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MEMANTINE IMPROVES SPATIAL LEARNING AND MEMORY IMPAIRMENTS BY REGULATING NGF SIGNALING IN *APP/PS1* TRANSGENIC MICE

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Abstract—Memantine (MEM) is used for improving the cognitive impairments of the patients suffering from Alzheimer's disease (AD) by multiple neuroprotective mechanisms. However, it is still not clear whether nerve growth factor (NGF) signaling is involved in the mechanisms of MEM. The present study investigated the neuroprotective effects of MEM treatment on the cognitive performance and amyloidosis in *APP/PS1* transgenic mice, and disclosed the NGF-related mechanism of MEM. We found that MEM treatment improved the cognitive performance by decreasing the escape latency and path length in the navigation test, by shortening the duration in target quadrant and reducing the frequency to pass through the target in probe trial, and by prolonging the latency and decreasing the frequencies of entering the dark compartment in passive avoidance test. The over-expressions of Aβ(1–42) and amyloid precursor protein (APP) were also decreased in the brains of *APP/PS1* mice. Interestingly, MEM treatment improved the decreased NGF levels in *APP/PS1* mice. Furthermore, NGF/TrkA signaling was activated by increasing the phosphorylation levels of tyrosine kinase (TrkA), proto-oncogene serine/threonine-protein kinase, Raf1 (c-Raf), extracellular regulated protein kinases (ERK)1/2 and cAMP-response element binding protein (CREB) after MEM treatment. Simultaneously, MEM also inhibited NGF/p75^{NTR} signaling via decreasing the cleavage substrate of p75^{NTR}, increasing the JNK2 phosphorylation and decreasing the levels of p53 and cleaved-caspase 3. Therefore, the dual-regulation on NGF signaling was attributed to the improvements of cognitive deficits and Aβ depositions in *APP/PS1* mice. In conclusion, MEM treatment activated the NGF/TrkA signaling, and inhibited the p75^{NTR} signaling in *APP/PS1* mice to ameliorate the behavioral deficits and amyloidosis, indicating that NGF signaling was a new potential target of MEM treatment for AD therapy.

Key words: memantine, nerve growth factor, p75 neurotrophin receptor, TrkA receptor, cognitive deficit, *APP/PS1* transgenic mice.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease, clinically characterized by cognitive impairments and pathologically characterized by deposits of β-amyloid (Aβ) peptides as senile plaques in the AD brain (Hsiao et al., 1996; Lesné et al., 2006; Walsh and Selkoe, 2007). Memantine hydrochloride (MEM) is a low- to moderate-affinity, uncompetitive, and voltage-dependent N-methyl-D-aspartate (NMDA) receptor antagonist. It is the first drug to be approved by the FDA in US for the treatment of moderate to severe AD for ameliorating the cognitive and behavioral impairments in AD patients (Olivares et al., 2012; Sinforiani et al., 2012; Lyseng-Williamson and McKeage, 2013). The principal neuroprotective mechanisms of MEM treatment are believed to be the blockade of NMDA receptors, the decrease of the over-excitation of NMDA receptors induced by glutamate, and the inhibition of the excitatory toxicity of glutamate. As a result, the memory and learning impairments are improved (Lipton, 2005; Johnson and Kotermanski, 2006). However, it has been suggested that there are some other mechanisms for neuroprotection besides the inhibition of the NMDA receptor, such as, the improvements of brain-derived neurotrophic factor (BDNF) and TrkB receptor, or direct stimulation of the muscarine receptors (Marvanová et al., 2001; Drever et al., 2007; Jantas et al., 2009; Nyakas et al., 2011). Moreover, MEM decreases Aβ levels in neuronal cultures and in the brains of AD animals, protecting the neurons from Aβ-induced toxicity (Alley et al., 2010; Martinez-Coria et al., 2010; Danysz and Parson, 2012; Colom et al., 2013).

Nerve growth factor (NGF), the first neurotrophin, is known for its stimulatory effects on differentiation, maintenance, survival and plasticity of neurons. NGF deficiency in the brain induces apoptosis, death and

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Abbreviations: Aβ, β-amyloid; AD, Alzheimer's diseases; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; c-Raf, proto-oncogene serine/threonine-protein kinase, Raf1; CREB, cAMP-response element binding protein; DAB, 3′-diaminobenzidine; ERK, extracellular regulated protein kinasesl; ITI, inter-trial interval; IOD, integrated optical density; LTP, long-term potential; MEM, memantine; MWM, Morris water maze; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; PAT, passive avoidance test; PBS, Phosphate-buffered solution; PMSF, phenylme-thylsulfonyl fluoride; PVDF, polyvinylidene difluoride; TrkA, tyrosine kinase; WT, wild type.

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dysfunction of neurons, and accelerates Aβ deposits and Aβ-induced toxicity (Counts and Mufson, 2005; Colafrancesco and Villoslada, 2011). However, it is unclear whether NGF signaling was one of potential targets of MEM treatment on the inhibition of Aβ deposits and improvement of cognitive performance. In the present study, we investigated the neuroprotective effects of MEM on cognitive performance and amyloidosis in *APP/PS1* transgenic mice and determined the involvement of NGF signaling in the mechanisms of MEM.

EXPERIMENTAL PROCEDURES

Animals

The original *APP/PS1* double-transgenic mice were obtained from the Jackson Laboratory [*B6.Cg-Tg (APPswe, PSEN1dE9) 85Dbo/J*]. The *APP/PS1* double-transgenic mice overexpress a chimeric mouse/human *APP* containing the *K595N/M596L* Swedish mutations and a mutant human *PS1* carrying the exon 9-deleted variant. The mice used in this study were derived from the original *APP/PS1* mice by backcrossing them with C57 BL/6J mice for 5–6 generations. These mice manifested a rapid accumulation of amyloid plaques in the hippocampus and cortex. The twenty offspring 12-month-old *APP/PS1* mice (20–28 g weight) were distributed equally into the vehicle-treated amyloid precursor protein (APP)/PS1 group (APP/PS1 group) and MEM-treated APP/PS1 group ($n = 10$, per group), compared with their age-matched wild-type (WT) littermates (C57 BL/6J mice, $n = 10$, 21–30 g weight). Male and female animals were equally distributed into each group in order to make each group gender-matched. All mice were housed in cages in a controlled environment (22 °C, 55% relative humidity, 12-h light/dark cycle) with free access to food and water, and maintained in a specific pathogen-free environment at the Laboratory Animal Center of the China Medical University. All animal care and experimental procedures were in compliance with the Standard Medical Laboratory Animals' Care and Use Protocols (Ministry of Health PR China, 1998) and the Laboratory Animal Ethical Standards of the China Medical University.

Drug treatment

MEM (Sigma–Aldrich Chemicals Pvt. Ltd., St. Louis, MO, USA) was dissolved in distilled water at a concentration of 0.5 mg/mL. Distilled water was used as vehicle. MEM or vehicle was administered by intragastric administration (5 mg/kg body weight) at 9:00 am once daily for 4 weeks. Mice in every group were age-matched and gender-matched, and 10 mice were used in every group ($n = 10$, per group). MEM-treated APP/PS1 group intragastically received MEM (5 mg/kg) once daily for 4 weeks, APP/PS1 group and WT group received the same volume of vehicle for 4 weeks.

Passive avoidance test (PAT)

After 4 weeks of the MEM treatment, mice were subjected to a PAT using a PAT apparatus (BA-200, Chengdu Taimeng Tech. Co. LTD, Chengdu, PR China), as previously described (Galeotti et al., 1998; Tsuji et al., 2003). In the training session, each mouse was placed in the light compartment and allowed to explore for 3 min, at which point the guillotine door was raised to allow the mouse to enter the dark compartment. When the mouse entered the dark compartment, the guillotine door was closed and an electrical foot shock (0.5 mA, 1 s duration) was delivered. Training session was conducted before the test session. The test session was performed 24 h after the training session. In the test session, each mouse was placed in the light compartment and allowed to explore 3 min, and then the guillotine door was raised. Latencies and frequencies for mice to enter the dark compartment were recorded during the whole testing period (300 s).

Morris water maze (MWM) test

After the PAT, mice were given another behavioral test, MWM, for consecutive 6 days including navigation tests and a probe trial test, as previously described with a few modifications (Morris, 1984; Vorhees and Williams, 2006).

The MWM is a stainless-steel circular water tank (120 cm diameter × 50 cm height) equipped with a platform (10 cm diameter) placed in the second quadrant and submerged 0.5–1 cm below the surface of water. In brief, mice were allowed to swim freely for 1 min without the platform to adjust themselves to the circumstances at the baseline day (day 0). From the 1st day to the 5th day, the platform was placed under the water in the tank for navigation tests, and each mouse was subjected to 4 trials per day at an inter-trial interval (ITI) of 60 s for spatial acquisition. Different start locations were used for each trial. If a mouse failed to find the platform within 60 s, it would be picked up and placed on the platform for 60 s. For each trial, the latency and the path length by which the mouse found the hidden platform were recorded. On the 6th day, a probe trial was performed to assess memory consolidation. In this trial, the platform was removed from the tank, and the mice were allowed to swim freely for 60 s. The start position was a novel one which was 180° from the original platform position to ensure that the spatial preference was a reflection of the memory of the goal location rather than for a specific swim path. Frequency that each mouse crossed the center of the quadrant (where the platform was previously located) and the percent of time that each mouse spent in the quadrant were recorded. All data were obtained by a video tracking system (Chengdu Taimeng Tech. Co. LTD, Chengdu, PR China).

Locomotivity test

Locomotivity test was conducted after MWM and PAT using a locomotivity testing paradigm (ZZ-6 system for mice, Chengdu Taimeng Tech. Co. LTD, Chengdu, PR China). Briefly, mice were placed in the system and the exploration was assessed for 10 min. Cages were routinely cleaned with ethanol following each session. The locomotivity and the frequency of stand-up for each mouse were recorded.