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Memantine selectively depresses NMDA receptor-mediated responses of rat spinal neurones in vivo

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The clinically used agent memantine (1-amino-3,5-dimethyladamantane) can act as an antagonist of NMDA (*N*-methyl-D-aspartate) when tested in vitro, but whether this applies with clinically relevant doses under in vivo conditions is not clear. In this study memantine has been compared with the known NMDA channel blocker ketamine, by intravenous administration in anaesthetized rats, for effects on the responses of spinal neurones both to iontophoretic administrations of excitatory amino acids and to peripheral noxious stimuli. Spontaneous activity, nociceptive responses and blood pressure were not significantly affected by memantine and ketamine, whereas both agents selectively reduced responses to NMDA.

Adamantane derivatives such as amantadine have long been known for their anti-spastic and anti-Parkinson actions. Memantine, the 1-amino-3,5-dimethyladamantane derivative, possesses locomotor stimulant effects in rats [5, 11] and mice [12], has been used in the clinical treatment of spasticity [8], and improves cognition and vigilance in patients with dementia-related deficits [6, 7].

Recent data indicate that the therapeutic actions of memantine may be related to inhibition of excitatory amino acid (EAA)-mediated activity. Results from patch-clamp recordings of NMDA-activated membrane currents in cultured neurones indicated that memantine, like the NMDA channel blocker MK-801, reduced NMDA-evoked activity [2, 4, 13]. Memantine, at therapeutic concentrations, has also been shown to displace [³H]MK-801 from binding sites in post-mortem human cortex [10].

It has not yet been demonstrated, however, whether memantine can indeed act as an NMDA antagonist under in vivo conditions (i.e. in the presence of Mg²⁺ and at body temperature), whether such an action is selective, and whether synaptic responses are affected. We now report that memantine does act as a selective NMDA antagonist when administered intravenously in experiments on rat spinal neurones in vivo.

Experimental methods were similar to those described

in detail previously [9]. Male Wistar rats (300–390 g, *n* = 10) were anaesthetized with halothane and the trachea, right jugular vein and carotid artery were cannulated. After a laminectomy (L₃–T₈), the spinal cord was sectioned at thoracic 9–10 level. Thereafter, anaesthesia was maintained with α -chloralose (50 mg/kg i.v. initially, supplemented with doses of 20 mg/kg as required). Body temperature was maintained close to 37°C; blood pressure was monitored continuously and systolic pressure remained above 100 mmHg throughout.

Extracellular records of neuronal spike activity and microiontophoretic ejection of amino acids were achieved using 7 barrel micropipettes. The recording barrel was filled with 3.5 M NaCl and other barrels with NMDA (100 mM in 100 mM NaCl), AMPA ((*RS*)- α -amino-3-hydroxy-5-methyl-4-isoxazole propionate, 10 mM in 200 mM NaCl), kainate (5 mM in 200 mM NaCl), AP5 (D-amino,5-phosphonovalerate, 50 mM in 150 mM NaCl), CNQX (6-cyano,7-nitroquinoxaline-2,3-dione, 1 mM in 50 mM NaCl), all at pH 7.5. The last barrel contained Pontamine sky blue (2% in 0.5 M Na acetate) which was ejected at recording sites for retrospective histology. Neurones were selected with receptive fields on the ipsilateral hind paw. Each cell was activated in 5 min cycles of four stimuli comprising NMDA, AMPA and kainate (10 neurones) plus either noxious heat (6 neurones) or pinch (3 neurones) applied to the receptive fields. Each iontophoretic administration lasted a minimum of 40 s in each cycle. Heat (47.0–49.5°C) or pinch

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MEMANTINE HCl

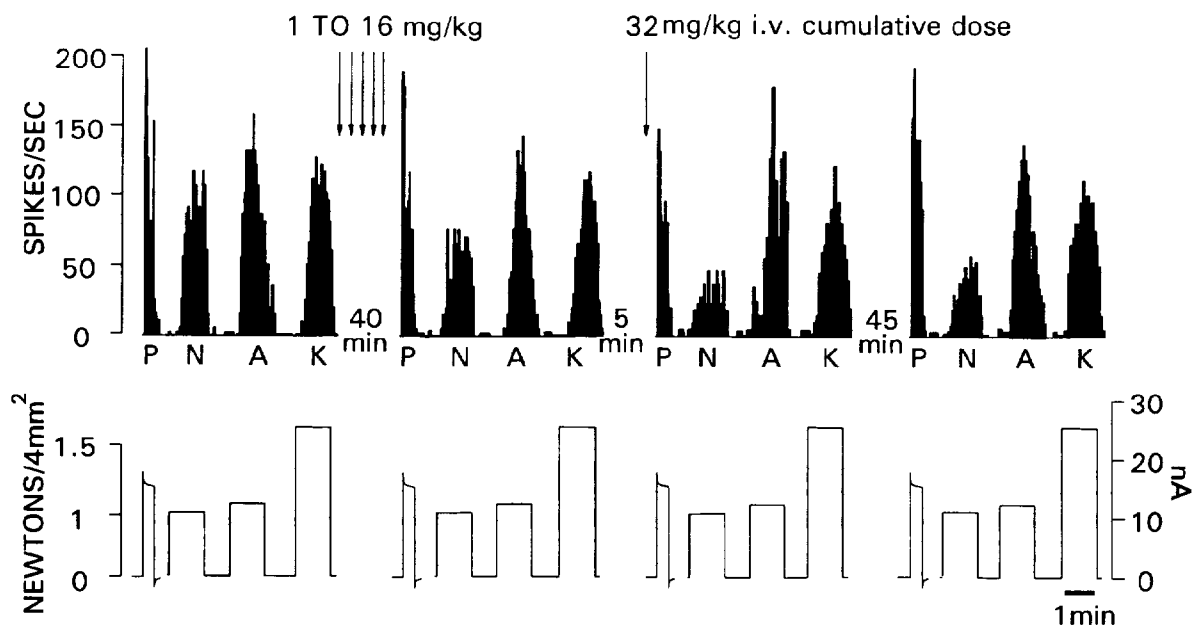


Fig. 1. Effects of intravenous memantine on the spike discharge of a rat spinal dorsal horn neurone to four stimuli applied in a regular 5 min cycle: noxious pinch (P) and microiontophoretic, NMDA (N), AMPA (A) and kainate (K). The diagram shows records of the last cycle before the injection of the first dose of memantine, immediately after the injection of the first 5 cumulative doses (1, 2, 4, 8 and 16 mg/kg; trace taken after a break in the record of 40 min), immediately after the last dose of memantine (32 mg/kg); and the last cycle studied, 50 min after the last dose of memantine. Pinch stimuli were applied to a toe of the ipsilateral hindlimb; force is expressed in Newtons applied over 4 mm². Microiontophoretic currents are shown for the amino acids. α -chloralose-anaesthetized spinalized rat.

(1.2–1.5 N over 4 mm²) stimuli were applied over 15 s; analysis was performed of the spike count over the last 10 s of these stimuli. Neuronal activity, stimulus details and physiological data were recorded continuously on a pen recorder. A trace of responses obtained with this cycle of stimuli is shown in Fig. 1. Counts of evoked activity, in epochs related to the stimuli, were analyzed by computer on-line and the effects were expressed as a percentage of pre-drug control (taken as the mean of 3 cycles).

Memantine HCl (Merz) was dissolved in saline and was injected i.v. in a volume of 0.3 ml, in a cumulative log₂ dose progression (1–32 mg/kg in 7 of 10 cells) given at 10 min intervals (i.e. every 2 stimulus cycles). Maximal effects occurred rapidly, and the analysis below is of the effect on the first cycle of responses after each dose. Other EAA antagonists were also tested on the same cells. Ketamine [1] was administered (at 1–4 mg/kg) following the same protocol, but before memantine; this NMDA channel blocker was chosen because of its rapid metabolism. The competitive NMDA antagonist D-AP5, and the AMPA-kainate antagonist CNQX, were tested by iontophoretic ejection on 9 of the 10 neurones

before the memantine test; these agents produced the expected selective reduction of amino acid-induced responses. Statistical significance was calculated by the non-parametric Mann-Whitney *U*-test, comparing the responses obtained after each dose of memantine with pre-drug control values.

The 10 cells tested were classified as either wide dynamic range (WDR, $n = 7$) or high threshold mechanoreceptive (HTMR) neurones. They all responded to the three amino acids as well as to noxious peripheral stimuli. Spontaneous activity was studied over periods of 60 s within each cycle. Three of the neurones showed no spontaneous activity and the other 7 discharged at a low rate (mean 1.5 spikes/s). All neurones were located in laminae III–VI in the dorsal horn.

Memantine dose-dependently reduced the responses to NMDA on all neurones studied. Fig. 1 shows a ratemeter record from a single experiment, illustrating the protocol and a typical dose-dependent effect; Fig. 2 shows pooled data for the 7 neurones tested with the full dose range. Results with lower maximal doses on a further 3 cells were consistent with this pattern. The lowest cumulative dose at which a significant ($P < 0.01$) mean

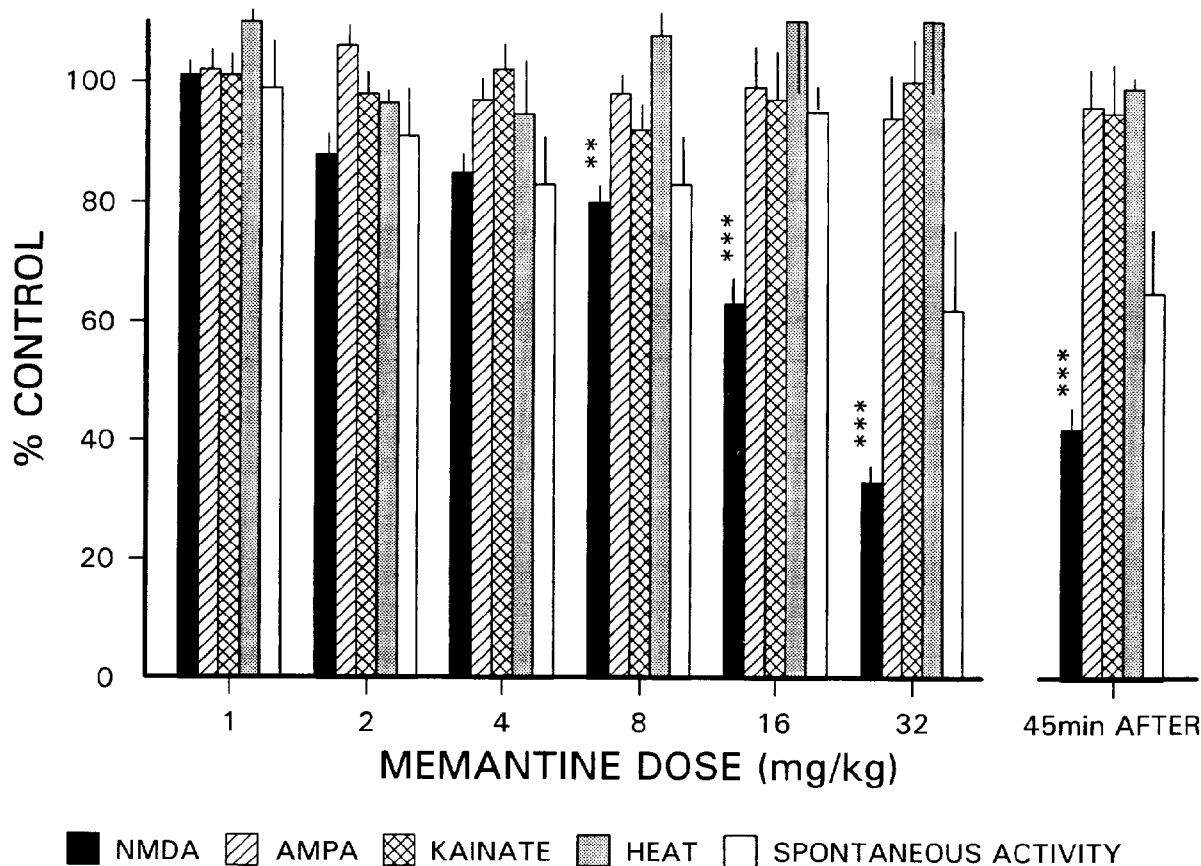


Fig. 2. Pooled data of the effects of memantine, 1 to 32 mg/kg i.v., on the responses of rat spinal neurones to iontophoretic administrations of NMDA (solid bars), AMPA (diagonally hatched bars), kainate (cross-hatched bars), to noxious heat stimuli (stippled bars) as well as on spontaneous activity (open bars). Responses of the neurones recorded 45 min after the last memantine injection (32 mg/kg) are also shown. The data are expressed as percentages of pre-drug control responses. Error bars show standard error of the mean; $n = 7$ for amino acids, 4 for heat. Original spike count data were compared with pre-drug values using the Mann-Whitney U -test (** $P < 0.01$; *** $P < 0.001$).

reduction occurred was 8 mg/kg, which caused a reduction to $69 \pm 8\%$ of control. The ID_{50} (calculated from mean data by regression) was estimated at 22 mg/kg. The effects of memantine were monitored for 45 ($n = 8$) to 90 min ($n = 2$) after the last dose. At 45 min there was no significant recovery of responses to NMDA (Fig. 2). Only slight further recovery (by 24% of the peak effect) was seen 90 min after the last dose.

Memantine did not significantly affect the responses of these neurones to the other stimuli tested (Fig. 2); after 32 mg/kg, responses to AMPA were $94 \pm 11\%$ control; to kainate $98 \pm 11\%$ control (both $n = 7$); to noxious heat $112 \pm 20\%$ ($n = 4$); and to noxious pinch 98% ($n = 3$). Although the low spontaneous activity was reduced, this was not statistically significant; in any case a reduction from these low values of activity (1.5 spikes/s) is unlikely to be pharmacologically important.

Memantine produced minimal variations of the mean arterial blood pressure. After 16 mg/kg, blood pressure

remained at $99 \pm 6\%$ control; with 32 mg/kg there was a reduction, to $80 \pm 9\%$ of control, that was not significant. Respiration generally remained normal (as judged by visual inspection) although in two animals it became fast and shallow with the highest dose.

Ketamine had effects similar to those of memantine on all 9 neurones tested. It caused a highly significant reduction of the NMDA-evoked activity with an ID_{50} of 3.5 mg/kg. This effect was selective, there being no significant reduction at the highest dose tested (4 mg/kg) of the activity evoked by AMPA ($88 \pm 7\%$ of control) or kainate ($93 \pm 7\%$ of control). Responses to noxious heat stimuli also remained unchanged at $91 \pm 6\%$ of control ($n = 6$).

The results obtained in these experiments are consistent with previous observations from in vitro preparations [2,4], in showing that memantine is an antagonist of NMDA. This antagonism is selective in living animals, there being no effect on responses to AMPA or kainate.

This result is consistent with the finding that memantine displaces MK-801 [10] which itself binds to the phencyclidine site in the NMDA ionophore complex [15].

Memantine is not, however, a potent NMDA antagonist. When compared on the same neurones with ketamine, itself a relatively weak PCP-site ligand [1], memantine was some 6 times weaker.

The lack of effect of memantine on the polysynaptic responses tested in the dorsal horn indicates that it does not have non-selective actions on synaptic transmission. The uncompetitive nature of memantine block, together with its relatively rapid off-rate kinetics, has been taken to imply that it should preserve normal levels of NMDA receptor-mediated activity whilst reducing pathological responses to enhanced levels of amino acids acting at NMDA receptors [4,13]. This might at first be taken as an explanation for the lack of effects on the excitatory synaptic responses tested. However ketamine, an NMDA channel blocker known to be able to affect synaptic responses of ventral horn neurones *in vivo* [9], was equally ineffective against these nociceptive responses. It will therefore be important to test memantine against a synaptic response *in vivo* that can be demonstrated with other antagonists to have a significant NMDA-receptor mediated component.

These experiments clearly demonstrate that memantine crosses the brain-blood barrier quickly and that it has long-lasting effects; the latter is consistent with the stimulation of motor activity in mice for up to 5 h [14]. The antagonism of NMDA occurs at doses similar to, or somewhat higher than, those at which behavioural effects are observed in rats (see for example ref. 3).

In conclusion, memantine *i.v.* in anaesthetized rats behaved like the NMDA channel blocker ketamine in selectively reducing NMDA-evoked neuronal activity. It had no effect on responses to AMPA, kainate or peripheral noxious stimulation. Its action lasted more than 1.5 h and side effects on blood pressure and respiration were minimal. The importance of these results lies in the fact that memantine is the first NMDA channel blocker not to have excessive side effects in man, presumably because of its unique channel blocking kinetics [4, 13].

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