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# Anti-amnesic effect of neurosteroid PREGS in $A\beta_{25-35}$-injected mice through $\sigma_1$ receptor- and α7nAChR-mediated neuroprotection

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ABSTRACT

A single intracerebroventricular injection of β-amyloid 25–35 peptide ($A\beta_{25-35}$) (9 nmol/mouse) induces the spatial cognitive deterioration and approximately 50% loss of pyramidal cells in hippocampal CA1 region within 1 week. The present study focused on exploring the effects of neurosteroid pregnenolone sulfate (PREGS), in comparison with the selective agonists of sigma-1 receptor ($\sigma_1$R) and α7 nicotinic acetylcholine receptor (α7nAChR), on the cognitive deficits and the death of pyramidal cells in $A\beta_{25-35}$-mice. Herein, we reported that the administration of PREGS (1–100 mg/kg) for 7 days after $A\beta_{25-35}$-injection could dose-dependently ameliorate the cognitive deficits and attenuate the apoptosis of pyramidal cells. Either the $\sigma_1$R antagonist NE100 or the α7nAChR antagonist MLA could block the neuroprotection of PREGS in $A\beta_{25-35}$-mice. Both the $\sigma_1$R agonist PRE084 and the α7nAChR agonist DMXB could mimic the PREGS-neuroprotection against the $A\beta_{25-35}$-neurotoxicity. The neuroprotection of PRE084 was attenuated by MLA, but the DMXB-action was insensitive to NE100. The neuroprotection of PREGS, PRE084 or DMXB was blocked by the phosphatidylinositol-3-kinase (PI3K) inhibitor LY294002, whereas only the effect of PREGS or PRE084 was sensitive to the MAPK/ERK kinase (MEK) inhibitor U0126. PREGS prevented $A\beta_{25-35}$-inhibited Akt (Serine/threonine kinase) phosphorylation leading to increase in caspase-3 activity, which was $\sigma_1$R- and α7nAChR-dependent. By contrast, PREGS-rescued reduction of extracellular signal-related kinase-2 (ERK2) phosphorylation in $A\beta_{25-35}$-mice only required the activation of $\sigma_1$R. Blockage of PREGS-neuroprotection by LY294002 significantly attenuated its anti-amnesic effect in $A\beta_{25-35}$-mice. The findings indicate that the anti-amnesic effects of PREGS in $A\beta_{25-35}$-mice depend on the $\sigma_1$R- and α7nAChR-mediated neuroprotection.



## 1. Introduction

Alzheimer's disease (AD) is characterized by a progressive neurodegeneration leading to dementia and death. The accumulation and deposition of β-amyloid (Aβ) within the brain is thought to be the primary force driving the pathogenesis of AD. Aβ has been reported to disrupt neuronal cells by calcium dyshomeostasis (Nelson et al., 2007) and enhancement of excitotoxicity (Rothman and Olney, 1995) and apoptosis (Forloni et al., 1993). At sites of Aβ-aggregation, hippocampal neuronal cells die leading to the rapid loss of learning and memory (Yan et al., 1996).

Cholinergic system is very sensitive to the Aβ-neurotoxicity. The Aβ-induced dysfunction of α7 nicotinic acetylcholine receptor (α7nAChR) results in the impairment of spatial memory (Chen et al., 2006, 2010). The α7nAChR agonists are able to attenuate the Aβ-neurotoxicity and glutamate toxicity in cultured neurons (Kihara et al., 1997; Zamani et al., 1997). The treatment with DMXB,

*Abbreviations:* AD, Alzheimer's disease; Aβ, β-amyloid; DMXB, 3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride; LY294002, 2-(4-morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one hydrochloride; MLA, methyllycaconitine; MAPK, mitogenic activated protein kinase; NMDAr, N-methyl-D-aspartate receptor; NE100, N,N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl] ethylamine hydrochloride; ERK, extracellular signal-related kinase; PI3K, phosphatidylinositol-3-kinase; PREG, pregnenolone; PREGS, pregnenolone sulfate; PRE084, 2-(4-morpholinethyl)-1-phenylcyclohexanecarboxylate hydrochloride; U0126, 4-diamino-2,3-dicyano-1-4-bis [2-aminophynylthio] butadiene; α7nAChR, α7 nicotinic acetylcholine receptor; $GABA_AR$, γ-aminobutyric acid type A receptor.

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a selective α7nAChR agonist, ameliorates the $A\beta_{25-35}$-induced deficits in spatial memory (Chen et al., 2010). Furthermore, nicotine not only inhibits beta-amyloidosis (Hellström-Lindahl et al., 2004) but also prevents the accumulation of Aβ in APP transgenic mice (Liu et al., 2007). On the other hand, the activation of sigma-1 receptor ($\sigma_1$R) may induce important neuroprotection against the $A\beta_{25-35}$-neurotoxicity (Li et al., 2010). The selective $\sigma_1$R agonists are potent neuroprotective drugs as observed in excitotoxicity models (Maurice and Lockhart, 1997; Nakazawa et al., 1998) and Aβ-induced toxicity in cortical neurons *in vitro* (Marrazzo et al., 2005). Meunier et al. (2006) have reported the potent anti-amnesic and neuroprotective effects of donepezil against $A\beta_{25-35}$-neurotoxicity through its cholinergic and $\sigma_1$R agonistic properties.

Steroids which are synthesized within the central or peripheral nervous system have been named "neurosteroids" (Baulieu, 1997). Recently, the correlation between decreased levels of neurosteroids and neuronal degeneration in AD patients has been paid close attention (Naylor et al., 2010). Pregnenolone sulfate (PREGS), a steroid synthesized de novo in the brain, is thought to relate with cognitive performance in senescent animals (Schumacher et al., 2008). Deficient cognitive performance in aged rats can be corrected by intrahippocampal injection of PREGS (Vallée et al., 1997). In addition to as a negative modulator of $GABA_A$ receptors (Akk et al., 2001) and positive allosteric modulator of N-methyl-D-aspartate receptor (NMDAr, Maurice et al., 2006), PREGS has been demonstrated to enhance the function of α7nAChR (Chen and Sokabe, 2005) and $\sigma_1$R (Monnet et al., 1995). Collectively, it is speculated that PREGS can antagonize the Aβ-neurotoxicity through its α7nAChR and $\sigma_1$R agonistic actions.

$A\beta_{1-42}$, a major constituent of senile plaques, is well known to be one of the candidates causing memory loss, because numerous studies have demonstrated a significant correlation between the number of senile plaques and the degree of cognitive deficits in AD brains. Among the Aβ fragments studied so far, peptide bearing the 11 amino acids (25–35) ($A\beta_{25-35}$) is the shortest fragment of Aβ processed *in vivo* by brain proteases (Kubo et al., 2002). This peptide retains the ability to self-aggregate and mediates the toxicity of the full-length peptide, though it lacks a hydrophobic C terminal sequence of five amino acids as compared to $A\beta_{1-40}$ (Burdick et al., 1992). It has been proposed that $A\beta_{25-35}$ represents the biologically active region of $A\beta_{1-42}$ (Pike et al., 1995). Experiments using transgenic and gene targeting mouse models have shown a close association between excess amounts of Aβ and the deficits in learning and memory. Thus, two nontransgenic rodent models of AD created by intracerebroventricular (i.c.v.) infusion of $A\beta_{1-40/42}$ (Chen et al., 2006) or $A\beta_{25-35}$ (Maurice et al., 1996) have been widely used to analyze the morphological and behavioral consequences of Aβ-neurotoxicity *in vivo*. Consistent with the $A\beta_{1-40/42}$ infusion (i.c.v.) in rats, the injection (i.c.v.) of $A\beta_{25-35}$ in mice induces, within 1 or 2 weeks after administration, histological and biochemical changes in cholinergic system (Kowall et al., 1991, 1992). A single injection (i.c.v.) of either $A\beta_{1-40/42}$ (Wu et al., 2008) or $A\beta_{25-35}$ (Chen et al., 2010) in rats and mice can impair the induction of long-term potentiation (LTP) in hippocampal CA1 region. A single injection (i.c.v.) of aggregated $A\beta_{25-35}$ (3 nmol/mouse) in mice induces amnesia in many kinds of behavior experiments such as in Y-maze, step-down type passive avoidance and Morris Water maze, although the low-dose of $A\beta_{25-35}$ does not produce neuronal cell loss (Maurice et al., 1996; Wang et al., 2007). By contrast, one single injection (i.c.v.) of $A\beta_{25-35}$ at high dose of 9 nmol/mouse can cause the death of hippocampal neuronal cells. Therefore, the present study focused on exploring the anti-amnesic and neuroprotective effects of PREGS, in comparison with the selective agonists of $\sigma_1$R and α7nAChR, after a single injection of $A\beta_{25-35}$ (9 nmol/mouse) in mice by examining the spatial memory behavioral, hippocampal morphological and biochemical changes. Our results showed that the administration of PREGS in $A\beta_{25-35}$-mice exerts a potent anti-amnesic effect through $\sigma_1$R- and α7nAChR-mediated PI3K–Akt and ERK neuroprotective mechanisms.

## 2. Materials and methods

### 2.1. Subjects

The present studies were approved by Animal Care and Ethical Committee of Nanjing Medical University. All procedures were in accordance with the guidelines of Institute for Laboratory Animal Research of the Nanjing Medical University. Male mice (ICR, Oriental Bio Service Inc., Nanjing), weighing 20–25 g (8 weeks old) at the beginning of the experiment, were used throughout the study. All animals were housed in a light controlled room under a 12-h light–dark cycle starting at AM 7:00 and kept at a temperature of 23 °C in the Animal Research Center of Nanjing University. They received food and water *ad libitum*. All efforts were made to minimize animal suffering and to reduce the number of animals used.

### 2.2. Preparation of an animal model of Alzheimer's disease

$A\beta_{25-35}$ (Sigma, St. Louis, MO, USA) was dissolved in distilled water at the concentration of 3 mM. "Aggregated" $A\beta_{25-35}$ was obtained by incubating at 37 °C for 4 days according to previous report (Maurice et al., 1996) and then diluted to the final concentration with saline just before the experiment. Mice were anaesthetized with intraperitoneal (i.p.) injection of chloral hydrate (400 mg/kg) and placed in a sterotactic device (Kopf Instruments, Tujunga, CA). For a single injection (i.c.v.) of $A\beta_{25-35}$, a 28-G stainless-steel needle (Plastics One, Roanoke, VA) was inserted into lateral ventricular (0.3 mm posterior, 1.0 mm lateral, and 2.5 mm ventral to bregma), and then the "aggregated" $A\beta_{25-35}$ (9 nmol/3 μl/mouse) was injected with a stepper-motorized micro-syringe (Stoelting, Wood Dale, IL, USA) at a rate of 0.5 μl/min. The injection site was confirmed in preliminary experiments by injecting Indian ink. Control mice were given an equal volume of vehicle.

### 2.3. Drug administration

PREGS, when intraperitoneally (i.p.) injected, may cross blood–brain barrier and can be taken up by the brain (Higashi et al., 2003). PREGS and PREG were dissolved in dimethyl sulfoxide (DMSO), and then diluted in sesame oil to a final concentration of 1.0% DMSO, because the high dose of PREGS (100 mg/kg) could not be dissolved in 1.0% DMSO diluted by saline. PREGS (1–100 mg/kg) or PREG (20 mg/kg) was subcutaneously (s.c.) injected at 100 μl once daily. $\sigma_1$R agonist PRE084 (1.0 mg/kg) and α7nAChR agonist DMXB (5 mg/kg, Taisho Pharmaceuticals, Tokushima, Japan) were dissolved by 0.9% saline and all of these drugs were intraperitoneally injected once daily on 1–7 days after $A\beta_{25-35}$-injection. NMDAr antagonist MK801 (2 mg/kg, i.p.) and $\sigma_1$R antagonist NE100 (3 mg/kg, i.p. Taisho Pharmaceutical Co. Ltd. Tokyo, Japan) were dissolved by distilled water, and α7nAChR antagonist methyllycaconitine (MLA) (0.1 nmol/mouse, i.c.v.), MAPK/ERK kinase (MEK) inhibitor U0126 (0.3 nmol/mouse, i.c.v.) and PI3K inhibitor LY294002 (0.3 nmol/mouse, i.c.v.) were dissolved in DMSO, and then in 0.9% saline to a final concentration of 1% DMSO. These antagonists were injected 30 min before treatment with PREGS or agonists. Chemicals, unless stated, all came from Sigma Chemical Company (St Louis, MO, USA). For repeated injection (i.c.v.) of drugs, a 26-G stainless-steel guide cannula (Plastics One, Roanoke, VA) was implanted into the right lateral ventricle (0.3 mm posterior to bregma, 1.0 mm lateral, and 2.3 mm ventral) and anchored to the skull with four stainless steel screws and dental cement. The drugs were prepared freshly on the day of experiment and were injected using a 28-G stainless-steel needle combining with stepper-motorized micro-syringe (Stoelting, Wood Dale, IL, USA) at a rate of 0.5 μl/min (final volume 3 μl/mouse). Control mice were given an equal volume of vehicle.

### 2.4. Behavioral analysis

For the Morris water maze task, a pool (diameter 90 cm) made of black-colored plastic was prepared, with water temperature maintained at 20 ± 1 °C. Swimming paths were analyzed using a computer system with a video camera (AXIS-90 Target/2, Neuroscience). In the hidden platform test, the platform (7 cm in diameter) was submerged 1 cm below the water surface. On the first and the last day of water maze training, the swimming speed was assessed in the absence of the platform. Mice were given 90 s to reach the hidden platform. Four starting positions were used and each mouse was trained with four trials per day. After reaching the platform, the mouse was allowed to remain on it for 10 s. If the mouse did not find the platform within 90 s, the trial was terminated and the animal was put on the platform for 10 s. The water maze task was consecutively performed on day 3–7 after $A\beta_{25-35}$-injection. Average swimming speed (cm/sec) and latency (sec) to reach the platform were scored on all trials and analyzed where appropriate.