 2a amplifier (Axon Instruments) and digitised using TIDA for Windows (Battelle) before being stored on an IBM PC for off-line analysis. The slope of the rising phase of the f.e.p.s.p. (mV ms^{-1}) was measured between 20–80% of the peak amplitude and was assessed semi-automatically by AUTESP for IBM (Garching Instruments, Munich). A single tetanic stimulation (100 Hz for 1 s, 20 μs pulse width) was used to evoke LTP. Field e.p.s.p. slopes were normalized with respect to the 30 min control period prior to tetanic stimulation.

'Blind' whole-cell patch clamp recording techniques were used to study synaptically-evoked NMDA receptor-mediated excitatory postsynaptic currents (e.p.s.cs) in the stratum radiatum of area CA1 (Boulton *et al.*, 1994). NMDA receptor-mediated e.p.s.cs were isolated by adding picrotoxin (50 μM) and CNQX (6-cyano-7-nitroquinoxaline.2,3-dione, Tocris, 10 μM) to block $GABA_A$ (γ-aminobutyric acid) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor-mediated synaptic transmission respectively (2 ml min^{-1}; room temperature). Patch electrodes (4.5–5 MΩ) contained (in mM): $CsMeSO_4$ 130, HEPES 5, NaCl 1, $MgCl_2$ 1, $CaCl_2$ 0.035, EGTA 0.05 and QX-314 5, pH was adjusted to 7.3 with CsOH or HCl. Synaptic responses were evoked by constant voltage stimulation of the Schaffer collaterals via bipolar tungsten electrodes (10–20 V, 200 μS duration).

Patch clamp experiments on cultured superior colliculus neurones

Patch clamp recordings from cultured superior colliculus neurones were performed as described previously (Parsons *et al.*, 1993). In brief, superior colliculi were isolated from embryonic rats (E20–21) and maintained in culture for 11–16 days in $NaHCO_3$/HEPES- buffered minimum essential medium supplemented with 5% foetal calf serum and 5% horse serum (Gibco) and incubated at 37°C with 5% CO_2 (95% humidity). The superior colliculus culture was chosen for these experiments as it provides very stable recording conditions which are an absolute prerequisite for voltage-dependency and kinetic experiments. Moreover, the relatively small neurones (soma 15–20 μm ∅) are ideally suited to minimize problems of buffered diffusion for concentration clamp experiments. Finally, our own unpublished data indicate that the somatic NMDA receptors expressed in cultured hippocampal and cortical neurones are similar.

Patch clamp recordings were made from these neurones with polished glass electrodes (4–8 MΩ) in the whole cell mode at room temperature (20–22°C) with the aid of an EPC-7 amplifier (List). Test substances were applied by switching channels of a custom made fast superfusion system with a common outflow (< = 10 ms exchange times). The contents of the intracellular solution were as follows (mM): CsCl 120, TEACl 20, EGTA 10, $MgCl_2$ 1, $CaCl_2$ 0.2, glucose 10, ATP 2, cyclic AMP (0.25); pH was adjusted to 7.3 with CsOH or HCl. The extracellular solutions had the following basic composition (mM): NaCl 140, KCl 3, $CaCl_2$ 0.2, glucose 10, HEPES 10, sucrose 4.5, tetrodotoxin TTX 3×10^{-4} M, glycine (1×10^{-3} M), (pH 7.3). All compounds were obtained from Sigma unless otherwise noted. Memantine was synthesised by Merz + Co.

Analysis and statistics

IC_{50}s were calculated according to the four parameter logistic equation using the Grafit computer programme (Erithacus Software, England). Pearson product moment correlation analysis (SigmaStat, Jandel Scientific) was used to compare equilibrium potencies against NMDA-induced currents at various membrane potentials after log transformation of IC_{50}s. Results are expressed as means ± s.e.mean.

The apparent depth (δ) of the channel blocking site for memantine and MK-801 was calculated according to Woodhill (1973):

$$\delta = \frac{\left(\ln \frac{Ki_{(V2)}}{Ki_{(V1)}}\right) * RT}{FVz}$$

where $Ki_{(V1)}$ = potency at voltage 1, $Ki_{(V2)}$ = potency at voltage 2, V = V1 – V2, F = Faraday's constant, R = universal gas constant, T = temperature (°Kelvin), z = valency.

Results

Secondary population spikes in hippocampal slices in Mg^{2+}-free aCSF

The first experiments investigated the effectiveness of memantine as an antagonist of NMDA receptor-mediated synaptic responses in the hippocampal slice preparation. In Mg^{2+}-free aCSF, stimulation of the Schaffer collateral commisural pathway elicits NMDA receptor-dependent multiple population spikes recorded in area CA1 (Coan & Collingridge, 1987a). With the use of appropriate stimulus intensities, secondary

population spikes can be selectively blocked by NMDA receptor antagonists whereas the first population spike is only sensitive to AMPA receptor blockade. This approach provides a simple means to test for antagonism of one form of NMDA receptor-mediated synaptic transmission. In agreement with recent data from Apland & Cann (1995), perfusion with memantine (10–100 μM) for up to 60 min reduced, but did not abolish, the secondary population spikes recorded in Mg^{2+}-free aCSF (Figure 1a; $n=4$). In contrast, the competitive NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (AP5; 50 μM) completely abolished these secondary spikes (data not shown). Recovery from the effects of memantine was very slow, i.e. was first achieved several hours after removal of memantine from the superfusion medium.

Comparative effects of short incubations with memantine and MK-801 on synaptic plasticity in hippocampal slices

The influence of memantine on synaptic plasticity was first investigated by testing the effects of 30 min pre-incubations with high concentrations (10–100 μM) on the induction of LTP in otherwise normal aCSF. On no occasion was the induction of LTP influenced by memantine. Thus, even at a very high concentration of 100 μM, the slopes of potentiated synaptic responses were 153±21% of baseline levels when measured 30 min after the tetanic stimulation (100 Hz for 1 s, $n=6$; Figure 1b). A similar lack of effect was evident for peak amplitude responses (data not shown). This level of LTP was similar to that seen in control slices (157±10% of control, $n=8$, data not shown). Likewise, no aspect of the normal synaptic response was altered by the presence of memantine (1–100 μM) i.e. slope, offset rate or peak.

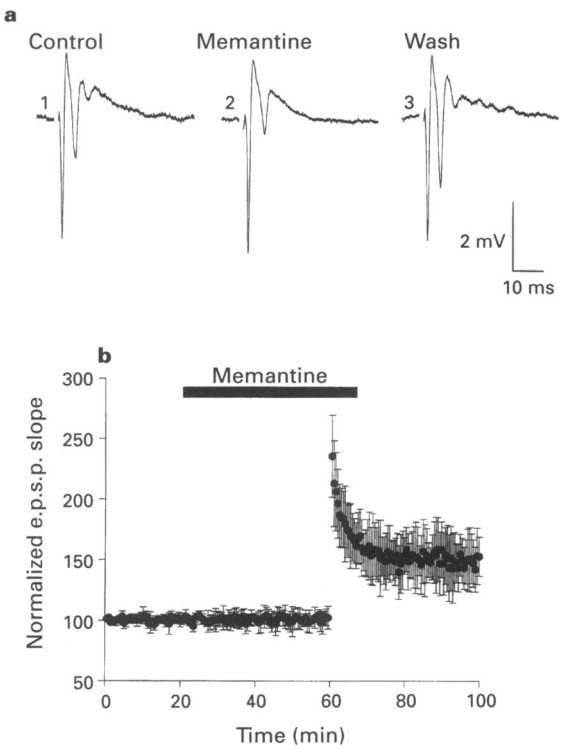

Figure 1 Memantine has very weak effects on NMDA receptor-mediated synaptic responses in hippocampal slices when administered for relatively short periods of time. (a) Synaptic NMDA receptor activation in the absence of Mg^{2+} was monitored by recording field potentials in area CA1 of hippocampal slices in response to electrical stimulation of the Schaffer collaterals (10–20 V, 200 μs, 0.033 Hz). Responses illustrated are the average of 4 consecutive samples. Responses developed secondary population spikes within a few minutes after switching to Mg^{2+}-free aCSF (1). Memantine (50 μM) partially reduced these NMDA receptor-mediated population spikes but this effect developed slowly; responses recorded 30 min after commencing memantine superfusion are illustrated in (2). Full recovery was first seen 4 h after washout of memantine (3). (b) The effect of short incubations with memantine on synaptic plasticity in the hippocampus. Preincubation with memantine (100 μM) for 30 min, as indicated by the bar, did not block the induction of long-term potentiation ($n=6$). Slopes of f.e.p.s.ps were normalized to those recorded in the 10 min control period prior to memantine administration and have been plotted as means±s.e.mean against time.

The lack of effect of memantine (100 μM) on the induction of LTP was compared to the actions of another NMDA receptor channel blocker, MK-801 (Wong *et al.*, 1987; Huettner *et al.*, 1988; Davies *et al.*, 1988). Since MK-801 is about 100 times more potent than memantine in binding studies, initial experiments were performed with 30 min incubations in the presence of MK-801 (1 μM). This concemtration of MK-801 was also unable to block the induction of LTP (slope = 149±11% of control; $n=3$). On both occasions tested, however, a 10 fold higher concentration of MK-801 (10 μM) did indeed block the induction of LTP (slope = 105 and 103% of control) as has been reported previously (Coan *et al.*, 1987).These concentrations of memantine and MK-801 are well above those known to block NMDA receptors at membrane potentials near resting levels in cultured neurones (e.g. Wong *et al.*, 1987; Huettner & Bean, 1988; Chen *et al.*, 1992; Parsons *et al.*, 1993). As such, it seems likely that problems of penetration into the slice by these highly lipophilic compounds and the nature of their NMDA receptor antagonistic activity, i.e. open channel blockade, prevented them from reaching equilibrium within the NMDA receptor channel following such 30 min incubations.

e.p.s.cs in hippocampal slices

Experiments to assess how quickly high concentrations of memantine can gain assess to open NMDA channels in the hippocampal slice were performed on pharmacologically-isolated NMDA receptor-mediated e.p.s.cs in area CA1, recorded by conventional 'blind' whole-cell recording (Boulton *et al.*, 1994). Memantine (100 μM) reduced the size of NMDA receptor-mediated e.p.s.cs (recorded at −35 mV) to 11±4% of control ($n=9$). The depression of NMDA receptor-mediated e.p.s.cs by memantine was slow to develop, reaching maximal levels only after 30–40 min (Figure 2b) and recovery following wash out was too slow to be assessed with this recording technique. Thus, even with the repetitive activation of NMDA receptor channels, high concentrations of memantine took considerably longer to achieve equilibrium blockade than e.g. APV or Mg^{2+} (Coan & Collingridge, 1987b). It should be noted that memantine had no discernible effect on the time course of NMDA receptor-mediated e.p.s.cs during the development of blockade. This has been illustrated by scaling 20 averaged responses recorded 15–20 min after starting the memantine application to averaged control responses (Figure 2a).

A prerequisite for the proposed mechanism of action of memantine is that its effects on the synaptic activation of NMDA receptors in hippocampal slices are voltage-dependent. This was indeed the case, even a high concentration of memantine (100 μM) showed a greater degree of blockade of NMDA receptor-mediated e.p.s.cs at a holding potential of −35 mV than at +40 mV (Figure 3a). In some experiments, however, there was a run down in the size of NMDA receptor-mediated e.p.s.cs during the course of recording. This precluded seeing a relief of the block by memantine of NMDA receptor-mediated e.p.s.cs at positive holding potentials. In such experiments, the stimulus intensity was increased to match the pre- and post-drug responses recorded at +40 mV, but responses were still blocked to the same extent at −35 mV (Figure 3b).

Comparative effects of prolonged incubations with memantine and MK-801 on synaptic plasticity in hippocampal slices

The above data indicate that MK-801 and memantine do not achieve equilibrium blockade of NMDA receptors following

30 min incubations prior to tetanic stimulation in LTP experiments. Further experiments were therefore performed under conditions where true equilibrium occupancy of NMDA receptors is more likely to occur. Slices were transferred to aCSF containing various concentrations of memantine or MK-801 immediately after preparation and were then incubated for at least 4 h in these solutions before starting recording. Control experiments with normal aCSF were interlaced with MK-801 (0.1, 0.3 and 1 μM) and memantine (3, 10 and 30 μM). MK-801 was much more potent when present for 6.8 ± 0.4 h prior to tetanic stimulation than following 30 min incubations and blocked the induction of LTP with an IC_{50} of 0.13 ± 0.02 μM (Figures 4 and 6). Moreover, memantine was also able to block the induction of LTP when present for 6.6 ± 0.4 h prior to tetanic stimulation, but was 90 times less potent that MK-801 in this regard (IC_{50} of 11.6 ± 0.53 μM, Figures 5 and 6).

Comparison of the effects of memantine and MK-801 on NMDA-induced currents

The relative potencies of memantine and MK-801 in blocking LTP were between those obtained in [^{3}H]-MK-801 binding experiments in membranes from area CA1 i.e. a 164 fold difference at 0 mV (Bresink *et al.*, 1995) and those seen against NMDA-induced inward currents in freshly dissociated area CA1 neurones at -100 mV i.e. an 8.7 fold difference (Parsons *et al.*, 1994; 1995a). In order to test whether such differences are related to a greater degree of voltage-dependency for memantine, the IC_{50}s of MK-801 and memantine against NMDA-induced currents in cultured superior colliculus neurones were compared at different membrane potentials (Figure

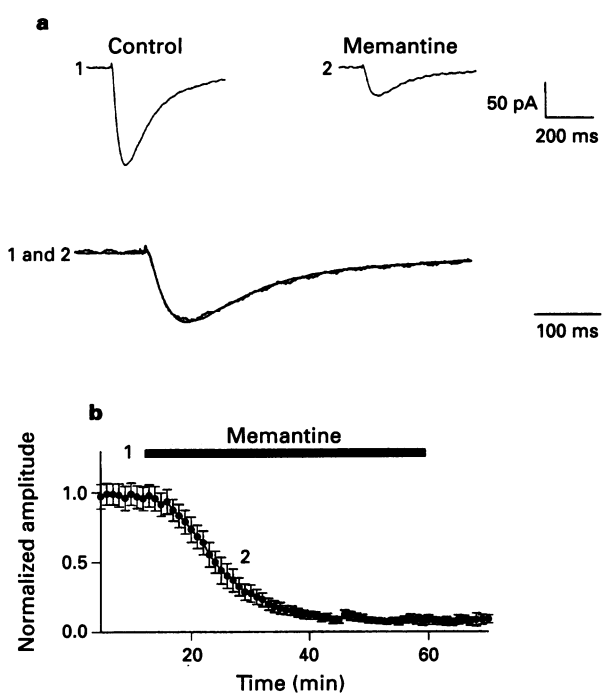

Figure 2 Memantine antagonism of pharmacologically-isolated NMDA receptor-mediated e.p.s.cs. (a) Traces (average of 20 responses) illustrate synaptically-evoked NMDA e.p.s.cs recorded at -35 mV before (1) and 15–20 min after (2) starting the application of memantine (100 μM). Traces 1 and 2 have also been re-scaled to the same peak amplitudes and superimposed to illustrate that e.p.s.cs in the presence of memantine have a similar response kinetics to control e.p.s.cs. (b) Time course of the effects of memantine in blocking e.p.s.cs recorded at -35 mV plotted as mean normalized amplitude $\pm$ s.e.mean against time ($n=9$). Memantine (100 μM) was present as indicated by the bar.

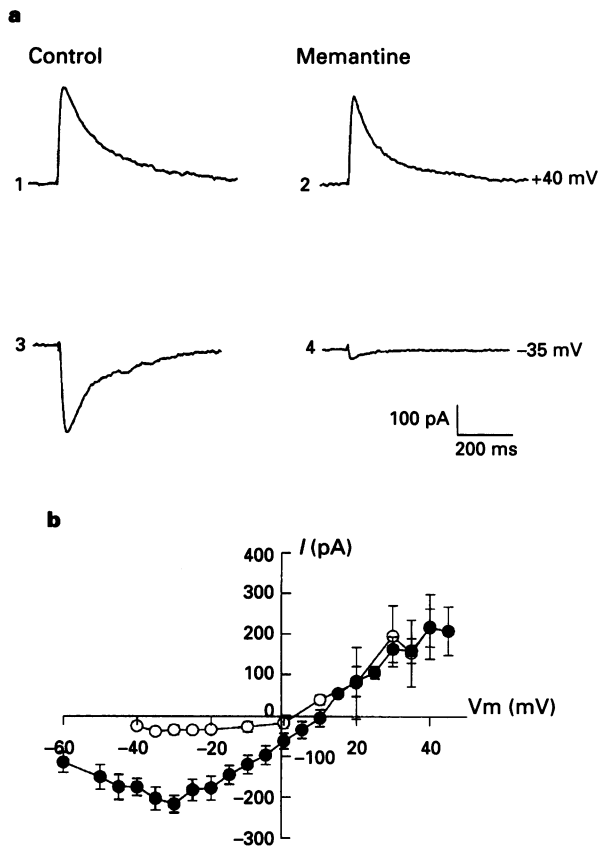

Figure 3 Voltage-dependence of the blockade of NMDA receptor-mediated e.p.s.cs by memantine (100 μM). (a) Traces (average of 20 responses) illustrate synaptically-evoked NMDA receptor-mediated e.p.s.cs recorded before (1 and 3) and 40 min after starting the application of memantine (100 μM, 2 and 4). Note the strong block at -35 mV (4 versus 3) but much less block at $+40$ mV (2 versus 1). (b) Mean $\pm$ s.e.mean currents for 9 cells plotted against membrane potential. NB: for 3 of these 9 cells the stimulus intensity was increased as described in the text. Mean $\pm$ s.e.mean peak e.p.s.c. amplitude before (●) and after (○) memantine (100 μM) respectively.

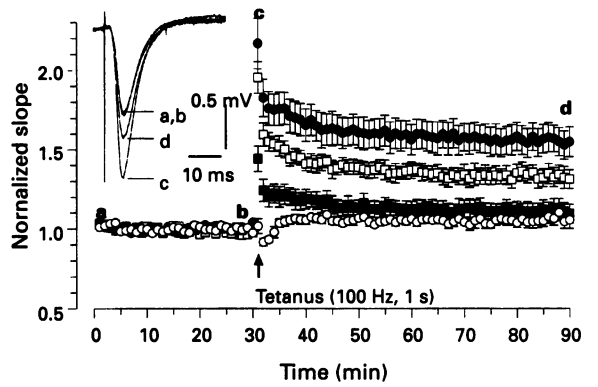

Figure 4 MK-801 is a potent antagonist of NMDA receptor-dependent LTP when assessed under conditions where true equilibrium blockade of NMDA receptors can be expected. When present immediately after preparing slices and thereafter for an average of 6.8 ± 0.4 h prior to tetanic stimulation, high nM concentrations of MK-801 blocked the induction of LTP in a concentration-dependent manner. Traces were averaged in groups of 4 consecutive responses (4×15 s $= 1$ min) and were then normalised with respect to the grouped average slope of responses during the 30 min prior to tetanic stimulation and have been plotted as means $\pm$ s.e.mean against time. The raw data presented illustrate a representative example of the level of LTP seen in the presence of MK-801 (0.1 μM). Each superimposed trace is the average of 4 consecutive responses. The relation of these responses to the time course of the recording session are given by (a through d) on the lower graph. The number of slices tested at each concentration were as follows: control (●) $n=10$; MK-801 (0.1 μM) (□) $n=7$; MK-801 (0.3 μM) (■) $n=8$; MK-801 (1.0 μM) (○) $n=8$.

7). The effects of memantine were highly voltage-dependent and indicated that its affinity decreased ε fold per 50 mV of depolarization (apparent $\delta = 0.71$). In contrast, under the conditions used, MK-801 appeared to much less voltage-dependent i.e. affinity decreased ε fold per 329 mV of depolarization (apparent $\delta = 0.15$). There was a strong correlation between potency and membrane potential for memantine ($r = 0.97$, $P < 0.0001$) whereas the much less pronounced voltage-dependence of MK-801 was reflected in a somewhat weaker correlation ($r = 0.77$, $P < 0.0001$). The relative differences in potencies for memantine and MK-801 can be interpolated at various membrane potentials and were e.g. 10.6 and 93.5 at −100 and 0 mV respectively. These values agree well with the respective patch clamp and binding data from area CA1 neurones. Furthermore, interpolation of the IC_{50} value for memantine in blocking the induction of LTP, namely 11.6 μM, indicates that the sub-synpatic membrane may have been depolarized to a value near to the predicted −18.7 mV (dashed line, Figure 7.)

This voltage-dependence of channel block could only account for the relatively weak effects of memantine on the induction of LTP if memantine can leave the NMDA channel rapidly, i.e. within the time course during which neurones are normally strongly depolarized during the induction of LTP. Further experiments to assess how quickly memantine can leave the NMDA channel were performed on cultured superior colliculus neurones. Memantine (10 μM) blocked NMDA (200 μM)-induced inward current responses to 16.3±1.9% of control at a holding potential of −70 mV ($n = 5$). On step depolarization of the cell membrane to +50 mV, the blockade was relieved to 78.2±1.8% of control. Moreover, the relief of this blockade was very rapid and was best fitted by a double exponential function (17.2±11.7 and 698±204 ms) the faster component of which was most pronounced, such that responses had reached 69±3% of control within 200 ms (Figure 8a). A similar rapid relief of blockade following −70 mV to +50 mV depolarizing steps was also seen with higher con-

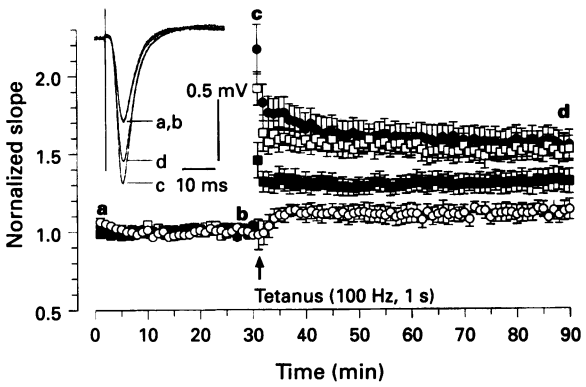

Figure 5 High concentrations of memantine can block the induction of NMDA receptor-dependent LTP when assessed under conditions where true equilibrium blockade of NMDA receptors can be expected. Presentation as in Figure 4. Pre-incubation time 6.6±0.4 h prior to tetanic stimulation. Raw data for memantine (3 μM). The number of slices tested at each concentration were as follows: control (●) $n = 10$; memantine (3 μM) (□) $n = 7$; memantine (10 μM) (■) $n = 7$; memantine (30 μM) (○) $n = 7$.

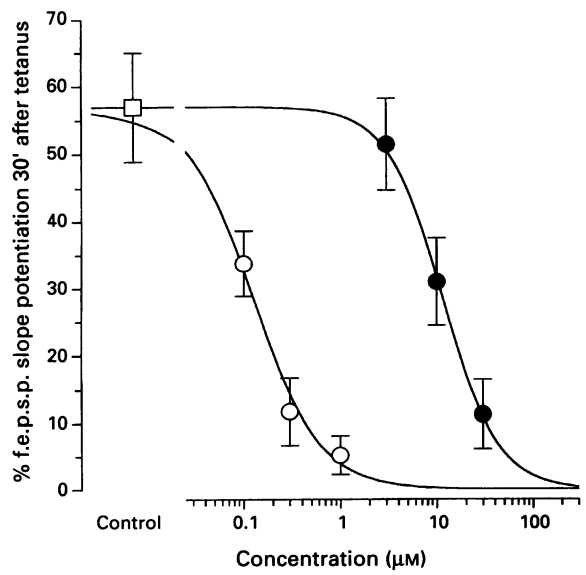

Figure 6 Concentration-dependent block of the induction of LTP by MK-801 (○) and memantine (●); control (□). Averaged normalized responses over 30 min, starting 30 min after tetanic stimulation, have been derived from the data presented in Figures 4 and 5 and plotted as % slope potentiation against antagonist concentration. Curves were fitted according to the 4 parameter logistic equation. MK-801 blocked the induction of LTP with an IC_{50} of 0.13±0.02 μM (Hill Coeff. 1.26±0.24). Memantine blocked the induction of LTP with an IC_{50} of 11.6±0.53 μM (Hill Coeff. 1.52±0.10).

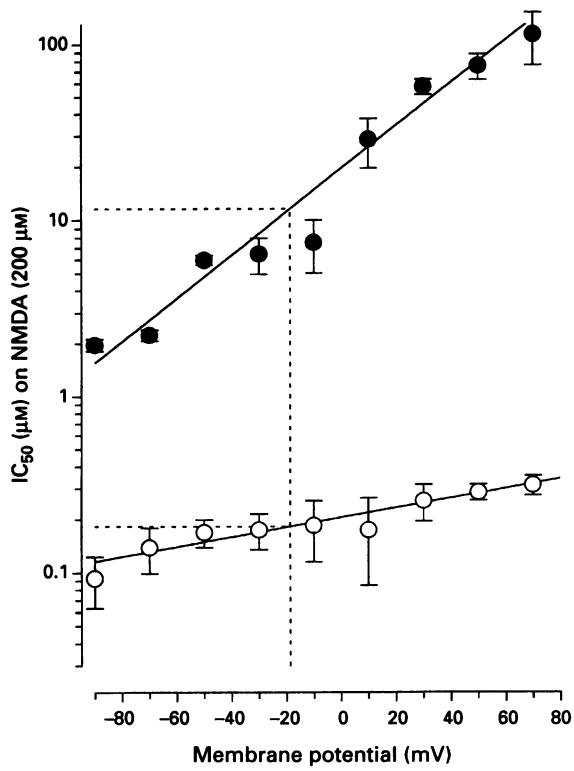

Figure 7 The antagonistic effects of memantine on NMDA-induced currents in cultured superior colliculus cells are highly voltage-dependent. The voltage-dependent effects of memantine (●) and MK-801 (○) were assessed on steady-state responses to NMDA 200 μM (glycine 1 μM) applied for 2.5 s every 30 s to cultured superior colliculus neurones. Equilibrium block was always established at −90 mV before commencing the voltage-progression with the antagonists. Voltage-dependency curves were assessed in 20 mV progressions for both control responses and currents in the presence of various concentrations of memantine or MK-801 i.e. 9 consecutive current responses to 2.5 s applications of NMDA were recorded over 4.5 min at the 9 different holding potentials. IC_{50}s were calculated according to the four parameter logistic equation and have been plotted against membrane potential. Estimates indicated by dashed line. Fits of the voltage-dependency of memantine and MK-801 were made by regression analysis.

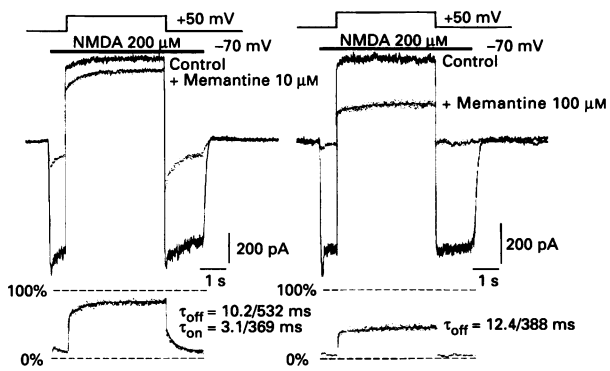

Figure 8 Memantine leaves the NMDA channel with very fast kinetics upon depolarization. NMDA (200 μM) was applied to cultured superior colliculus neurones for 6 s every 30 s at different holding potentials (solid bars above each trace) in the continuous presence or absence of memantine. The membrane potential was then clamped to various +ve potentials for 3.7 s. (square wave above each trace) during the NMDA application. (a) Memantine (10 μM) antagonized NMDA responses at −70 mV to around 10% of control but this block was rapidly relieved upon switching to +50 mV. This effect is even clearer when the ratio of memantine (upper dotted trace) to control (solid trace) was calculated (bottom dotted trace). The unblocking rate (τ_{off}) was best fitted by a double exponential, the major component of which was very fast (10.2 ms). The re-blocking rate of memantine (τ_{on}) was also very fast and best fitted by a double exponential. (b) Memantine (100 μM) was, as expected, less voltage-dependent but showed equally fast unblocking kinetics. NB: re-blocking kinetics were too fast to be resolved at the 1 kHz sampling rate. All currents were corrected for simultaneous passive and active currents (e.g. voltage-activated Ca^{2+} channels) by performing identical voltage steps without NMDA. Series resistance and membrane capacitance were always compensated to the highest degree possible.

centrations of memantine (Figure 8b) although the degree of relief was, as expected, inversely related to concentration, i.e. memantine 30 μM (8.3±2.0 to 67.7±1.2%; τ_{off} 17.5±2.7/292±144 ms, $n=3$); memantine 100 μM (2.6±0.7 to 45.5±11.5%; τ_{off} 18.3±6.7/228 ms±21.2 ms, $n=3$).

Discussion

It has previously been suggested that the good clinical utility of memantine in the treatment of chronic neurodegenerative diseases is due to its strong voltage-dependency and rapid unblocking kinetics which might allow therapeutically relevant concentrations to block the pathological activation of NMDA receptors whilst leaving their physiological activation relatively intact (Parsons *et al.*, 1993). The primary aim of the present study was to test whether these biophysical properties of memantine do indeed influence its effects on the physiological activation of NMDA receptors, namely on the induction of LTP in hippocampal slices.

The hypothesis of Parsons *et al.* (1993) was made on the basis of the fact that NMDA receptors are activated physiologically by mM concentrations of synaptically-released glutamate which transiently strongly depolarize the postsynaptic membrane (Clements *et al.*, 1992; Pongracz *et al.*, 1992) whereas, even during acute excitotoxic insults like ischaemia, relatively low μM concentrations of interstitial glutamate cause only moderate membrane depolarization but for much longer periods of time (Benveniste *et al.*, 1984; Globus *et al.*, 1989; 1991; Andine *et al.*, 1991; Buisson *et al.*, 1992; Mitani *et al.*, 1992; Hashimoto *et al.*, 1994). It is well accepted that the strong voltage-dependency and rapid unblocking kinetics of Mg^{2+} (Nowak *et al.*, 1984; Mayer *et al.*, 1984) allow this cation to leave the NMDA receptor channel upon transient physiological activation during the induction of LTP. Indeed, precisely these properties make the NMDA receptor channel complex inherently suited for its role in mediating synaptic plasticity (Herron *et al.*, 1986; Bliss & Collingridge, 1993). However, the voltage-dependency of Mg^{2+} block is so pronounced that it also leaves the NMDA channel upon moderate depolarization under excitotoxic conditions (for review see Danysz *et al.*, 1995a). In contrast, the rapid unblocking kinetics, but somewhat less pronounced voltage-dependency of memantine, were hypothesized to allow it to antagonize the pathological effects of the sustained, but relatively small increases in extracellular glutamate concentration but, like Mg^{2+}, leave the NMDA receptor channel during physiological activation (Parsons *et al.*, 1993).

The present data are consistent with this hypothesis and support the idea that memantine could show neuroprotective activity in chronic neurodegenerative diseases at doses devoid of negative side effects. Thus, relatively high concentrations of memantine were required to block the induction of LTP in area CA1 of hippocampal slices whereas therapeutically relevant concentrations blocked NMDA-induced inward currents in cultured cells at membrane potentials closer to resting levels. This ability of memantine to differentiate between different forms of NMDA receptor activation is supported by its ability to block NMDA receptor-mediated pathology *in vivo* at doses having little or no side effects i.e. allowing their physiological activation (Misztal *et al.*, 1994; Wenk *et al.*, 1994; 1995; Barnes *et al.*, 1995; for review see Danysz *et al.*, 1995b). Moreover, the 5–10 fold difference in potency agrees very well with the therapeutic index of 8 obtained with memantine in these *in vivo* studies (e.g. Wenk *et al.*, 1995).

This effect does indeed seem to be related to the relatively strong depolarization of postsynaptic membranes by synaptically-released glutamate during the tetanic stimulation used to induce LTP and the ability of memantine to leave the NMDA receptor channel rapidly upon depolarization. Firstly, the voltage-dependent relief of blockade of NMDA receptor channels in the continuous presence of memantine was very rapid upon a depolarizing step from −70 to +50 mV in cultured superior colliculus neurones. Secondly, even the effects of high concentrations of memantine (100 μM) on NMDA receptor-mediated e.p.s.cs in hippocampal slices were voltage-dependent and, in contrast to previous reports with MK-801 (Hessler *et al.*, 1993; Rosenmund *et al.*, 1993), showed no obvious effects on response kinetics. This indicates that the onset rate of open channel blockade by memantine (100 μM), like that of Mg^{2+}, is too quick to be resolved on NMDA receptor-mediated e.p.s.cs, an assumption which is supported by the predicted τ_{on} for memantine (100 μM) of 30 ms or faster (interpolation of data presented in Parsons *et al.*, 1993, Figure 7). However, one caveat in the interpretation of the present kinetic data is that the time course of these effects was not assessed in the presence of equilibrium concentrations of memantine in the slice. Finally, comparison of the IC_{50} value for memantine in blocking the induction of LTP with its potency against NMDA-induced currents at various membrane potentials would predict that the sub-synaptic membrane is depolarized to a value of around −20 mV during tetanic stimulation. Obviously, such interpolations have many caveats as they do not take several important factors into account e.g. the glutamate concentration and duration in the synaptic cleft (Clements *et al.*, 1992) and the rate at which memantine blockade is relieved upon depolarization. Nonetheless, this estimated value agrees reasonably well with that modelled to occur in dendrites upon tetanic stimulation (Pongracz *et al.*, 1992) and is also close to the membrane potentials at which NMDA receptors should permit a maximum flux of Ca^{2+} into the postsynaptic neurone in the presence of physiological levels of Mg^{2+}.

In contrast, whilst the high affinity uncompetitive NMDA receptor antagonist, MK-801, was 90 fold more potent then memantine in blocking the induction of LTP, it was only 8–16 times more potent than memantine in blocking NMDA-induced currents at membrane potentials closer to resting levels (see also Parsons *et al.*, 1993; 1994; 1995a, b). This reflects its very low voltage-dependency and slow unblocking kinetics. As

a result, the potency of MK-801 in blocking LTP and NMDA-induced currents is essentially the same and shows much less dependence on membrane potential. Once again, this poor separation of different forms of NMDA receptor activation by MK-801 agrees well with the poor therapeutic indices, i.e. around one, seen in *in vivo* models of NMDA receptor-mediated pathology (Danysz *et al.*, 1994b; Misztal *et al.*, 1994; Wenk *et al.*, 1994; 1995; see Danysz *et al.*, 1995a, b for review).

The present voltage-dependency data agree well with previous reports on the strong voltage-dependency of memantine (Chen *et al.*, 1992; Parsons *et al.*, 1993; 1995a) and weak voltage-dependency of MK-801 (Wong *et al.*, 1987; Huettner & Bean, 1988; Karschin *et al.*, 1988; Halliwell *et al.*, 1989). Moreover, the δ value for memantine, indicating that the binding site experiences 71% of the membrane field (Woodhill, 1973), agrees entirely with the data of Chen *et al.* (1992). However, it should be noted that previous interpretations that such δ values really reflect the depth of the binding site for uncompetitive antagonists in the NMDA receptor channel have been considered doubtful (MacDonald & Nowak, 1990). This is especially true for MK-801 which almost certainly binds to the same site as memantine and therefore cannot be located so near to the extracellular side of the membrane as indicated by its very low δ of 0.15. It is almost certain that the voltage-dependence of MK-801 would be somewhat more pronounced if assessed under true equilibrium conditions at each membrane potential. However, such experiments were not feasible and, more importantly, not relevant to the questions addressed in the present study on the basis of kinetic data (e.g. Huettner & Bean, 1988; Parsons *et al.*, 1995b) slow blockers such as MK-801 would have little chance to attain new equilibrium binding states during the induction phase of LTP.

One caveat in the interpretation of the present kinetic data is that the voltage-dependent unblocking rates of MK-801 were not assessed and that the kinetics of all channel blockers are likely to be faster at the higher temperatures used for the LTP experiments which were closer to those found *in vivo* (Davies *et al.*, 1988), the biophysical experiments were performed at room temperature. However, the relative differences for memantine and MK-801 are likely to be similar to those reported previously for τ_{off} upon removal of these antagonists (Huettner & Bean, 1988; Karschin *et al.*, 1988; Chen *et al.*, 1992; Parsons *et al.*, 1993; 1995b). As such, it can be assumed that the very slow open channel blocker MK-801 would still be able to leave the NMDA channel only after several seconds of depolarization and that the degree of relief would, in any case, be very small.

An important observation was the fact that the apparent potency of both MK-801 and memantine in blocking LTP in hippocampal slices was dramatically reduced when these agents were applied only for 30 min incubations prior to tetanic stimulation. This effect is probably related to problems of penetration into the slice by these highly lipophilic compounds and the nature of their NMDA receptor antagonistic activity, i.e. open channel blockade. When maintained in the presence of physiological concentrations of Mg^{2+}, healthy hippocampal slices were essentially devoid of NMDA receptor-mediated synaptic activity during the 30 min incubation period prior to delivering the tetanic stimulus i.e. uncompetitive NMDA receptor antagonists would have little opportunity to gain access to open NMDA receptor channels. In contrast, when slices are exposed for several hours to such antagonists starting immediately after preparation, access to the channel is much more likely to reach equlibrium, especially in view of the fact that slices probably have spontaneous NMDA receptor-mediated synaptic activity for several minutes after cutting. Poor access to the NMDA receptor channel is likely to have been the case in most previous studies testing the effects of such compounds on LTP *in vitro* with short incubation times (see e.g. Coan *et al.*, 1987) and may also underlie the dearth of literature on the effects of substances like ketamine on LTP in hippocampal slices. As such, previous reports that memantine does not block LTP *in vitro* must be interpreted with some degree of caution (Stieg *et al.*, 1993; Dimpfel, 1995).

In contrast, systemic administration of uncompetitive NMDA receptors *in vivo* is likely to achieve equilibrium blockade much faster due to the rapid penetration of the blood brain barrier by these generally highly lipophilic compounds, higher temperatures and a much greater level of ongoing NMDA receptor activity (Davies *et al.*, 1988). Indeed, there is ample evidence that relatively low doses of many uncompetitive NMDA receptor antagonists block LTP *in vivo* and cause memory deficits in rats at similar doses (see Danysz & Archer, 1994; Danysz *et al.*, 1995a, b for review). As such, the fact that chronic treatment with therapeutically-relevant doses of memantine has no negative effects on LTP in the rat dentate gyrus *in vivo* or in area CA1 of hippocampal slices *ex vivo* (Barnes *et al.*, 1995; Misztal *et al.*, 1995) can be taken as providing further support for the results of the present study.

Interestingly, in the study of Barnes *et al.* (1995) memantine actually prolonged the duration of LTP *in vivo* and also showed a trend to improve retention of memory in the Morris maze in the same rats. This finding is probably related to the semi-chronic nature of memantine administration used (30 mg kg^{-1} per day for >8 weeks) and agrees well with data from Danysz *et al.* (1994b) and Zajaczkowski *et al.* (1995) where chronic infusion of memantine via osmotic mini pumps actually reversed memory deficits previously induced by quinolinic acid lesions of the entorhinal cortex. In both of these *in vivo* studies, therapeutically-relevant serum concentrations of memantine were achieved for prolonged periods of time (i.e. around 1 μM, Kornhuber *et al.*, 1994; Kornhuber & Quack, 1995) and the results correlate well with clinical reports of improved cognition in demented patients within a few weeks of treatment (Ditzler, 1991). Although the mechanism of action for this facilitatory effect has not yet been established, it is not necessarily related to the acute effects of memantine assessed in the present study (see Danysz *et al.*, 1995b for discussion). In contrast, the acute facilitatory effect of memantine on hippocampal synaptic transmission *per se* reported by Dimpfel (1995) was not observed in the present study or in the studies of Stieg *et al.* (1993) or Barnes *et al.* (1995). The reasons for this discrepancy remain elusive.

In conclusion, the present study provides strong support for our working hypothesis on the mode of action of memantine. This uncompetitive NMDA receptor antagonist can be likened to a potent Mg^{2+} ion. Under resting conditions, and in their continuing presence, both Mg^{2+} and memantine occupy the NMDA receptor channel. Likewise, both are able to leave the NMDA receptor channel upon strong synaptic depolarisation due to their pronounced voltage-dependency and rapid unblocking kinetics. However, memantine contrasts to Mg^{2+} in that it does not leave the channel so easily upon moderate prolonged depolarization during chronic excitotoxic insults.

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