Memantine improves spatial learning in a transgenic mouse model of Alzheimer’s disease†

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List of nonstandard abbreviations:
Aβ = beta amyloid
AD = Alzheimer’s disease
FAD = familiar Alzheimer's disease
APP = amyloid precursor protein
APPswe = amyloid precursor protein with Swedish mutation
5-HT = 5-Hydroxytryptamine = serotonin
LTP = long-term potentiation
NMDA = N-methyl-D-aspartate
NT = non-transgenic
PS = presenilin

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Abstract

Memantine, a low to moderate affinity, uncompetitive NMDA receptor antagonist, has been shown to improve learning and memory in several pharmacological models of Alzheimer’s disease (AD). In the present study, the effect of memantine on locomotor activity, social behavior and spatial learning was assessed in a transgenic mouse model of AD. Eight-month-old male C57BL/6J mice carrying mutated human APP and PS1 genes (APP/PS1) and their non-transgenic littermates (NT) were administered a therapeutic dose of memantine (30 mg/kg/day; p.o.) for 2-3 weeks. At this age, APP/PS1 mice show elevated levels of β-amyloid peptides in several brain regions. APP/PS1 mice exhibited less exploratory rearing and increased aggressive behavior compared to NT mice. In the water maze test for spatial learning, APP/PS1 mice had longer escape latencies to both hidden and visible platforms, but they did not differ from NT mice in their swimming speed. Memantine significantly improved the acquisition of the water maze in APP/PS1 mice without affecting swimming speed. Memantine did not affect either locomotor activity or aggressive behavior in either genotype. These data indicate that memantine improves hippocampus-based spatial learning in a transgenic mouse model of AD without producing nonspecific effects on locomotion/exploratory activity.
In the mammalian brain, NMDA receptors are involved in important physiological functions such as synaptic plasticity and synapse formation, which play important roles in memory, learning and the formation of neural networks during development (Mayer and Westbrook, 1987). NMDA receptors are also thought to be involved in a variety of neuropathological states caused by excitotoxic neuronal injury such as ischemia, epilepsy and several neurodegenerative diseases (e.g., Alzheimer’s disease, Parkinson’s disease and Huntington’s disease). In fact, any central nervous system disorder in which neuronal loss is caused by glutamate-induced excitotoxicity has the potential to be treated by NMDA receptor antagonists. However, given the critical role of NMDA receptors in learning and memory (Morris, 1989; Tsien et al., 1996), it may appear counter-intuitive that an NMDA receptor antagonist could improve the symptomatology of Alzheimer's disease (AD).

Several NMDA receptor antagonists possessing high affinity for NMDA receptors [e.g., (+) MK-801] have been found to cause neurobehavioral adverse effects such as hallucination and cognitive impairment (Benvenga and Spaulding, 1988; Abi-Saab et al., 1998). These adverse events have largely limited the clinical development of high affinity NMDA receptor antagonists. An alternative approach to avoid such side effects is to produce a partial rather than complete blockade of the NMDA receptor. Partial receptor blockade can be achieved, for example, by low affinity NMDA receptor antagonists, which typically possess a better therapeutic window than high affinity NMDA receptor antagonists (Rogawski, 2000). Memantine, a low to moderate affinity NMDA receptor antagonist, has been shown to improve performance in several pharmacological models of impaired learning and memory (Zajaczkowski et al., 1996; Wenk et al., 1997), in aged rats with impaired baseline memory function (Barnes et al., 1996) and in patients with moderate to severe AD (Reisberg et al., 2003; Tariot et al., 2004).
One of the most distinct pathological hallmarks of AD is extracellular deposition of β-amyloid (Aβ) plaques in select brain regions. A subset of AD cases exhibit early onset and are familial (FAD). FAD is caused by mutations in the presenilin 1 (PS 1), presenilin 2 (PS 2) or amyloid precursor protein (APP) genes. Such mutations lead to enhanced production of highly fibrillogenic Aβ1-42 peptides (Borchelt et al., 1997; Holcomb et al., 1998). Several lines of evidence suggest that Aβ toxicity may be related to elevated levels of glutamate and/or overactivity of NMDA receptors. For example, APP is expressed by glutamatergic neurons (Ouimet et al., 1994), and the cellular damage in the brains of AD patients is found predominantly in areas that display glutamatergic synaptic plasticity (Arendt et al., 1998). Infusion of Aβ in rat brains produces deficits in learning and memory (Sweeney et al., 1997) and impairment in long-term potentiation (LTP), a model of activity-dependent synaptic plasticity that may underlie some forms of learning and memory (Stephan et al., 2001; Walsh et al., 2002). Transgenic mice overexpressing Aβ and APP also exhibit age-dependent cognitive decline (Chapman et al., 1999; Puoliväli et al., 2002), and glutamate is known to exacerbate Aβ-induced impairment of LTP (Nakagami and Oda, 2002). Moreover, in a recent study, memantine protected rat hippocampal cells from Aβ-induced apoptosis (Miguel-Hidalgo et al., 2002). Even in the absence of either Aβ or APP, overactivation of NMDA receptors can decrease synaptic plasticity and learning. For example, the generation of LTP can be impaired by a high concentration of NMDA (Katagiri et al., 2001), and systemic administration of a non-convulsive dose of NMDA has been shown to impair passive avoidance learning in rats (Zajaczkowski et al., 1997).

The finding that down-regulation of the glial glutamate transporter, GLT-1 (EAAT-2) occurs in AD patients also supports the idea that synaptic levels of glutamate and therefore NMDA receptor activity may increase in AD (Masliah et al., 1996). Interestingly, mice lacking GLT-1 also show elevated synaptic levels of glutamate and impaired hippocampal LTP, which are partially restored.
to normal levels by a low dose of NMDA receptor antagonist (Katagiri et al., 2001), and APP transgenic mice show impaired glial glutamate transporter activity (Masliah et al., 2000). Collectively, these findings suggest that the overactivation of NMDA receptors and/or elevated levels of glutamate in the synapse can exacerbate the neurotoxic and memory-impairing effects of Aβ and APP.

In the present study, the effect of sub-chronic oral administration of memantine on hippocampus-based spatial learning and other general behaviors was determined in mice carrying mutated human APP(swe) and PS1 (A246E) genes. These mice develop age-dependent memory impairment and exhibit age-related increases in Aβ levels in several brain regions (Liu et al., 2002; Puoliväli et al., 2002).

METHODS

Animals

Transgenic mice expressing either human PS1 harboring the familial AD-linked A246E mutation or chimeric mouse/human APP695 harboring a human Aβ domain and mutations (K595N, M596L) linked to Swedish familial AD pedigrees (APPswe) (Borchelt et al., 1997) were back-crossed to C57BL/6J for 16 generations and then crossed together to generate double transgenic mice co-expressing both transgenes. In all tests, 8-month-old double-mutant male mice (APP/PS1; n=45) and their non-transgenic littermates (NT; n=36, ) were used. At this age, APP/PS1 mice exhibit increased brain levels of β-amyloid peptides (Wang et al., 2003). The therapeutic dose of memantine was defined as the dose producing a steady-state plasma drug level of ~1 μM and was determined in 8-month-old male C57 BL/6J mice (background strain of the transgenic mice).
Throughout the experiment animals were housed individually in a controlled environment (temperature 21 ± 1°C, humidity 50 ± 10%, light period 07:00-19:00 h). Food and water were available ad libitum. The experiments were conducted according to the Council of Europe (Directive 86/609) and Finnish guidelines, and approved by the State Provincial Office of Eastern Finland.

**Drug treatment**

*Dose-finding pilot study:* A pilot study was undertaken to determine the therapeutic dose of memantine [i.e., the dose of memantine producing a steady-state plasma drug level of around 1µM, which several preclinical and clinical studies have indicated is therapeutic (Kornhuber and Quack, 1995; Zajaczkowski et al., 1996)] to be used in subsequent experiments in transgenic mice. Memantine (Forest Research Institute, Jersey City, NJ) was administered orally (via drinking water) to male C57BL/6J mice at the doses of 10 mg/kg/day (n = 10), 30 mg/kg/day (n = 10) and 100 mg/kg/day (n = 10) for 4 weeks. The placebo group (n = 10) had drinking water without memantine. Blood samples were taken from the femoral vein 4 weeks after the initiation of drug treatment to determine the steady-state plasma concentration of memantine. Plasma samples were analyzed at Merz Pharmaceuticals GmbH (Frankfurt am Main, Germany) using a gas chromatograph system coupled with a mass selective detector (Kornhuber and Quack., 1995).

*Memantine treatment:* Based on the pilot study, the dose of 30 mg/kg/day (see Results section for details) was chosen for the behavioral study and administered in drinking water for 3 weeks. Memantine was administered to 23 APP/PS1 mice and 19 NT mice. The placebo group (APP/PS1: n = 22; NT: n = 17) received drinking water without memantine. Behavioral testing started 2 weeks after treatment onset and continued for one week.
Behavioral testing

After two weeks of treatment, mice were tested for exploratory activity, isolation-induced aggression, and performance in the Morris water maze.

*Exploratory activity:* TruScan® (Coulbourn Instruments, CO, USA) automated activity monitor based on infrared photo detection was used for monitoring exploratory activity. The system consists of a transparent observation cage (26x26x39 cm) and two rings of photo detectors enabling separate monitoring of horizontal (XY-movement over time) and vertical activity (rearing). Activity was measured for 10 min in two separate sessions separated by 48 h.

*Isolation-induced aggression:* All test mice (‘residents’) had been housed in individual cages for at least 3 weeks prior to the start of this test. ‘Intruders’ were NT male C57Bl/J6 mice, 16-20 weeks old at the time of testing, housed in groups of 4-8 since weaning. A randomly chosen intruder was placed in the resident’s cage, and aggression of the resident was assessed by measuring attack latency, i.e. the time in seconds between the introduction of the intruder into the cage and the first attack by the resident. The experimenter was blind to genotype and drug treatment.

*Morris water maze:* The Morris water maze was used to measure spatial learning and memory. The apparatus was a black plastic pool with a diameter of 120 cm. A black escape platform (square, 14 x 14 cm) was located 1.0 cm below (hidden) the water surface. The temperature of the water was kept constant throughout the experiment (20 ± 0.5°C), and a 10-min recovery period was allowed between the training trials. First, the mice were pre-trained to find and climb onto the platform for two days by using an alley (1 m x 14 cm x 25 cm) leading to the platform located 1 cm below the water. The training consisted of 8 consecutive days of testing, with 5 trials per day. If the mouse failed to find the escape platform within the maximum time (60 seconds), the animal was placed on
the platform for 10 s by the experimenter. During the first 5 days of testing the mice were trained with a hidden platform. The platform location was kept constant and the starting position varied between four constant locations at the pool rim. Mice were placed in the water with their nose pointing towards the wall at one of the starting points in a random manner. On the sixth day, the platform was removed and the mice were allowed to swim for 60 s to determine their search bias. On testing days 7 and 8, a black curtain was hung around the swimming pool in order to conceal all extra-maze visual cues. The mice were trained to find a visible platform, which had a 10 cm high pole with a white flag and which was changed every trial to a new position. Timing of the latency to find the submerged platform was started and ended by the experimenter. A computer connected to an image analyzer (HVS Image®, Hampton, UK) monitored the swim pattern. During the water maze training, we measured swimming speed and latency to find the platform. The wall-swimming tendency (thigmotaxis) was assessed by dividing the pool into 3 concentric zones of equal surface area and calculating the time spent in the outer zone. Search bias during the probe trial was measured by calculating the time the mice spent in the vicinity of where the platform was previously located. We defined this as a target area centered on the platform with a diameter of 30 cm. This target area comprised 6.25 % of the total surface area, thus a random swim for 60 s in the pool would yield a dwell time of 3.75 s in the target area during the probe trial.

**Statistical analysis**

All statistical analyses were performed using SPSS for Windows software, version 11.5.1 (SPSS, Chicago, IL). The effects of genotype, treatment, training day, and their interaction with the behavioral parameters of exploratory activity and performance in the Morris water maze were evaluated by analysis of variance (ANOVA) for repeated measures. Attack latency from the isolation-aggression test was analyzed by two-way ANOVA with genotype and treatment as factors.
RESULTS

Memantine plasma concentrations: The steady-state plasma levels following oral administration of 10, 30 and 100 mg/kg/day memantine were 0.49 ± 0.06, 1.14 ± 0.07 and 5.54 ± 0.40 µM (mean ± SEM), respectively. Based on these data, the dose of 30 mg/kg/day, which produces the therapeutic steady-state plasma level of around 1µM, was chosen for all behavioral studies.

Exploratory activity: APP/PS1 and NT mice were first tested in an automated activity monitor to detect genotype and drug effects on motor and exploratory activity. ANOVA revealed a significant genotype effect. The APP/PS1 mice exhibited less horizontal activity (Fig. 1A; F(1, 77) = 13.0, p = 0.001) and less rearing (Fig. 1B; F(1, 77) = 35.0, p < 0.001) than NT mice. Memantine did not significantly affect either measure of exploratory activity (Fig. 1A and Fig. 1B).

Isolation-induced aggression: When confronted with an intruder mouse, APP/PS1 mice exhibited a shorter latency to attack the intruder than NT controls (Fig. 2; F(1, 56) = 3.9, p = 0.05). The increased aggressive behavior observed in APP/PS1 mice was not significantly modified by memantine (Fig. 2).

Morris water maze: The overall ANOVA revealed both a genotype (F(1, 77) = 12.5, p = 0.001) and a drug effect (F(1, 77) = 8.0, p = 0.006) in spatial learning. Placebo-treated APP/PS1 mice were slower than NT controls in finding the hidden platform (Fig. 3A; F(1,40) = 8.9, p = 0.005). Treatment with memantine reduced the escape latency in APP/PS1 mice compared to placebo-treated APP/PS1 mice (Fig. 3B; F(1,43) = 6.0, p = 0.02). In fact, the performance level of memantine-treated APP/PS1 mice did not differ from that of placebo-treated NT mice (one-way ANOVA with four groups, followed by Tukey’s post-hoc test, p = 0.96). There was also a trend towards improved performance in NT mice treated with memantine; however, this effect was not
significant (Fig. 3C; F(1,34) = 3.0, p = 0.09). APP/PS1 mice were also slower than their NT littermates in finding the visible platform (Fig. 3A; F(1, 77) = 15.8, p < 0.001). However, memantine did not show a significant improvement in this paradigm (Fig. 3B). Swimming speed was not affected by genotype (F(1, 77) = 0.27, p > 0.6) or drug treatment (F(1, 77) = 0.94, p > 0.3).

Initially, the natural tendency of mice is to remain close to the pool wall to find an escape from the water. However, they soon realize there is no escape through the wall and begin to search for the platform in the middle of the pool. To further analyze search pattern, we separately measured the time spent in the outer zone of the pool. The total time spent in the outer zone for APP/PS1 mice was significantly greater than for NT mice (Fig. 3D; F(1, 77) = 18.3, p < 0.001). Memantine significantly reduced the time spent in the outer zone (F(1, 77) = 11.7, p = 0.001), and this effect was significant for both APP/PS1 mice (Fig. 3E; F(1,43) = 4.7, p = 0.04) and NT mice (Fig. 3F; F(1,34) = 9.8, p = 0.004).

The strength of the learned spatial search bias was assessed during a probe trial on the sixth day without the platform. Mice in all groups spent more time in the vicinity of the platform location than would be expected by random swimming (3.75 s out of 60 s; see Methods). The time spent in the target area for the different groups was as follows: NT (placebo): 36.0 ± 2.0 s (mean ± sem), NT (memantine): 36.1 ± 2.6 s, APP/PS1 (placebo): 35.5 ± 2.3 s, APP/PS1 (memantine): 39.0 ± 2.0 s. Group differences were not significant.

**DISCUSSION**

Several preclinical and clinical studies have indicated that the therapeutic plasma concentration of memantine ranges between 0.5-1 µM (Kornhuber and Quack, 1995; Zajaczkowski et al., 1996). Therefore, in order to mimic a clinically relevant condition, an oral dose of 30 mg/kg/day was
selected from a pilot study that produced a steady-state plasma level of 1.14 µM in C57BL/6J mice (the background strain of the transgenic mice). Following 3-4 weeks of oral administration, memantine significantly improved the learning phase of spatial navigation in APP/PS1 mice, which exhibit age-dependent impairment in spatial learning (Fig. 3B). Memantine did not affect spontaneous locomotor activity or special motor patterns such as swimming in the water maze.

Sensorimotor disturbances are frequently associated with dissociative anesthetic-type NMDA receptor antagonists [e.g., (+)MK-801, ketamine or PCP] (Rogawski, 2000). Memantine is a non-dissociative anesthetic-type NMDA receptor antagonist, and it exhibits pharmacological properties that are different from dissociative anesthetic antagonists. For example, memantine has low to moderate affinity for the NMDA receptor channel, and it exhibits strong voltage-dependent channel blocking characteristics and fast channel unblocking kinetics (Parsons et al., 1995). Due to these unique biophysical and pharmacological properties, memantine can selectively block the pathological activation of NMDA receptors without affecting physiological NMDA receptor transmission, which is critical for learning and memory (Zajaczkowski et al., 1996). Some of the characteristic effects of (+)MK-801 in rodents are dose-dependent hyperactivity (French et al., 1991; Hargreaves and Cain, 1995), impairment in water maze performance and increased wall-clinging (thigmotaxis) (Cain et al., 1996). In the present study, we did not observe any changes in spontaneous rearing or horizontal locomotion in either APP/PS1 or NT mice treated with memantine. In contrast, memantine treatment improved water maze learning in APP/PS1 mice and reduced thigmotaxis. These effects of memantine are in agreement with the high tolerability profile of memantine observed in clinical trials.

It is possible that learning deficits observed in APP/PS1 mice are due to overactivation of NMDA receptors. The development of learning impairment in APP/PS1 mice correlates with age-dependent
increases in Aβ levels in the brain (Puoliväli et al., 2002). There is evidence that Aβ impairs synaptic plasticity (Chapman et al., 1999; Stephan et al., 2001; Walsh et al., 2002), elevates extracellular levels of excitatory amino acids and increases intracellular accumulation of calcium (Harkany et al., 2000). In addition, APPswe mice exhibit increased susceptibility to excitotoxicity and ischemic brain damage (Zhang et al., 1997; Fitzjohn et al., 2001). These findings are indicative of possible aberrant functioning of NMDA receptors in APP/PS1 mice.

In the present study, memantine treatment resulted in a significant improvement in water maze acquisition in APP/PS1 mice. One earlier study in Fisher 344 rats also reported improved water maze learning with memantine treatment of 30 mg/kg/day for 8 weeks (Barnes et al., 1996). However, in that study, the effect of memantine was apparent at the later stages of water maze learning by improved search bias in the probe tests, whereas in our study the effect was most pronounced at the early stages of learning. It is generally believed that activation of NMDA receptors plays an important role in fast learning of several simultaneous aspects of complex tasks such as the Morris water maze (e.g., learning that there is a platform to provide escape from the water, that there is no escape through the wall, and determining the location of the platform with respect to extra-maze spatial cues). Therefore, improved learning observed in the early phase of water maze acquisition in memantine-treated APP/PS1 mice compared to untreated transgenic mice indicates that the physiological functioning of NMDA receptors is restored by memantine under pathological conditions.

Another explanation for differences in the effects of memantine on early- versus late-phase learning between the present and the study of Barnes et al could be the difference in species tested (rat versus mouse). In general, mice have a much stronger tendency to swim near the pool wall (thigmotaxis) and show much poorer search bias (Wolfer et al., 2001). Furthermore, the present data
indicate that thigmotaxic behavior is even more accentuated in APP/PS1 transgenic mice. This raises the question whether the beneficial effect of memantine in the water maze task can be explained, at least in part, by reduced thigmotaxis. This is unlikely because the escape latency of memantine- and placebo-treated APP/PS1 mice differed more than the time spent in the outer pool zone (Fig. 3).

Behavioral disturbances are often the leading cause for institutionalization of AD patients. Aggression towards caregivers is a significant problem in caring for these patients and is often present without psychotic symptoms (Lopez et al., 2003). Increased aggressiveness has been reported in transgenic mice expressing mutated human APP (Kumar-Singh et al., 2000). The present findings further support the link between APP (and PS1) transgenes and aggression as APP/PS1 mice exhibited increased isolation-induced aggression. Aggressive behavior in rodents either can be increased (by high affinity, dissociative anesthetic-type NMDA receptor antagonists like MK-801) or decreased (by low to moderate affinity antagonists like memantine) (McAllister, 1990). In the present study, we did not observe any significant anti-aggressive effects of memantine, although it may be noted that memantine did not increase aggressive behavior in either APP/PS1 or NT mice.

In addition to its affinity for NMDA receptors, memantine has been reported to reduce serotonergic 5-HT3 receptor-mediated inward currents in a non-competitive manner with an IC₅₀ of 2.3 µM, the same order of magnitude as its potency for blocking NMDA receptors (Rammes et al., 2001). The antagonism of 5-HT3 receptors by memantine may account for, at least partially, the observed behavioral (cognition-enhancing) effects of memantine. Although it is not clear how 5-HT3 receptor antagonists offer beneficial effects to cognition, 5-HT3 receptor antagonists can improve
water maze learning in aged rats (Pitsikas et al., 1993) and in rats with neurotoxic lesions of the 
basal forebrain (Hodges et al., 1996).

In conclusion, subchronic oral administration of memantine mimicking its clinical use improves the 
impaired spatial learning of APP/PS1 transgenic mice but does not affect the increased aggression 
or reduced exploratory activity observed in these mice. These observations warrant further studies 
on the effect of memantine in AD and other neurodegenerative conditions.

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Footnotes

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Figure legends

Fig. 1. Measures of exploratory activity during a 10-min recording in a closed transparent cage. (A) Horizontal activity (distance traveled), (B) vertical activity (number of rearings). The filled and the open columns denote mean ± SEM on Day 1 and Day 3, respectively. ***APP/PS1 mice differed significantly from the NT littermates in the overall ANOVA (p< 0.001).

Fig. 2. Isolation-induced aggression test. The columns denote mean ± SEM latency of the resident mouse to attack the intruder mouse (filled columns for NT mice and open columns for APP/PS1 mice). *APP/PS1 mice differed significantly from the NT littermates in the overall ANOVA (p<0.05).

Fig. 3. Morris water maze test for memantine and placebo-treated NT and APP/PS1 mice. The mean escape latency (A, B, C) and mean % time in the outer zone of the pool (D, E, F) are given for different test days. Days 1-5: hidden platform test; days 7-8, visible platform test. The asterisks denote differences between the given two groups over all testing days (five for hidden platform, two for visible platform): *p < 0.05, **p < 0.01, ***p < 0.001 (ANOVA for repeated measures).
Figure 2

Latency to attack (min)

-/- Placebo
-/- MEM
APP/PS1 Placebo
APP/PS1 MEM

* *
Figure 3

A  
Escape latency (s)
- NT Placebo
- APP/PS1 Placebo

B  
Escape latency (s)
- APP/PS1 Placebo
- APP/PS1 MEM

C  
Escape latency (s)
- NT Placebo
- NT MEM

D  
Time in outer zone (%)
- NT Placebo
- APP/PS1 Placebo

E  
Time in outer zone (%)
- APP/PS1 Placebo
- APP/PS1 MEM

F  
Time in outer zone (%)
- NT Placebo
- NT MEM