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Enhancement of long-term spatial memory in adult rats by the noncompetitive NMDA receptor antagonists, memantine and neramexane

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Abstract

Memantine and neramexane are noncompetitive NMDA receptor antagonists which have been investigated for their promising effects in aiding memory in people with dementia. Memantine is approved for the treatment of Alzheimer's disease, and neramexane is currently under development for this indication. Therefore, the present study provided a comparative assessment of the effects of equimolar doses of memantine and neramexane on spatial (hippocampus-dependent) memory. Adult male rats were given only 3 training trials to learn the location of a hidden platform in a water maze. In control (vehicle-injected) rats, this minimal amount of training produced intact short-term (15 min), but poor long-term (24 h), memory. Pre-training administration of memantine or neramexane produced a dose-dependent enhancement of long-term memory. Pharmacokinetic experiments with equimolar doses of both agents indicated that lower plasma levels of neramexane were more effective than memantine at enhancing memory. The effective doses of both agents in the current study produced plasma levels (and extrapolated brain CSF levels) within a range of activity at NMDA receptors and plasma levels seen in patients with Alzheimer's disease. These findings provide support for the use of neramexane as a pharmacological intervention in the treatment of dementia.

Keywords: NMDA receptor antagonist; Trace learning; Radial-arm water maze; Spatial memory; Dementia; Alzheimer's disease

1. Introduction

Glutamate acts predominantly as an excitatory neurotransmitter within the central nervous system and plays a major role in synaptic transmission. While rapid transmission is mediated via ionotropic glutamate receptors such as the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate, and NMDA (*N*-methyl-D-aspartate) receptors, transmission via the G-protein coupled metabotropic glutamate receptors (mGluRs) is of a more modulatory nature (Parsons et al., 1998; Pin and Acher, 2002). Most AMPA receptors are impermeable to Ca^{2+} and contribute to fast synaptic transmission. In contrast, NMDA receptors are highly permeable to Ca^{2+}, but their voltage-dependent block by Mg^{2+} tends to result in slow gating kinetics. These features make NMDA receptors more suitable for mediating plastic changes in the brain, such as those involved in learning (Collingridge and Singer, 1990). Indeed, NMDA receptor activation is obligatory for some forms of long-term potentiation (LTP) (Hrabetova et al., 2000; Malenka and Bear, 2004; Malinow et al., 2000) and learning and memory (Baker and Kim, 2002; Danysz et al., 1995; de Lima et al., 2005; Flood et al., 1990; Kawabe et al., 1998; Li et al., 1997; Morris et al., 1986; Parada-Turska and Turski, 1990; Pitkanen et al., 1995; Roesler et al., 1998; Ward et al., 1990).

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The finding that NMDA receptor activation is an important component of the induction of neural plasticity and memory formation appears to contradict the observation that an NMDA receptor antagonist, memantine (1-amino-3,5-dimethyladamantane), can be effective in the treatment of Alzheimer's disease (Danysz and Parsons, 2003; Parsons et al., 1999a,b). However, many systems, and the NMDA receptor system in particular, work optimally within a certain range, and either excessive activation or extreme inhibition results in the impairment of plasticity (Coan et al., 1989; Collingridge and Singer, 1990; Danysz et al., 1995; Morris et al., 1986; Zajaczkowski et al., 1997). Moreover, the resting block of NMDA channels by Mg^{2+} ions is a necessary element of their co-incidence detection function, and removal of these ions results in impaired plasticity as well (Coan et al., 1989; Frankiewicz and Parsons, 1999). Extensive work has linked dementia with a hyperactive glutamatergic system, which results in the over-activation of NMDA receptors, excessive Ca^{2+} influx, and eventual cell death (Greenamyre, 1991; Hynd et al., 2004). In fact, dementia severity correlates reasonably well with the amount of neuronal death induced by an excess of glutamate (Sonkusare et al., 2005). Individuals with Alzheimer's disease tend to display aberrant glutamate transporter activity, which may lead to an excessive accumulation of glutamate at the synaptic cleft (Masliah et al., 1996; Scott et al., 2002). Investigators have shown that CSF levels of glutamate are significantly greater in Alzheimer's patients than in control subjects (Pomara et al., 1992), and the accumulation of Aβ in these individuals, which is a cardinal characteristic of Alzheimer's disease, dramatically increases the susceptibility of neurons to excitotoxic insult (Gegelashvili and Schousboe, 1997; Smith-Swintosky and Mattson, 1994). Researchers have thus hypothesized that the positive effects of memantine on dementia involve a "damping down" of NMDA receptor function during these conditions, when the strength of Mg^{2+} is insufficient to maintain a resting block of the channel (Danysz and Parsons, 2003; Parsons et al., 1999a,b). Memantine blocks NMDA receptors in a use-dependent manner, and this block cannot be overcame by an increase in glutamate concentration (Doraiswamy, 2003). Thus, memantine can, in theory, inhibit the pathological features of NMDA receptor activation, while permitting the necessary physiological and cognitive functions to remain intact.

Conventional pharmacotherapy for dementia (e.g., Alzheimer's disease) has involved the prescription of cholinesterase inhibitors, which prevent acetylcholinesterase (AChE) from breaking down acetylcholine (ACh) in the brain (Standridge, 2004). Although these agents can improve individuals' cognition in mild cases of dementia, they are incapable of terminating the process of neurodegeneration (Sonkusare et al., 2005). In addition, AChE inhibitors often lead to several adverse side effects, including nausea, vomiting, diarrhea, and dizziness (Hake, 2001). These side effects are avoided, for the most part, when noncompetitive NMDA receptor antagonists, such as memantine, are the treatment of choice, providing another reason for the development of these substances as a therapeutic option for dementia.

As a neuroprotective agent, memantine has been shown to normalize the impairments in synaptic plasticity and cognition that typically follow excitotoxic neuronal injury. For instance, memantine normalized LTP that was impaired either by the administration of NMDA or the reduction of Mg^{2+} concentration (Frankiewicz and Parsons, 1999; Zajaczkowski et al., 1997) and improved LTP in aged rats *in vivo* (Barnes et al., 1996). Additionally, the administration of memantine following global ischemia resulted in significant improvements in rat spatial memory and an attenuation of ischemia-induced damage to hippocampal CA1 cells (Block and Schwarz, 1996). Others have shown that memantine-treated rats exhibit a significant decline in hippocampal cell death after traumatic brain injury (Rao et al., 2001). Memantine also prevented damage to rat hippocampal CA1 neurons after the administration of Aβ (Miguel-Hidalgo et al., 2002) and has been shown to improve spatial memory in mice carrying mutant APP and PS1 genes (Minkeviciene et al., 2004). Although memantine has shown positive effects on cognition in experimental animals with conditions leading to cellular atrophy and glutamatergic hyperactivity, it has never been found to enhance memory processes in intact animals (Minkeviciene et al., 2004; Zajaczkowski et al., 1997).

For the present study, we examined whether memantine and neramexane, two noncompetitive NMDA receptor antagonists, would improve learning and memory in rats trained in the radial-arm water maze (RAWM), a well-described spatial (hippocampus-dependent) learning and memory task (Diamond et al., 1999; Sandi et al., 2005; Woodson et al., 2003). Memantine is approved for the treatment of Alzheimer's disease, and neramexane is currently under development for the same indication (Danysz et al., 2002). Thus, we have provided a comparative assessment of the effects of administration of each agent on memory in normal adult rats. We also performed pharmacokinetic experiments to assess whether the doses which were effective in enhancing memory in water maze-trained rats would lead to plasma concentrations that are typically observed in patients.

2. Materials and methods

2.1. Subjects

2.1.1. Pharmacokinetic study

Experimentally naïve adult male Sprague-Dawley rats (220–260 g; *Janvier*, France) were housed in groups of four per cage. Colony room temperature and humidity were maintained respectively at 20±1 °C and 60±3%. Food and water were available *ad libitum*, and the animals were maintained on an alternating 12 h/12 h light-dark cycle (lights on at 0700) for at least 6 days before the experiments began. All manipulations were conducted during the light phase.

The study was approved by the Ethical Committee, Regierungspraesidium Darmstadt, Hessen and performed in accordance with the recommendations and policies of the U.S. National Institutes of Health Guidelines for the Use of Animals.

2.1.2. Spatial memory study

Experimentally naïve adult male Sprague-Dawley rats (225–250 g; Charles River Laboratories, Wilmington, Massachusetts, USA) were housed on a 12 h/12 h light/dark schedule (lights on at 0700) in Plexiglas cages (two rats per cage) with *ad libitum* access to food and water. Colony room temperature and humidity were maintained respectively at 20±1 °C and 60±3%. All rats were given 1 week to acclimate to the housing room environment prior to water maze training. During the last 3 days of the 1-week acclimation period, all rats were transported to the laboratory's water maze training room and handled for 2–3 min each. All behavioral manipulations were conducted between 0800 and 1400 h of the light phase.

The study was approved by the Institutional Animal Care and Use Committee (IACUC), Division of Comparative Medicine and Division of Research Compliance at the University of South Florida. All procedures were conducted in accordance with the policies of animal care and treatment established by the IACUC.

2.2. Pharmacological agents

Memantine hydrochloride and neramexane mesylate were provided by Merz Pharmaceuticals, Frankfurt, Germany. They were dissolved in 0.9% saline solution and administered at a volume of 1 mL/kg via intraperitoneal (i.p.) injections.

2.3. Pharmacokinetic experiment

2.3.1. Plasma sampling

For each substance, 36 experimental and 4 control animals were used. Each animal was injected once with either memantine (2.5, 5 or 10 mg/kg) or an equimolar dose of neramexane mesylate (3.1, 6.2, 12.3 mg/kg). Plasma samples were then obtained from each animal at three time points post-injection. Thus, 18 rats (6 rats/dose/substance) were sampled 30, 120, and 420 min post-injection, and 18 other rats (6 rats/dose/substance) were sampled 60, 240, and 600 min post-injection. For blood sampling, animals were anaesthetized with chloroform. The first two blood samples from each animal were taken from the left and right retrobulbar spaces using micro-haematocrit-tubes (Heiland, Hamburg; Germany) and collected into Lithium-Heparin tubes (Microvette® 500 LH, Sarstedt, Nuembrecht, Germany). The third blood sample was taken via heart puncture into a Lithium-Heparin S-Monovette® 9 mL LH.

All blood samples were centrifuged (3000 rpm) for 2 min. Plasma was decanted and kept in prepared glasses. The plasma samples were stored in the freezer (−18°) until analyzed. Assays were performed by AAI (Neu-Ulm, Germany) in compliance with GLP using GC/MS (GC HP 5890 and Fisons Trio 1000 respectively). Amantadine was used as an internal standard.

2.3.2. Extraction

In each case (standards, quality control, and study samples), 50 pL of plasma were aliquoted into a clean, screw-cap vial with a Teflon-lined cap. The plasma was spiked with 25 pL of the internal standard, which was dissolved in methanol (0.20 ng 1 pL), or, in the case of the standards, with 25 pL of the corresponding working solution of the sample. 0.1 mL of 2 M HCl was added to the solution, which was then horizontally shaken for 3 min. After an incubation period of 30 min at 70 °C and subsequent cooling, 0.1 mL of 32% NaOH were added to the solution, which was again horizontally shaken for 3 min. 700 pL of n-Hexane were added to the samples, which were then extracted horizontally for 30 min. After the samples were centrifuged and frozen in an ice bath, the organic phase was transferred into a conical glass tube.

2.3.3. Derivatization

10 pL of MBTFA were added to the samples, the conical glasses were screwed, and the samples were derivatized at 70 °C for 30 min. The samples were evaporated to dryness with a gentle stream of nitrogen at 35O °C and reconstituted in 40 pL of Ethyl acetate1Toluene (80120).

2.3.4. Measurement

The analysis was performed on a GC/MS-system. A fused silica open tubular capillary column of cross-linked Cyanopropylphenyl-dimethylpolysiloxane type was used for the GC-separation. The MS was operated in the positive ion chemical ionization mode with ammonia as the reagent gas. Selected ion monitoring was performed for m/z 293, the base peak of the mass spectrum for memantine, and m/z 265, the base peak for 1-aminoadamantane. Data acquisition and integration of the peak areas were achieved using the instrument's standard software.

2.4. Spatial memory task

2.4.1. Apparatus

Training took place in a black, galvanized round tank (168 cm in diameter, 56 cm height, 43 cm deep) filled with clear water (21±2 °C) and located in a light- and sound-attenuated room. Using 6 V-shaped stainless steel walls (54 cm height, 56 cm length), the tank was divided into six arms (each of which had a width of approximately 25 cm) radiating from an open central area (56 cm in diameter). A hidden, black, plastic platform (12 cm in diameter) was located 2 cm below the surface of the water at the end of one arm (the goal arm).

2.4.2. Procedure

Prior to water maze training, all rats were transported to the laboratory and left undisturbed in the water maze room for a 30-min acclimation period. Thereafter, rats received i.p. injections of memantine hydrochloride (2.5, 3.75, 5, or 7.5 mg/kg), neramexane mesylate (3.1, 4.65, 6.2, or 9.3 mg/kg), or saline vehicle (1 mL/kg). Thirty minutes later, rats began water maze training. Each rat was released in one of the arms (the start arm), at the point where the arm entered the central open area, and it was given 60 s to find the hidden platform. If the rat could not locate the platform within the 60-s period, it was gently guided to the platform by the experimenter. Once the rat found, or was guided to, the platform, it was allowed to stand on it for 15 s before the

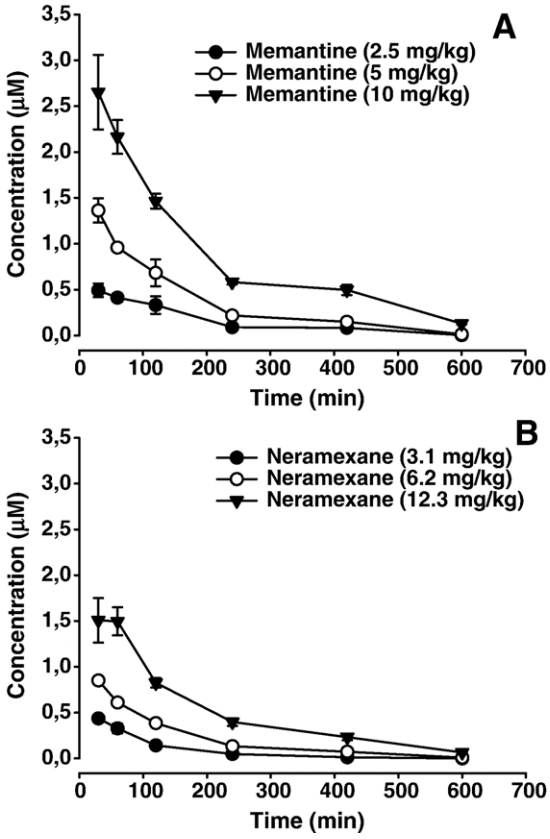

Fig. 1. Plasma concentration of memantine (A) and neramexane (B) following i.p. administration in rats. Values are expressed as means±SEM (n=6 rats/dose).

next trial began. For each trial, the experimenter recorded the number of arm entry errors made by each rat and the length of time it took each rat to find the platform. An arm entry was operationally defined as a rat passing halfway down the arm. An arm entry error occurred when a rat entered an arm that did not contain the hidden platform, or entered the arm that contained the hidden platform but was unable to locate it. The latter type of entry error was extremely rare – over 98% of arm entry errors were entries into arms that did not contain the hidden platform. The goal arm was different across rats to prevent the build-up of odour cues in any one arm. The start arms varied randomly across trials, and for every trial, a different start arm was employed.

All rats received 3 acquisition trials (T1–T3) to learn the location of the hidden platform, followed by a 15-min delay period, which terminated with a memory test trial (short-term memory). Then the rats were returned to their home cages and transported back to the housing room. Twenty-four hours later, all rats were brought back to the laboratory and left undisturbed in the water maze room for a 30-min acclimation period, after which they were given a single memory test trial to assess their long-term (24 h) spatial memory. We developed this training protocol with pilot work, which indicated that under control conditions rats had intact memory at 15 min but no evidence of memory at 24 h. Thus, this procedure provided the opportunity to assess the ability of memantine and neramexane to enhance long-term memory.

2.5. Statistical analysis

The behavioral data for memantine and neramexane were analyzed separately (SigmaStat, SPSS). For each drug, arm entry errors on the acquisition trials (T1–T3) were analyzed with a mixed-model ANOVA, with group (vehicle, dose 1, dose 2, dose 3, dose 4) serving as the between-subjects factor and trials (T1, T2, T3) serving as the within-subjects factor. Separate one-way ANOVAs were employed to examine arm entry errors made on the 15-min short-term memory test trial and the 24-h long-term memory test trial. In both cases, group served as the between-subjects factor. Behavioral data were expressed as means±SEM, and Holm-Sidak post hoc comparisons were employed as indicated.

Outlier data (values greater than 3 standard deviations from the exclusive mean) were removed from the analyses. After removal of outlier data, all n=7 or 8 rats per time point, with the exception of the vehicle group in the neramexane component, where n=11 for all time points. Less than 2% of all data were classified as outliers.

3. Results

3.1. Plasma pharmacokinetics

Memantine hydrochloride (2.5, 5, 10 mg/kg) and neramexane mesylate (3.1, 6.2, 12.3 mg/kg) were injected i.p. and measured in plasma 30, 60, 120, 240, 420, and 600 min thereafter. Administration of increased doses of memantine hydrochloride and neramexane mesylate produced increases in plasma concentrations of each agent (Fig. 1).

Administration of neramexane (3.1, 6.2, 12.3 mg/kg) at the doses equimolar to memantine (2.5, 5, 10 mg/kg), resulted in lower plasma concentrations of neramexane. This finding is consistent with pharmacokinetic parameters such as Cmax and AUC (Table 1). However, there were no differences in Tmax and T1/2.

3.2. Effects on spatial memory performance

3.2.1. Memantine

The analysis of arm entry errors during acquisition (T1–T3) revealed a significant main effect of trial, indicating that rats made fewer errors as the trials progressed, $F_{2,70}$=3.57, P<.05. There was no effect of group on acquisition in the RAWM,

Table 1
Pharmacokinetic plasma parameters of memantine hydrochloride and neramexane mesylate after i.p. administration in rats

Substance (mg/kg)	T max (h)	$T½$ (h)	C max (ng/ml)	AUC (hr∗ng/mL)
Memantine (2.5/5/10)	0.5/0.5/0.5	1.6/1.6/2.4	88/244/474	275/616/1483
Neramexane (3.1/6.2/12.3)	0.5/0.5/0.5	1.4/1.6/2.1	72/143/254	137/342/827

Values are means of 6 animals.

$F_{4,35}=1.07$, $P>.38$, and there was no significant Group x Trial interaction, $F_{8,70}=1.39$, $P>.21$ (Fig. 2A).

Memantine had no effect on performance in the 15-min short-term memory test, $F_{4,33}=1.09$, $P>.37$. The analysis of arm entry errors on the 24-h retention trial revealed that memantine produced a dose-dependent enhancement of long-term spatial memory, $F_{4,33}=3.27$, $P<.05$ (Fig. 2B). Post hoc comparisons demonstrated that 5 and 7.5, but not 2.5 or 3.75, mg/kg of memantine resulted in significantly fewer arm entry errors on the 24-h memory test trial (P's$<.05$).

3.2.2. Neramexane

The analysis of arm entry errors during acquisition (T1–T3) revealed a significant main effect of trial, indicating that rats made fewer errors as the trial progressed, $F_{2,76}=12.46$, $P<.001$ (Fig. 3A). In contrast to memantine, there was an effect of group on acquisition in the RAWM, $F_{4,38}=3.63$, $P<.05$. Rats that received 9.3 mg/kg of neramexane made significantly more errors during learning than controls. There was no significant Group x Trial interaction, $F_{8,76}=0.45$, $P>.88$.

There was a significant effect of neramexane on short-term memory, $F_{4,35}=18.74$, $P<.001$. Rats that received 9.3 mg/kg of neramexane made significantly more entry errors on the 15 min memory test trial than controls. In contrast, there was a dose-dependent enhancing effect of neramexane on long-term spatial memory, $F_{4,37}=2.73$, $P<.05$ (Fig. 3B). Post hoc comparisons demonstrated that 4.65 and 6.2, but not 3.1 or 9.3, mg/kg of neramexane resulted in significantly fewer arm entry errors on the 24-hr memory test trial (P's$<.05$).

3.2.3. Behavioral observations

Rats that were injected with 9.3 mg/kg of neramexane appeared to be hyperactive. They tended to jump back into the water after reaching the platform. These rats also exhibited perseverative errors, in that they repeatedly swam into the same

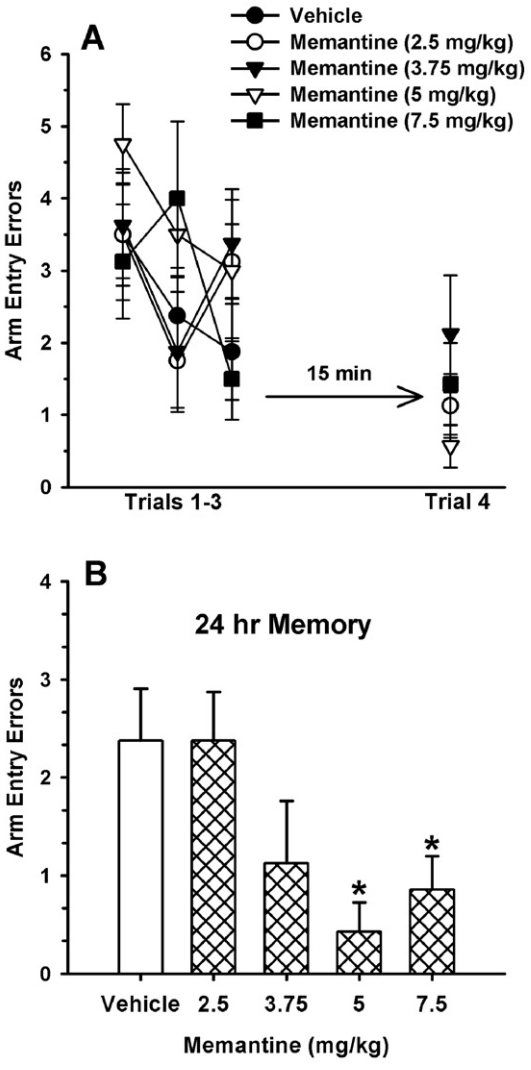

Fig. 2. Effects of memantine on acquisition and short-term memory (A) and 24 h retention (B) in the RAWM. Memantine (i.p.) was administered 30 min prior to training. Values are expressed as means±SEM. $N=7$. *$p<.05$ vs. Vehicle (Holm-Sidak post hoc comparisons).

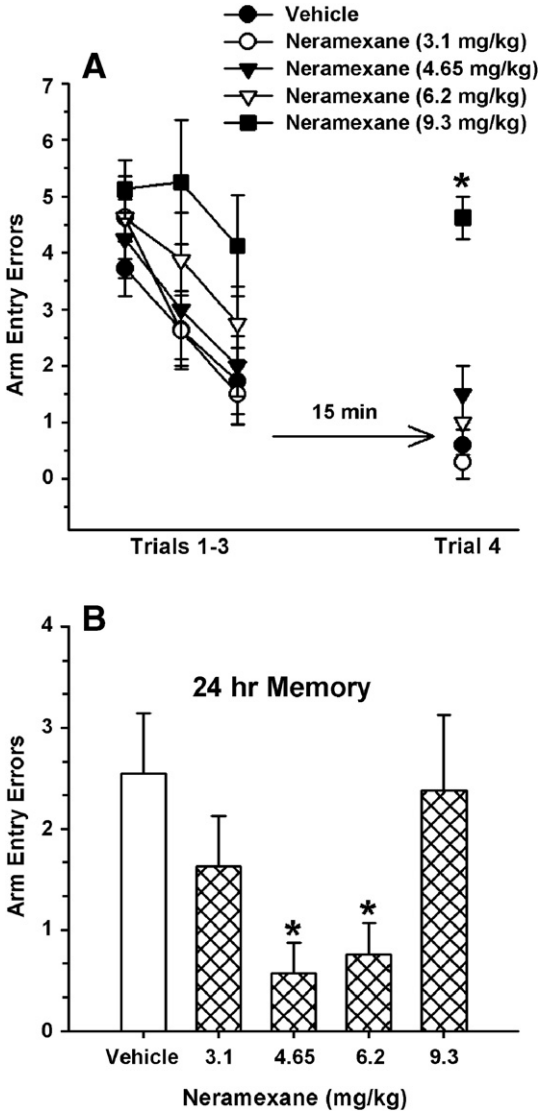

Fig. 3. Effects of neramexane on acquisition and short-term memory (A) and 24 h retention (B) in the RAWM. Neramexane (i.p.) was administered 30 min prior to training. Values are expressed as means±SEM. $N=11$ for vehicle group and 7 or 8 for the other groups. *$p<.05$ vs. Vehicle (Holm-Sidak post hoc comparisons).

(incorrect) arms. Thus, it is not clear from this work if the high dose of neramexane was ineffective at enhancing memory or if the increased motor activity interfered with the ability of the rats to perform the task.

4. Discussion

The primary finding of this work is that pre-training administration of memantine or neramexane dose-dependently enhanced long-term (24 h) spatial memory. This is the first study we are aware of to show that the administration of these particular NMDA receptor antagonists can enhance long-term memory in normal adult animals which don't have a treatment-induced deficit. This long-lasting, memory-enhancing effect is unlikely to be related to the influence of either drug at the time of memory testing; plasma levels dropped to approximately 0.1 μM concentrations 10 h after administration, a level that results in only minor activity at NMDA receptors. Neramexane appeared to be more potent than memantine, as it enhanced long-term memory at a dose (4.65 mg/kg) that was equimolar to an ineffective dose of memantine (3.75 mg/kg). Consistent with this finding is the observation that [^{3}H]MK-801 binding and patch clamp experiments performed *in vitro* have shown that neramexane is up to two times more potent than memantine at NMDA receptors (Parsons et al., 1999a,b).

We did find that the highest dose (9.3 mg/kg) of neramexane produced poor behavioral performance at 15 min with no improvement in performance at 24 h. The apparent impairment of learning at the 15 min time point at the highest dose of neramexane may have resulted from the increased hyperactivity produced by this dose, rather than an inability of the rats to learn and remember the platform location. This interpretation of the findings is supported by research that has demonstrated increased locomotor activity in rats after the administration of a comparable dose of neramexane (10 mg/kg) (Sukhotina et al., 2004). A U-shaped dose response curve has also been shown for memantine in a passive avoidance task (Zajaczkowski et al., 1997), and work from our group (unpublished observations) has shown that a high dose of memantine (e.g., 10 mg/kg) also produces hyperactivity, which prevents rats from performing well in the water maze. Thus, there appears to be a U-shaped relationship between memantine and long-term memory which is similar to that of neramexane and memory, but the dose response curves for memantine and neramexane are not identical; the dose response curve for neramexane, relative to memantine, is shifted to the left. This finding is supported not only by the memory enhancement observed at a lower dose of neramexane, but by the onset of behavioral abnormalities which occurred at a lower dose of neramexane as well.

A salutary effect of NMDA receptor antagonists on memory seems difficult to reconcile with the known obligatory role of this receptor subtype in forms of neural plasticity and learning (Danysz et al., 1995; Morris et al., 1986). However, over-activation of NMDA receptors has been connected with deficits in LTP and learning. For example, the application of NMDA impairs LTP *in vitro* (Coan et al., 1989; Izumi et al., 1992) and learning *in vivo* (Jones et al., 1989; Si et al., 2004; Zajaczkowski et al., 1997), and knocking out the glutamate transporter GLT1 leads to the impairment of LTP in hippocampal slices (Katagiri et al., 2001). Even further, Aβ suppresses LTP and can exacerbate the impairment produced by excessive glutamate (Nakagami and Oda, 2002; Ye and Qiao, 1999). Lastly, anti-sense of the glutamate transporter EAAT3 (rEAAC1 in rats) impairs Morris water maze learning and with high frequency stimulation, shifts hippocampal LTP to LTD (Beckman et al., 2005). These findings relate well to the glutamatergic deficiencies observed in Alzheimer's disease, which have been suggested to be associated with the severe memory impairments that are characteristic of the disorder.

In accordance with these observations, investigators have shown that NMDA receptor antagonists can normalize plasticity and learning under such conditions. For example, the competitive NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5) reverses the impairment of LTP produced by the administration of NMDA (Coan et al., 1989). Similarly, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), another competitive NMDA receptor antagonist, reverses LTP impairments produced in GLT1 knockouts (Katagiri et al., 2001). Moreover, positive effects of memantine have been reported in the Morris water maze in double transgenic (APP+PS1) mice and in APP23 mice (Minkeviciene et al., 2004; Van Dam et al., 2005). This is not unexpected bearing in mind that Aβ enhances glutamatergic function and, in particular, NMDA receptor activation (Danysz and Parsons, 2003; Mattson et al., 1992).

The issue arises, therefore, whether similar mechanisms could be operative in the task used in the present study. In the conditions of RAWM training, there is a clear stress component (immersion in cold water) which activates the glutamatergic system. Memantine and neramexane exerted positive effects on long-term memory, perhaps, in part, because of their ability to regulate water maze training-induced NMDA receptor activity and its corresponding Ca^{2+} influx. Thus, the intermediate doses of memantine and neramexane may have optimized NMDA channel functioning to produce favorable circumstances for memory consolidation. In comparable preliminary work on related NMDA receptor modulators, we have shown that low doses of tianeptine or CPP enhanced long-term (24 h) spatial memory in rats (Munoz et al., 2005). Tianeptine has been shown to normalize stress-induced increases in NMDA channel currents (Kole et al., 2002) and CPP is a well-studied NMDA receptor antagonist. Thus, the current work, in conjunction with our preliminary findings, is consistent with the view that memantine- and neramexane-induced enhancements of long-term memory occur via the modification of NMDA receptor activity.

This is not the first evidence for an enhancement of long-term memory via the administration of NMDA receptor antagonists. For instance, Lederer et al. (1993) found that the administration of CGP 37849, a competitive NMDA receptor antagonist, dose-dependently enhanced rats' long-term memory in a social learning paradigm. Specifically, the lowest dose of CGP 37849 (0.3 mg/kg) led to better social recognition memory 24 h later, while higher doses (1 and 3 mg/kg) had no effect on

performance. In addition, Puma and Bizot (1998) found that intraseptal infusions of AP5 (DL-2-amino-5-phosphonopentanoic acid) enhanced long-term object recognition memory in rats. Mondadori et al. (1989) further argued that the effects of NMDA receptor antagonists on learning and memory are dependent on the type of task employed by the experimenter. These investigators found that pre-training administration of AP7 (DL-2-amino-7-phosphonoheptanoate) or MK-801 facilitated retention in a step-down passive avoidance task but impaired retention in place navigation and step-through dark avoidance tasks. Mondadori and Weiskrantz (1993) replicated these findings when they administered CGP 37849 and MK-801 prior to step-down passive avoidance and step-through dark avoidance tasks. However, these investigators extended the original findings by showing that the enhancement of step-down passive avoidance memory was dependent on steroid manipulations, while the impairment of step-through dark avoidance memory was not. Collectively, these findings support the arguments that (a) NMDA receptor antagonists differentially affect performance on different behavioral tasks and (b) their memory-enhancing and memory-impairing effects are mediated by different neurobiological mechanisms.

Additional work by Creeley et al. (2006) questioned the efficacy of memantine in terms of facilitating learning and memory. In this study, low doses of memantine, comparable to the ones presented in the current study, disrupted rats' locomotor activity and led to 24 h memory impairments on a spatial memory, hole-board task. The inconsistency between the findings of Creeley et al. (2006) and the ones presented here could be attributable to methodological differences between the two studies. First, Creeley et al. (2006, p. 3924) used female rats in their study because "female rats are more sensitive than males to [the] adverse side effects of NMDA antagonists." Most, if not all, of the work that has demonstrated memory-enhancing effects of NMDA receptor antagonists has utilized male rats, and since females are more sensitive to the adverse side effects of NMDA receptor antagonists, it is not surprising that memantine led to memory impairments in this experiment.

Such greater sensitivity in older female rats [6–8 months old, as used by Creeley and colleagues (2006)] than those typically used in pharmacological studies is not surprising. Older rats exhibit higher plasma levels of memantine than young rats after the administration of an equivalent dose of the drug [e.g., in 2½-month-old rats, infusion of memantine (24 mg/kg/d) leads to plasma levels of approximately 1.4 μM, while in 24-month-old rats, it leads to plasma levels of about 4 μM (unpublished observations)]. Similarly, following the infusion or acute injection of memantine, female rats display serum levels that are almost twice as high as those observed in male rats (Zajaczkowski et al., 2000). In turn, the treatment regime employed by Creeley et al. (2006) resulted in higher levels of memantine despite the fact that the doses, per se, appeared to be low.

It is also possible that NMDA receptor antagonists differentially affect learning and memory, depending on the stressfulness of the task involved. For instance, Creeley et al. (2006) employed what might seem like a less stressful task (hole-board task) than the one employed in the present study (water maze). However, Marquez et al. (2005) found that a version of the hole-board task, similar to the one employed by Creeley et al. (2006), produced significant elevations of serum corticosterone (~20 μg/dL) in rats, which were greater than those produced by exposure to an elevated plus maze or circular corridor. These levels of serum corticosterone (~20 μg/dL) are comparable to those that we have recently found in rats exposed to 12 repeated training trials in the RAWM (unpublished observations). Thus, it is not clear as to whether or not these two tasks are *quantitatively* different in terms of their stressfulness. Furthermore, it is important to consider the minimal amount of training that rats were exposed to in the present study. The rats endured only 3 consecutive acquisition trials, which took approximately 3–4 min to complete. Even if these two tasks are different in terms of their aversiveness, it does not explain the findings that NMDA receptor antagonists can enhance memory in both aversive (Mondadori et al., 1989; Mondadori and Weiskrantz, 1993; Zajaczkowski et al., 1997) and non-aversive (Lederer et al., 1993; Puma and Bizot, 1998; Zajaczkowski et al., 1996) tasks.

In addition to the modulation of NMDA receptor activity, other mechanisms may have been involved in the memantine- and neramexane-induced enhancement of long-term memory. For instance, clinically relevant doses of memantine (e.g., 5 mg/kg) induce a significant increase in brain-derived neurotrophic factor (BDNF) mRNAs in cortical regions (Marvanova et al., 2001). This neurotrophin has been shown to acutely facilitate synaptic plasticity, and its application enhances the induction of hippocampal LTP (Figurov et al., 1996; Patterson et al., 1996). Indeed, a weak stimulation of nerve fibers results in greater LTP when it is paired with BDNF than when it is induced in isolation (Figurov et al., 1996). BDNF also has a long-term effect on synaptic development and function. In addition to playing an important role in axonal and dendritic outgrowth, BDNF is involved in activity-dependent synaptic competition and the development of late-phase LTP (L-LTP) (Lu, 2003). Perhaps the most important finding in relation to the current work is that the interaction between BDNF/trkB signaling and NMDA receptors is key for the development of spatial memory in the hippocampus (Mizuno et al., 2003). Together, these findings suggest that, in the present study, memantine and neramexane could have affected BDNF concentrations, which aided in the consolidation of long-term memory. It is also possible that the cognitive-enhancing effects of memantine and neramexane act preferentially on one mechanism (e.g., by affecting BDNF and NMDA receptors), while their hypermotoric effects occur via activation of a different mechanism.

In clinical trials, memantine has been shown to improve cognition and daily skills in patients diagnosed with moderate to severe Alzheimer's disease (Mobius et al., 2004; Reisberg et al., 2003). Neramexane, which belongs to a different chemical class of NMDA receptor antagonists, is currently under development for the treatment of neurodegenerative dementia (Danysz et al., 2002; Parsons et al., 1999a,b), among other indications. In the present study, pre-training administration of memantine or neramexane enhanced long-term spatial memory tested 24 h later. Neramexane, a more potent modulator of NMDA receptors, produced memory enhancements at a lower

equimolar dose than memantine. In summary, the current findings provide the first indication that neramexane and memantine can both enhance long-term memory in normal animals, which support the use of neramexane as a pharmacological intervention for the treatment of Alzheimer's disease.

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