

PATCH CLAMP STUDIES ON THE KINETICS AND SELECTIVITY OF *N*-METHYL-D-ASPARTATE RECEPTOR ANTAGONISM BY MEMANTINE (1-AMINO-3,5-DIMETHYLADAMANTAN)

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Summary—Memantine (1-amino-3,5-dimethyladamantan) was tested as an antagonist of *N*-methyl-D-aspartate (NMDA) receptors on cultured superior collicular and hippocampal neurones using the patch clamp technique and its actions were compared to those of Mg²⁺ ions, ketamine, dextrorphan, dextromethorphan, phencyclidine and dizocilpine (MK-801). Memantine (2–33 μM) concentration-dependently antagonized responses to NMDA 100 μM with an IC₅₀ of 2.92 ± 0.05 μM. In contrast, current responses to (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (L-AMPA 50–100 μM) and γ-amino butyric acid (GABA 10 μM) were unaffected by Memantine 8 μM. Memantine 8 μM caused a non-parallel shift of the NMDA concentration-response curve to the right in a manner indicative of uncompetitive open channel block. The effects of memantine were similar to ketamine in that both antagonists were weakly use- and strongly voltage-dependent. In contrast, MK-801, phencyclidine and dextrorphan showed much slower kinetics that was reflected in their marked use- and weaker voltage-dependency. The antagonistic effects of memantine were not reversed by increasing concentrations of glycine (0.1–100 μM) ruling out the possibility of an interaction of memantine with the strychnine-insensitive glycine modulatory site associated with the NMDA receptor-channel complex. Memantine (1–100 μM) also selectively antagonized responses to NMDA (40 μM) in the cortical wedge preparation with IC₅₀ of 12.9 ± 1.5 μM.

Key words—*N*-methyl-D-aspartate (NMDA), (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), dizocilpine (MK-801), γ-amino butyric acid (GABA), memantine (1-amino-3,5-dimethyladamantan), ketamine, phencyclidine (PCP), dextrorphan, dextromethorphan, Mg²⁺, patch clamp, culture, superior colliculus, hippocampus.

Memantine (1-amino-3,5-dimethyladamantan) has similar beneficial effects to classical *N*-methyl-D-aspartate (NMDA) receptor antagonists in animal models of spasticity and Morbus Parkinson (Richter, Fredow and Löscher, 1991; Schmidt, Zadow, Kretschmer and Hauber 1991) and is used clinically in Europe in the treatment of these disorders (Grossmann and Schutz 1982; Wesemann, Sontag and Maj, 1983; Schneider, Fischer, Clemens, Balzereit, Fünfgeld and Haase, 1984). It has also recently been shown to improve cognition and vigilance in patients with dementia-related deficits (Ditzler, 1991; Görtelmeyer and Erbler, 1992).

There is considerable *in vitro* evidence that memantine acts as an antagonist of NMDA receptors by interacting with the phencyclidine (PCP) recognition site in the channel of the NMDA receptor complex. Thus, high nanomolar concentrations of memantine displace the binding of [³H]dizocilpine (³H-MK-801) to the PCP site (Kornhuber, Bormann, Retz, Hubers and Riederer, 1989; Kornhuber, Bormann, Hubers,

Rusche and Riederer, 1991) and low micromolar concentrations block current responses of cultured neurones to NMDA in a use- and voltage-dependent manner and prevent NMDA-induced increases in intracellular Ca²⁺ (Bormann, 1989; Chen, Pellegrini, Aggarwal, Lei, Warach, Jensen and Lipton, 1992). Furthermore, memantine concentration-dependently, and non-competitively decreases glutamate- and NMDA-stimulated acetylcholine release from rabbit striatal slices in a Mg²⁺-sensitive manner with an IC₅₀ of about 4 μM (Lupp, Lücking, Koch, Jackisch and Feuerstein, 1992). Finally, NMDA receptor-mediated toxicity in cultured neurones is blocked by low micromolar concentrations of memantine (Erdö and Schäfer, 1991; Chen *et al.*, 1992; Osborne and Quack, 1992).

Similar concentrations to those effective in the above mentioned *in vitro* models have been measured in the central nervous system of humans and rats after systemic administration of therapeutically relevant doses of memantine (Wesemann *et al.*, 1983; Spanagel, Eilbacher and Wilke, 1992). Moreover, memantine selectively reduces single unit responses

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of rat spinal cord neurones to NMDA applied micro-electrophoretically *in vivo* with an IC_{50} of 25 mg/kg *i.v.* (Herrero, Headley and Parsons, *in press*). As such, it seems likely that the beneficial effects seen with memantine in the treatment of dementia could, at least in part, be due to blockade of the effects of sustained, increased levels of excitatory amino acids hypothesized to be involved in the pathology of this disorder [see Greenamyre and Young (1989); Palmer and Gershon (1990)]. However, in contrast to classical NMDA antagonists, memantine does not cause memory deficits in man (Ditzler, 1991) and long term potentiation (LTP) can be induced in the CA₁ region of the hippocampus *in vitro* in the presence of memantine 1–100 μ M (G. L. Collingridge, personal communication).

Chen *et al.* (1992) have demonstrated that the open channel blockade of NMDA receptors by memantine was much faster than that of MK-801 when tested on cultured retinal ganglion cells and have suggested that the much faster kinetics of action in comparison to classical uncompetitive antagonists of NMDA could account for the promising clinical profile of memantine. We used whole cell patch clamp recordings to characterize further the selectivity, kinetics and voltage-dependency of the NMDA blockade by memantine and compared these effects to those of classical NMDA channel blockers under similar conditions.

METHODS

Patch clamp recordings were made from cultured superior collicular and hippocampal neurones (10 to 21 days *in vitro*) in whole cell mode or "perforated patch" cell attached mode (Horn and Marty, 1988) at room temperature (20–22°C) with the aid of an EPC-7 amplifier (List). Most recordings were made at a membrane potential of -70 mV. Patch clamp electrodes were pulled with a horizontal plunger (DMZ) and had an internal tip diameter between 1.2 and 1.6 μ M and a tip resistance of 4–10 M Ω . Cells were continuously superfused via one of eight channels of a fast superfusion system (Konnerth, Lux and Morad, 1987). Test substances then were applied by rapidly switching channels. Complete exchange of the superfused solution was achieved within 10–20 msec—confirmed by the speed of block of voltage-activated calcium channels when switching to solutions containing cadmium. The application of solutions and the synchronized on-line electronic acquisition of data were controlled by the IBM program PCLAMP. Subsequently, AUTESP for IBM (Zucker, Max Planck Institute Munich) was utilized to analyze the data semi-automatically off-line. Only results from stable cells were accepted for inclusion in the final analysis *i.e.* following recovery of responses to NMDA by at least 50% of their depression by memantine.

Superior colliculus cells were cultured according

to a modified protocol from the Max Planck Institute in Munich. Superior colliculi were obtained from rat embryos (E20 to E21) and were then transferred to calcium and magnesium free Hank's buffered salt solution (Gibco) on ice. Cells were mechanically dissociated in 0.05% DNAase/0.3% ovomucoid (Sigma) following a 15 min pre incubation with 0.66% trypsin/0.1% DNAase (Sigma). The dissociated cells were then centrifuged at 18 g for 10 min, re suspended in minimum essential medium (Gibco) and plated at a density of 200,000 cells cm^{-2} onto poly-L-lysine (Sigma)-precoated plastic petri dishes (Falcon). The cells were nourished with NaHCO₃/HEPES-buffered minimum essential medium supplemented with 5% foetal calf serum and 5% horse serum (Gibco) and incubated at 37°C with 95% O₂/5% CO₂. The medium was exchanged completely following inhibition of further glial mitosis with cytosine- β -D-arabinofuranoside (20 μ M Sigma) after about 7 days *in vitro*. Thereafter the medium was exchanged partially twice weekly. The protocol for the preparation of hippocampal cells was essentially similar to that utilized for superior colliculus except for the omission of trypsin pre-incubation and the use of younger (E17) rat embryos.

The contents of the intracellular (electrode) solution for whole cell recordings were as follows (mM): CsCl (120), TEACl (20), EGTA (10), MgCl₂ (1), CaCl₂ (0.2), Glucose (10), ATP (2), cAMP (0.25). For "perforated patch" recordings in cell attached mode nystatin (100 μ g/ml) was added to this solution [for details see Horn and Marty (1988)]. The extracellular solutions had the following basic salt composition (mM): NaCl (140), KCl (3), Glucose (10), HEPES (10), CaCl₂ (0.2), Sucrose (4.5). Neurones were pharmacologically isolated from one another by the inclusion of 0.3 μ M tetrodotoxin (TTX) to block voltage-activated sodium currents. Test substances were added to this basic solution in concentrations detailed in results. In addition, the bath solution and one superfusion solution contained 1.5 mM CaCl₂ to aid formation of a "giga Ohm seal". Experiments on the possible interaction of memantine with the strychnine-insensitive glycine site associated with the NMDA receptor-channel complex (Johnson and Ascher, 1987)—hereafter called the glycine_B site—were performed in various concentrations of glycine (100 nm to 100 μ M) and in the continuous presence of bicuculline methiodide (25 μ M) to block spontaneous γ -amino butyric acid (GABA) mIPSPS and strychnine (2 μ M) to circumvent activation of inhibitory glycine receptors by the highest concentrations of glycine used. Otherwise, experiments were performed in the continuous presence of glycine 1 μ M; a concentration sufficient to cause around 70–80% activation of glycine_B receptors.

Depolarization of rat cortical wedges was recorded using a grease-seal technique modified from Harrison and Simmonds (1984). Briefly, wedge-shaped slices of cerebral cortex were placed in two compartment baths so that the grey matter was separated from the

white by a greased Perspex barrier. The grey matter was superfused with a magnesium-free modified Krebs solution at a rate of 2 ml/min and the DC signal between the two halves of the bath monitored continuously. Depolarizations were produced by 5 ml aliquots of buffer containing various agonists (NMDA 40 μ M, AMPA 40 μ M, quisqualate 40 μ M or kainate 10 μ M) at intervals of 15–20 min. Once stable responses were attained, drugs of interest were added to the superfusate and the agonists re-tested.

The following pharmacological compounds (with sources) were used: NMDA (Sigma), glycine (Sigma), bicuculline methiodide (Sigma), GABA (Sigma), strychnine (Sigma), ketamine (Sigma), dizocilpine (MK-801, RBI), dextrorphan (RBI), dextromethorphan (RBI), phencyclidine (PCP), AMPA (Tocris), *dl*-amino-phosphonovaleric acid (AP5, Tocris), quisqualate (Tocris), 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline (NBQX), a kind gift from Novo Nordisk, Måløv, Denmark).

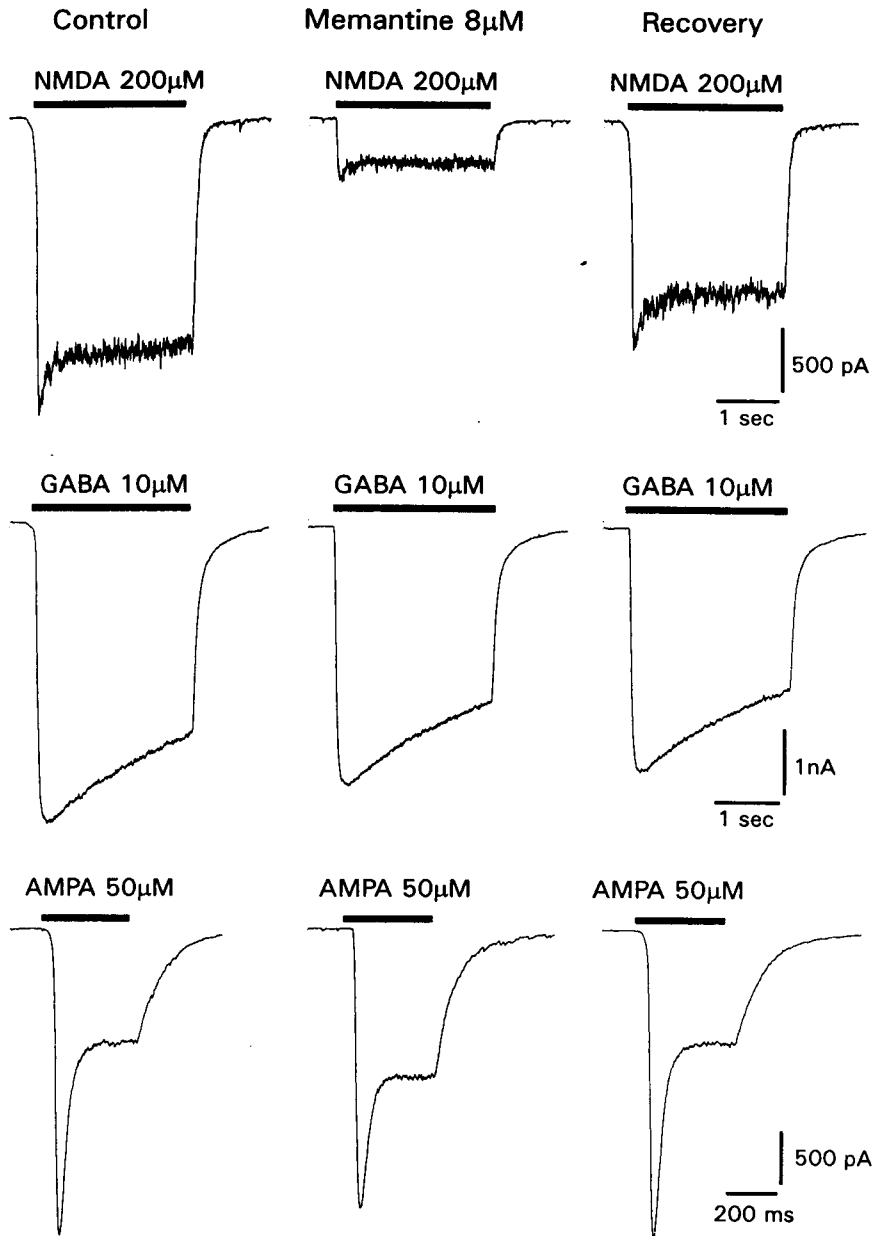


Fig. 1. Selective NMDA antagonistic effect of memantine on current responses of a single hippocampal neurone. NMDA (200 μ M, for 2.5 sec), GABA (10 μ M, for 2.5 sec) or AMPA (50 μ M, for 350 msec) were applied every 30 sec in the continuous presence of glycine 1 μ M and at a constant membrane potential of -70 mV. The left and right panels show control and recovery responses to the agonists tested. The middle panels show equilibrium agonist responses in the continuous presence of memantine 8 μ M. NB: different time scales.

RESULTS

Pilot experiments comparing the sensitivity of superior colliculus and hippocampal cells to AMPA (10–100 μM), GABA (1–100 μM) and NMDA (3–330 μM) revealed no obvious differences between the two cell types and confirmed the reproducibility and reliability of the techniques used. Responses to NMDA 200 μM , GABA 100 μM and AMPA 50 μM were selectively-antagonized by AP5 (30–300 μM), bicuculline methiodide 25 μM and NBQX (0.5–4 μM) respectively (data not shown).

Memantine 8 μM selectively-reduced whole cell current responses of hippocampal cells to NMDA 200 μM whilst having no effect on responses to GABA 10 μM or AMPA 50 μM (Fig. 1). On this illustrated cell, memantine seemed to modify slightly the kinetics of the response to AMPA with the peak response being somewhat reduced and the plateau response somewhat potentiated. Responses to all agonists were therefore divided into peak and plateau phases for quantitative analysis. However, as can be seen from the pooled data presented in Fig. 2 this effect was not consistent—neither plateau nor peak responses to AMPA were modified by memantine. The same was true for higher concentrations (100 μM) and longer applications of AMPA (data not shown). There were no apparent differences in the effects of memantine on agonist-induced current responses of

hippocampal and superior colliculus neurones. Data obtained from the two cell types were therefore pooled.

The blockade of whole cell current responses to NMDA 100 μM by memantine was concentration-dependent, the IC_{50} being $2.92 \pm 0.05 \mu\text{M}$ [Fig. 3(A)]—a value which fits well with those calculated from the above mentioned *in vitro* studies.

Further experiments were performed to assess the competitive, non- or uncompetitive nature of this antagonism. The NMDA concentration-response curve was shifted to the right by memantine 8 μM in a manner suggestive of an uncompetitive mechanism of action [Fig. 3(B)]. Moreover, the kinetics of the NMDA antagonism seen with memantine suggest that it is mediated by open channel block. Thus, the first response to NMDA following a 30 sec preincubation with memantine showed a profound change in kinetics—the peak current being less affected than the plateau current and stable antagonism in the continuous presence of memantine first being achieved after 3–5 applications of NMDA [Figs 4(A), 5(A) and 6(E)]. It seems unlikely that this effect reflects slow association/dissociation kinetics of memantine to a site outside of the NMDA channel as the use-dependency, kinetics and potency of the NMDA blockade by memantine were not altered in experiments where pre perfusion of memantine was omitted i.e. where memantine and NMDA were coapplied

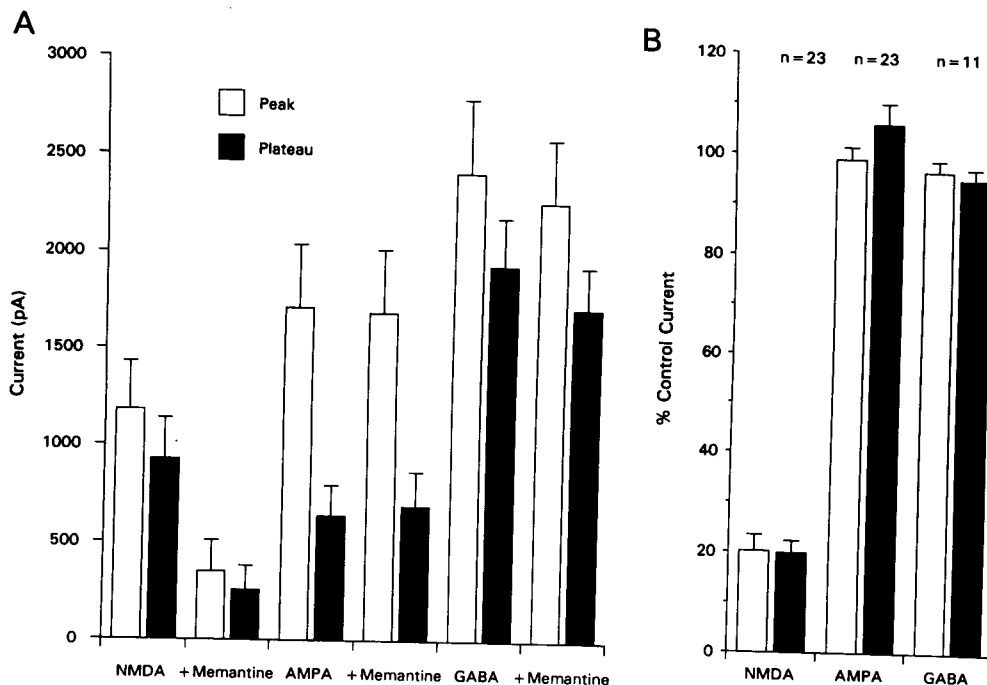


Fig. 2. Selective effects of memantine on pooled NMDA current responses. NMDA (200 μM), GABA (10 μM) and AMPA (50 μM) were applied as in Fig. 1. Current responses were divided into peak and plateau components, after subtraction of any leak current, to reveal possible effects on responses kinetics. (A) Control current responses and equilibrium current responses in the continuous presence of memantine 8 μM . (B) Equilibrium current responses in the continuous presence of memantine 8 μM as a percentage of control. Error bars represent the SEM.

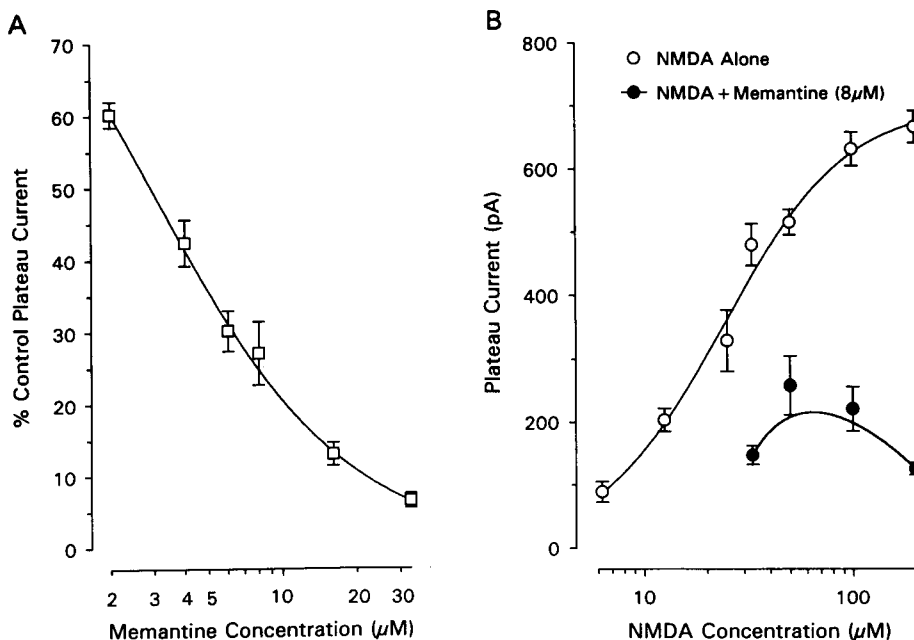


Fig. 3. (A) Concentration-dependent effects of memantine on pooled NMDA current responses of superior colliculus neurones. NMDA $100 \mu\text{M}$ was applied as in Fig. 1. Pooled responses were quantified as plateau current (pA) after subtraction of any leak current and plotted, after normalization to control, against log concentration of memantine. The number of cells tested with each concentration of memantine was as follows ($2 \mu\text{M}$ $n = 26$, $4 \mu\text{M}$ $n = 13$, $6 \mu\text{M}$ $n = 16$, $8 \mu\text{M}$ $n = 31$, $16 \mu\text{M}$ $n = 17$, $33 \mu\text{M}$, $n = 8$). The IC_{50} of memantine ($2.92 \pm 0.05 \mu\text{M}$) was calculated according to the 4 parameter logistic equation. (B) Noncompetitive nature of the antagonism of NMDA responses by memantine in hippocampal neurones. NMDA (6.25 – $200 \mu\text{M}$, $n = 18$) was applied as in Fig. 1. Pooled data of responses to NMDA in the absence and presence of memantine $8 \mu\text{M}$ were plotted as means against log concentration of NMDA. Error bars represent SEM.

[Fig. 5(A) and (B)] whereas little antagonism was seen when memantine was only present between NMDA applications [Fig. 5(C)].

The kinetics of blockage of current responses to NMDA $200 \mu\text{M}$ with memantine $16 \mu\text{M}$ ($n = 18$) were much faster than for approximately equieffective concentrations of MK-801 $1 \mu\text{M}$ ($n = 5$), dextrorphan $3.3 \mu\text{M}$ ($n = 4$) and PCP $3.3 \mu\text{M}$ ($n = 3$) but were comparable to ketamine $10 \mu\text{M}$ ($n = 8$) and dextromethorphan $10 \mu\text{M}$ ($n = 5$, data not shown). In contrast, the kinetics of NMDA antagonism seen with Mg^{2+} (1.5 mM , $n = 5$) were faster still. Typical examples of these differences in kinetics are illustrated in Fig. 6 and the pooled data for MK-801 are compared to memantine in Fig. 4. The IC_{50} values of these uncompetitive antagonists against NMDA $200 \mu\text{M}$ (glycine $1 \mu\text{M}$) at equilibrium were as follows: MK-801 $0.137 \pm 0.04 \mu\text{M}$ ($n = 12$); PCP $1.04 \pm 0.16 \mu\text{M}$ ($n = 15$); dextrorphan $1.34 \pm 0.26 \mu\text{M}$ ($n = 12$); ketamine $1.56 \pm 0.01 \mu\text{M}$ ($n = 20$); memantine $2.25 \pm 0.31 \mu\text{M}$ ($n = 49$); dextromethorphan $6.10 \pm 3.6 \mu\text{M}$ ($n = 9$). The higher potency of memantine against NMDA $200 \mu\text{M}$ than NMDA $100 \mu\text{M}$ is a reflection of the uncompetitive nature of the antagonism.

The kinetics of this fast open channel blockade were studied further by applying memantine 4 – $33 \mu\text{M}$ for 5 sec in the continuous presence of NMDA

$200 \mu\text{M}$ (Fig. 7). These studies indicated that memantine exhibits relatively fast, concentration-dependent open channel blocking kinetics (k_{on} $2.88 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$) whereas the offset rate was concentration-independent (k_{off} 0.20 sec^{-1}). Calculation of the ratio $k_{\text{off}}/k_{\text{on}}$ revealed an apparent K_d value of $0.69 \mu\text{M}$ which is comparable to the IC_{50} calculated at equilibrium. For comparison PCP and dextrorphan had k_{on} s of $0.135 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$ and $0.035 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$ and k_{off} s of 0.05 sec^{-1} and 0.075 sec^{-1} respectively.

Further support for an open channel-blocking effect of memantine was provided by the voltage-dependency of this effect. These recordings were performed with the "perforated patch" technique which allowed stable responses to be recorded for 1 hr or more. Thus, whilst the current-voltage relationship for responses to NMDA was as expected for the solutions used i.e. almost linear in the absence of Mg^{2+} and with a reversal potential around 0 mV , the antagonist effects seen with memantine $10 \mu\text{M}$ on inward NMDA currents recorded at -70 mV ($23.5 \pm 1.6\%$ of control) were not apparent for outward currents recorded at $+70 \text{ mV}$ ($101.7 \pm 7.8\%$ of control, $n = 4$). This voltage-dependency was not absolute, as a higher concentration of $33 \mu\text{M}$ was able to reduce outward current responses at positive

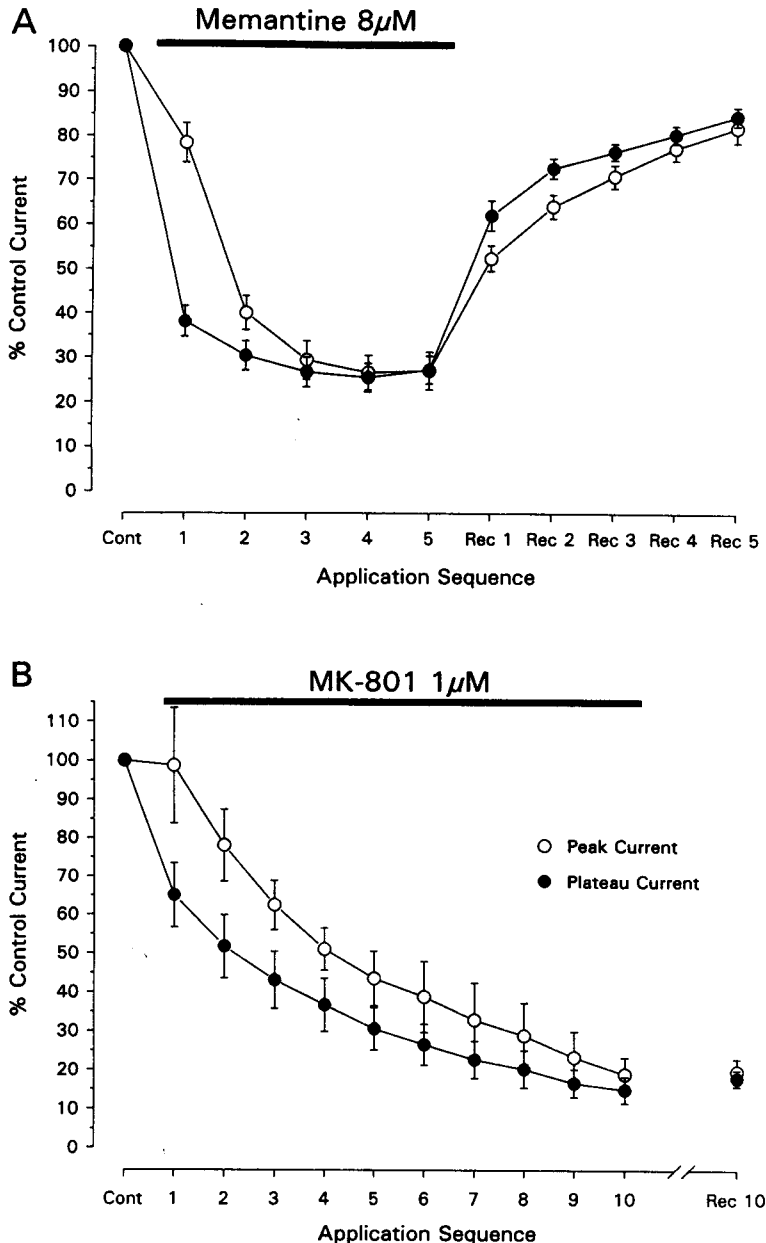


Fig. 4. Use-dependent effects of memantine $8 \mu\text{M}$ and MK-801 $1 \mu\text{M}$ on pooled NMDA current responses. NMDA $200 \mu\text{M}$ was applied as in Fig. 1. Current responses were divided into peak and plateau components after subtraction of any leak current and plotted against application sequence. (A) On the left are control responses to NMDA immediately before 5 successive applications in the continuous presence of memantine ($8 \mu\text{M}$, $n = 25$): note the rapid use-dependency of the blockade which is most evident for the greater blockade of the plateau component than the peak component of the first response to NMDA in the presence of memantine. The use-dependency of the recovery was slower than that of the blockade—maximal recovery was first obtained after 5 NMDA applications after removal of memantine (Rec 1 to Rec 5). (B) On the left are control responses to NMDA immediately before 10 successive applications in the continuous presence of MK-801 ($1 \mu\text{M}$, $n = 4$): note the pronounced use-dependency of the blockade. No recovery was seen even after 10 NMDA applications following removal of the antagonist (Rec 10). Depolarization to $+70 \text{ mV}$ was able to release the blockade albeit with a very slow time course (data not shown).

potentials to some degree [Fig. 8(B)]. In contrast, the voltage-dependency of blockade by Mg^{2+} (0.3 – 3.3 mM) was apparent at all concentrations tested [Fig. 8(A), 1 mM : $-70 \text{ mV} = 5.2 \pm 0.7\%$ of control,

$+70 \text{ mV} = 101.4 \pm 1.4\%$ of control, $n = 3$]. The voltage-dependency of ketamine was similar to memantine [Fig. 9(B), $10 \mu\text{M}$: $-70 \text{ mV} = 14.0 \pm 3.3\%$ of control, $+70 \text{ mV} = 77.6 \pm 4.2\%$ of control, $n = 4$].

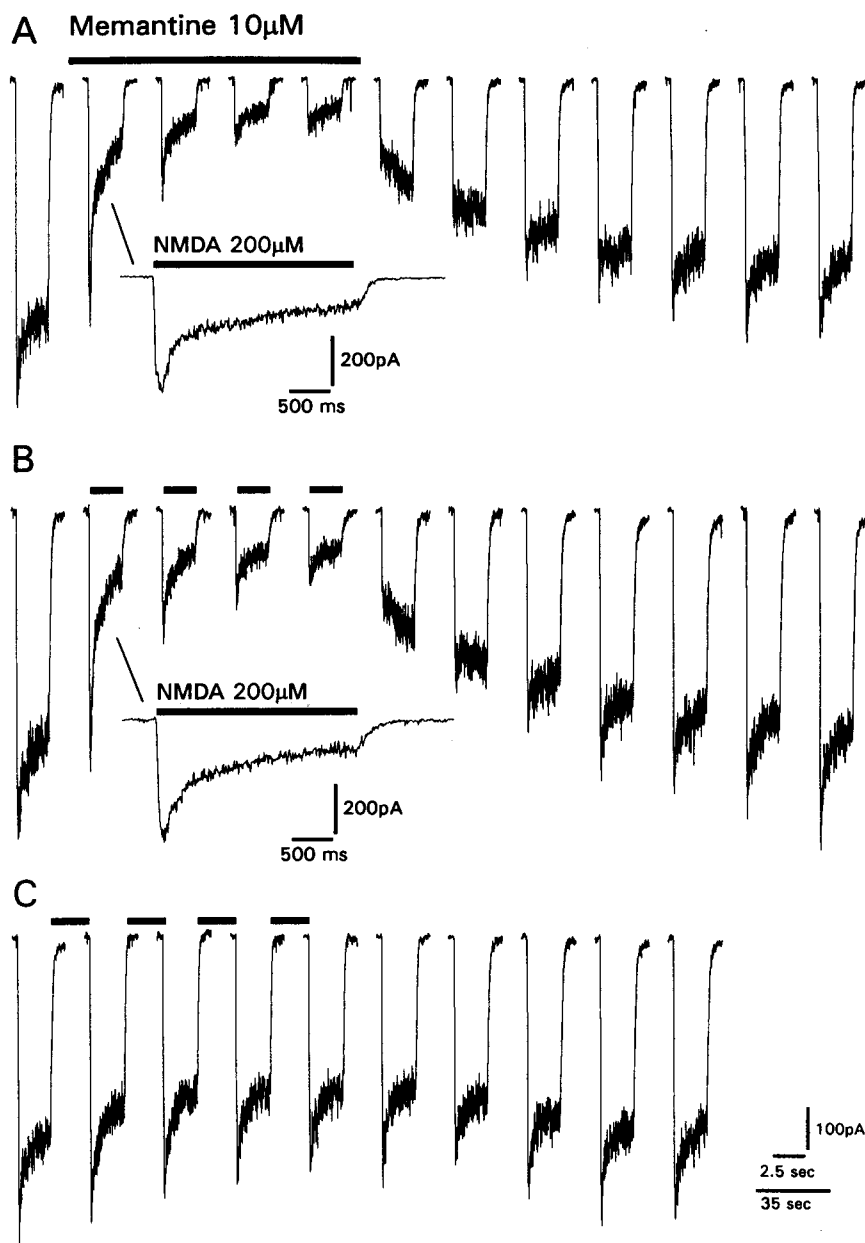


Fig. 5. Evidence for open channel blockade by memantine. NMDA $200 \mu\text{M}$ was applied for 2.5 sec every 30 sec but this inter response interval has been omitted to allow better resolution of the kinetics of individual responses (blank spaces in trace). (A) Memantine $10 \mu\text{M}$ was continuously present for 2 min as indicated by the bar. Insert shows the first response to NMDA in the presence of memantine on an expanded time scale. (B) Memantine $10 \mu\text{M}$ was only coapplied with NMDA for 2.5 sec every 30 sec. The insert shows the similar time course of the blockade of the first response to that seen in A. (C) Memantine $10 \mu\text{M}$ was only applied between applications of NMDA.

In this regard it should be noted that ketamine was somewhat more potent than memantine. In contrast, the voltage-dependency of dextrorphan [Fig. 9(A), $3.3 \mu\text{M}$: $-70 \text{ mV} = 13.7 \pm 2.2\%$ of control, $+70 \text{ mV} = 58.2 \pm 3.4\%$ of control, $n = 3$] and PCP [Fig. 10(A), $3 \mu\text{M}$: $-70 \text{ mV} = 22.0 \pm 1.4\%$ of control, $+70 \text{ mV} = 70.9 \pm 4.8\%$ of control, $n = 3$] was less pronounced and MK-801 was practically voltage-independent [Fig. 10(B), $0.3 \mu\text{M}$: $-70 \text{ mV} =$

$19.3 \pm 4.6\%$ of control, $+70 \text{ mV} = 19.6 \pm 3.9\%$ of control, $n = 3$].

An alternative, although unlikely explanation for the weaker use-dependency of the blockade by memantine in comparison to MK-801 is that memantine might additionally modulate the activity of NMDA receptors by interacting with the glycine_B site. If this were true then it should have been possible to reverse the antagonistic effects of memantine by increasing

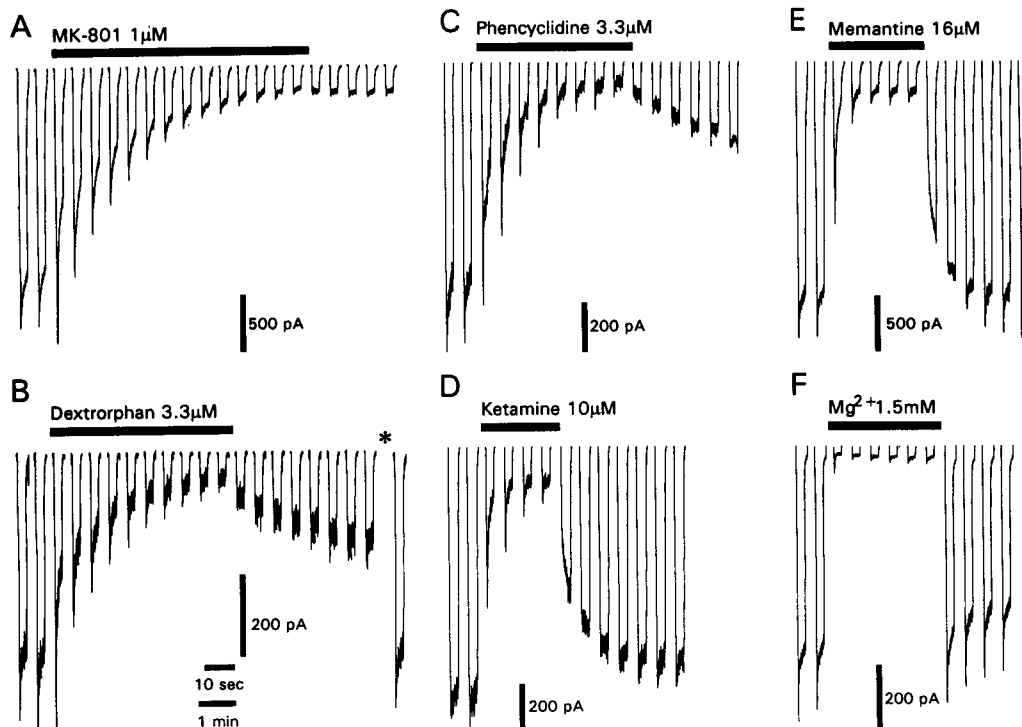


Fig. 6. Comparison of the use-dependency of MK-801, dextrorphan, phencyclidine, ketamine, memantine and Mg^{2+} in blocking NMDA current responses. Presentation and experimental paradigm as in Fig. 5(A). (A) MK-801 $1 \mu M$ was continuously present for 7 min as indicated by the bar. No recovery from the effects of MK-801 were seen even after 10 NMDA applications in the absence of this antagonist at -70 mV but reversal could be obtained at $+70$ mV. (B) Dextrorphan $3.3 \mu M$ was continuously present for 5 min. Partial recovery was seen even after 8 NMDA applications in the absence of antagonist at -70 mV and full reversal was achieved after prolonged application of NMDA $+70$ mV and then returning to -70 mV (see asterisk). (C) Phencyclidine (PCP, $3.3 \mu M$) was continuously present for 4 min. (D) Ketamine $10 \mu M$ was continuously present for 2 min. (E) Memantine $16 \mu M$ was continuously present for 2.5 min. Note the rapid use-dependency of the blockade which is most evident for the greater blockade of the plateau component than the peak component of the first response to NMDA in the presence of memantine. (F) Mg^{2+} 1.5 mM was continuously present for 3 min. Note the almost instantaneous onset of, and recovery from, the channel blocking effects of Mg^{2+} . NB: responses to MK-801, memantine and Mg^{2+} were recorded from the same neurone.

concentrations of glycine. This was not the case, the NMDA antagonistic effects of memantine were not dependent on glycine concentration (0.1 – $100 \mu M$), ruling out a possible interaction with glycine_B sites (Table 1).

Similar effects of memantine were seen in the cortical wedge preparation. Thus memantine 1 –

Table 1. Failure of glycine to reverse the antagonistic effects of memantine $8 \mu M$ on pooled current responses of neurones to NMDA.

Glycine concentration (μM)	n	% Control	SEM
0.1	12	20.2	1.5
0.3	24	21.8	1.6
1.0	40	25.3	3.1
2.5	14	22.5	2.6
10.0	10	19.1	1.1
100.0	6	17.7	1.9

NMDA $200 \mu M$ was applied for 2.5 sec every 30 sec in the continuous presence of various concentrations of glycine. Pooled responses were quantified as plateau current (pA) after subtraction of any leak current. The antagonistic effects of memantine $8 \mu M$ were then normalized with respect to control (% Control). SEM represents the standard error of the mean.

$100 \mu M$ antagonized responses to NMDA $40 \mu M$ with an IC_{50} of $12.9 \pm 1.5 \mu M$ ($n = 23$). Since the slow onset of antagonism was reminiscent of that seen with MK-801 (Fig. 11) this aspect was studied by comparing the NMDA antagonistic effects of memantine $31.6 \mu M$ after 1 hr in two pairs of slices maintained at 18 – $19^\circ C$ after frequent or infrequent application of NMDA. There was no difference within the pairs of slices (33, 35 and 24, 27% of control respectively). Against AMPA $40 \mu M$ there was little evidence of antagonism except at memantine $100 \mu M$ which reduced AMPA responses to $56.8 \pm 16.1\%$ of control ($n = 4$). At lower concentrations of memantine there was evidence of a small increase in the amplitude of AMPA responses. Thus at $31.6 \mu M$ ($n = 6$) and $10 \mu M$ ($n = 4$), AMPA responses were $106 \pm 0.8\%$ and $112 \pm 2\%$ of control respectively. In Mg^{2+} and TTX-free Krebs, these cortical slices show epileptiform activity which was abolished rapidly by memantine $100 \mu M$, slowly by $31.6 \mu M$ and almost by $10 \mu M$. Thus after 20 min perfusion with memantine $10 \mu M$ the bursts per minute were reduced to $49 \pm 1\%$ and

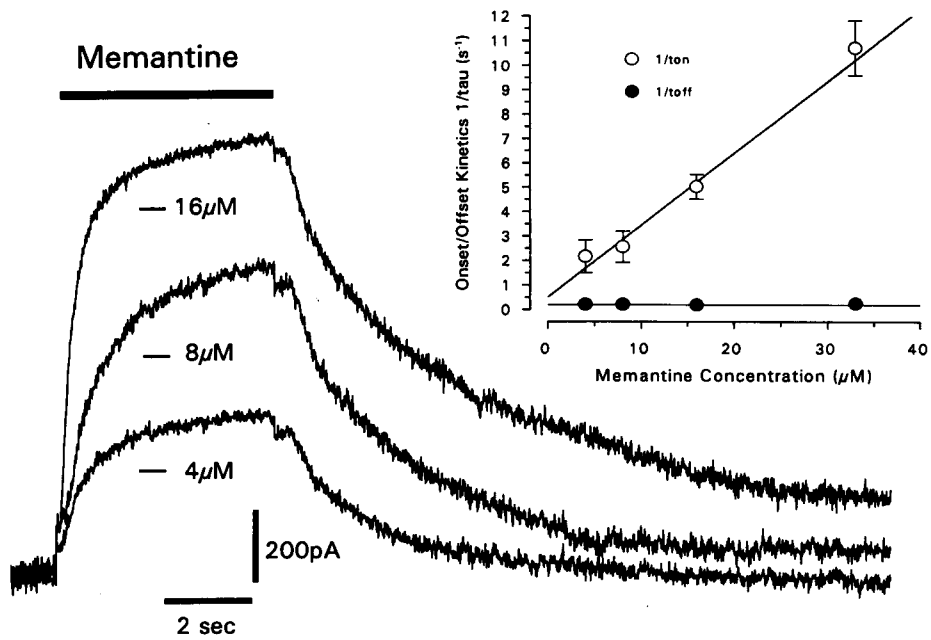


Fig. 7. Kinetics of NMDA open-channel block by memantine. NMDA 200 μM was applied continuously. Memantine 4–16 μM was then coadministered for 5 sec with NMDA as indicated by the bar. The insert shows the concentration-dependence of the rate of onset of memantine blockade and the concentration-independence of the offset rate (4–33 μM , $n = 8$).

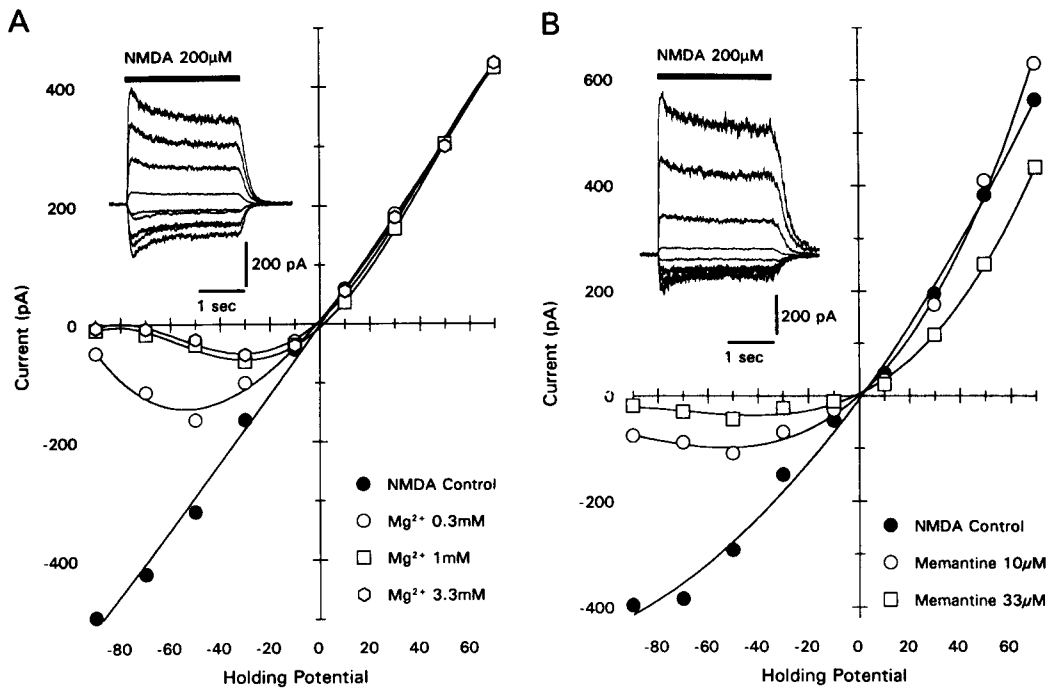


Fig. 8. Voltage-dependence of the blockade of NMDA receptors by Mg^{2+} and memantine. NMDA was applied for 2.5 sec every 30 sec in the continuous presence of glycine 1 μM at various membrane potentials. Recordings were made with the "perforated patch" technique i.e. in cell attached mode with nystatin filled electrodes. (A) NMDA responses were recorded in the absence and presence of Mg^{2+} (0.3, 1 and 3.3 mM). Plateau current responses have been plotted against membrane potential. Insert shows original data for the i.v. curve in the presence of Mg^{2+} 0.3 mM. (B) NMDA responses were recorded in the absence and presence of memantine (10 and 33 μM). Insert shows original data for memantine 10 μM .

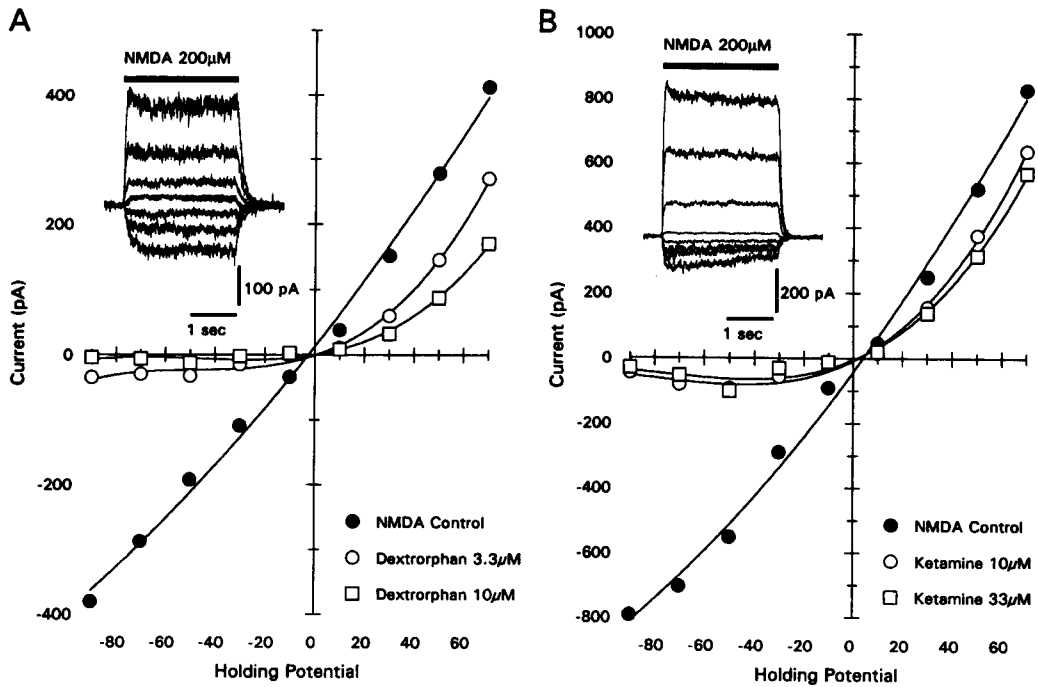


Fig. 9. Voltage-dependence of the blockade of NMDA receptors by dextrorphan and ketamine. Presentation and experimental paradigm as in Fig. 8. (A) NMDA responses were recorded in the absence and presence of dextrorphan (3.3 and 10 μM). Insert shows original data for dextrorphan 3.3 μM . (B) NMDA responses were recorded in the absence and presence ketamine (10 and 33 μM). Insert shows original data for ketamine 10 μM .

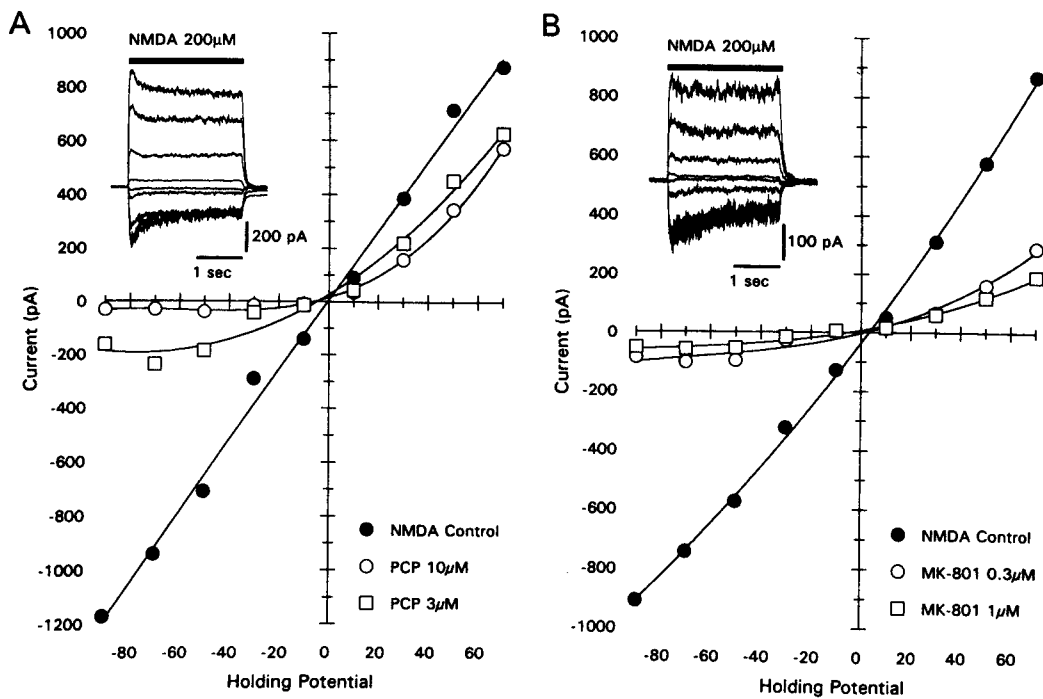


Fig. 10. Voltage-dependence of the blockade of NMDA receptors by phencyclidine (PCP) and MK-801. Presentation and experimental paradigm as in Fig. 8. (A) NMDA responses were recorded in the absence and presence of PCP (3 and 10 μM). Insert shows original data for PCP 3 μM . (B) NMDA responses were recorded in the absence and presence MK-801 (0.3 and 1 μM). Insert shows original data for MK-801 0.3 μM .

of NMDA receptor agonists *in vitro* (Erdö and Schäfer 1991; Chen *et al.*, 1992; Osborne and Quack, 1992; Weller, Finielsmarlier and Paul, 1993) and *in vivo* (Keilhoff and Wolf, 1992) and reduce neurotoxic damage in *in vivo* models of forebrain ischaemia (Seif el Nasr, Peruche, Rossberg, Mennel and Kriegelstein, 1990; Chen *et al.*, 1992).

In fact, removal of Mg^{2+} from the bathing solution can prevent the induction of LTP in hippocampal slices by allowing continuous low frequency activation of NMDA receptors (Coan, Irving and Collingridge, 1989) and can, after prolonged periods of time, induce epileptic activity which is blocked by memantine and other NMDA antagonists (Apland and Cann, 1991; Aram, Martin, Tomczyk, Zeman, Millar, Pohler and Lodge, 1989). Indeed, low concentrations of APV can restore LTP under such conditions (Coan *et al.*, 1989). A similar low frequency, but prolonged, activation of NMDA receptors due to relatively small but sustained increased extracellular levels of glutamate is likely to underlie the neuropathological changes seen in animal models of ischaemia (Globus, Busto, Martinez, Valdes, Dietrich and Ginsberg, 1991) and has been suggested to occur in dementia (see Greenamyre and Young, 1989). Blockade of such on-going activity may therefore not only prevent further pathological changes but may also have symptomological effects by restoring the physiological activation of NMDA receptors. This assumption is supported by the symptomological cognitive enhancement seen with memantine in clinical trials of dementia (Ditzler, 1991; Görtelmeyer and Erbler, 1992).

MK-801, dextrorphan and PCP antagonized responses to NMDA with much slower, strongly use-dependent kinetics and less marked voltage-dependency (Huettner and Bean, 1988; Karschin, Aizenman and Lipton, 1988; Halliwell *et al.*, 1989; Ffrench-Mullen and Rogawski, 1989). These biophysical properties might be expected to prevent both the pathological and physiological activation of NMDA receptors and probably account for the block of LTP *in vitro* seen with both PCP (Stringer, Greenfield, Hackett and Guyenet, 1983; Stinger, Hackett and Guyenet, 1984; Diamond, Dunwiddie and Rose, 1988; Bourne, Capek and Esplin, 1989) and MK-801 (Coan, Saywood and Collingridge, 1987; Okada, Miyamoto, Tomita, Sakurai and Shibata, 1989; Schwartzwelder, Ferrari, Anderson and Wilson, 1989). Thus, the slow dissociation kinetics and less marked voltage-dependency of MK-801 and PCP probably prevent them from leaving the NMDA receptor channel following its transient activation by high concentrations of synaptically released glutamate (Clements, Lester, Tong, Jahr and Westbrook, 1992) even though the postsynaptic membrane is strongly depolarized by current flow through AMPA receptors. These effects have been confirmed for LTP *in vivo* (Stringer and Guyenet, 1983; Gilbert and Mack, 1990; Morimoto, Katayama, Inoue and

Sato, 1991) and probably underlie the memory deficits observed in rats tested in the radial maze (Danysz, Wroblewski and Costa, 1988; Butelman, 1989, 1990; Ward, Mason and Abraham, 1990).

As ketamine and dextromethorphan blocked NMDA responses with similar kinetics to memantine it is clearly pertinent to compare the effects of these substances in models of synaptic plasticity. Both ketamine and dextromethorphan can block LTP *in vivo* (Stringer and Guyenet, 1983; Zhang and Levy, 1992; Krug, Matthies, Wagner and Brodemann, 1993) and disrupt learning in animal models (Sanger and Joly, 1991; Lalonde and Joyal, 1991). Surprisingly, data on their effects on LTP *in vitro* are lacking. It is therefore plausible that pharmacokinetic or metabolic effects may be responsible for their observed effects *in vivo*. This is most likely for dextromethorphan which is rapidly metabolized to the more active metabolite dextrorphan (Silvasti, Karttunen, Tukiainen, Kokkonen, Hänninen and Nykänen, 1987) which had similar effects to PCP and MK-801 in this study and can itself disrupt learning in rats (Sierocinska, Nikolaer, Danysz and Kaczmarek, 1991). In contrast, memantine is poorly metabolized in man and none of the known metabolites is a potent NMDA antagonist (data not shown).

An important aspect in relation to the interpretation of data on the use-dependent kinetics of NMDA receptor open channel blockers is the finding that the onset of blockade by MK-801 shows little use-dependence *in vivo* (Davies *et al.*, 1988). These authors also showed that the significant difference between *in vivo* and *in vitro* models was temperature. However, recovery from this blockade was use-dependent. As the recovery from blockade was slower than the onset for all substances tested at higher concentrations in the present *in vitro* study it may be that the *in vivo* microelectrophoretic and *in vitro* slow bath application used in the study of Davies and colleagues was too slow to resolve use-dependency of the onset of antagonism. Thus, whilst it is clear that the kinetics of antagonism are faster at higher temperatures, the relative differences in the kinetics of substances like MK-801 and memantine are still likely to occur *in vivo*, especially at the level of fast synaptic transmission.

In conclusion, memantine selectively and use-dependently blocked current responses of cultured neurones to NMDA. The fast kinetics and voltage-dependency of this effect may account for the ability of memantine to block the neurotoxic effects of NMDA receptor activation at concentrations which have little or no effect on NMDA receptor-mediated physiological processes such as LTP. These properties are likely to underlie the beneficial therapeutic effects of memantine.

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