Varenicline Ameliorates Learning and Memory Deficits in Amyloid β(25–35) Rat Model of Alzheimer’s Disease

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Abstract

Introduction: Alzheimer’s disease (AD) is an enfeeble neurodegenerative disorder characterized by increased β-amyloid (Aβ) deposition and neuronal dysfunction leading to impaired learning and recall. Among proposed risk factors, impaired cholinergic transmission is a main cause for incidence of disease.

Methods: In the present study, effects of the intracerebroventriculally administration of an agonist of nicotinic cholinergic receptors, varenicline (0.5 and 2 µg/µl), on learning and memory impairments induced by intrahippocampal Aβ(25–35) injection was assessed in rats.

Results: The results showed that the intrahippocampal Aβ(25–35) injected rats exhibit lower spontaneous alternation score in Y-maze tasks (p<0.05), impaired retention and recall capability in the passive avoidance test (p<0.05), and fewer correct choices (p<0.001) and more errors (p<0.001) in the RAM task. Varenicline, almost in both doses, significantly improved alternation score in Y-maze task (p<0.001), impaired retention and recall capability in the passive avoidance test (p<0.05), and correct choices in the RAM task (p<0.001).

Discussion: This study indicates that varenicline pretreatment attenuates Aβ-induced impairment of short-term spatial memory in rats probably due to its agonist activity at nicotinic receptors.

Key Words: Aβ(25–35), Varenicline, Passive Avoidance, Y Maze, Radial Maze, Rat

1. Introduction

Alzheimer’s disease (AD) is an age-related neurodegenerative disorder that has a profound effect on learning and memory, judgments, communication and daily activities (Stuchbury & Munch, 2005). AD is described by two main neuropathological characteristics: the extracellular accumulation of senile plaques whose major component is the amyloid-β (Aβ) peptide and intraneuronal neurofibrillary tangles made up of hyperphosphorylated tau protein. Other aspects of AD pathology are impaired cholinergic transmission, mitochondrial malfunction, neuronal stress oxidative damage, increased inflammatory mediators, synapse deprivation, deficiencies in steroid hormones, and glutamate-excitotoxicity and neuronal degeneration (Shah et al., 2008). Among these, it seems that cholinergic transmission impairment has a key role in development and progression of disease.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels exhibiting high cation permeability. The nAChRs can be homopentamers or...
heteropentamers, composed mainly of the α2-α10 and β2-β4 subunits (Albuquerque et al., 2009). These receptors greatly distributed in the CNS and underlie diverse neuronal processes such as those involved in learning/memory (Chan, Wong, & Sheu, 2007; Davis, Kenney, & Gould, 2007). Recent studies on the effects of Aβ on nAChRs, have focused on receptor activity in neurotransmission (Selkoe, 1998; Bossy-Wetzel et al., 2004; Marcello et al., 2008). β-amyloid binds with low to ultra-high affinity to neuronal nicotinic acetylcholine receptors (Wang et al., 2000a, 2000b). Aβ-nAChR complexes accumulate intraneuronally (Nagele, D’Andrea, Anderson, & Wang, 2002; Wang, Li, Benedetti, & Lee, 2003) and cause neuronal cell death. Accordingly, chronic perturbation of the nAChRs by Aβ in aged and AD brains might cause neuronal dysfunctions and the formation of Aβ-rich plaques and neurofibrillary tangles (NFTs) (Wang et al., 2000a; Lee & Wang, 2003). Since, Aβ severely restricts nAChR activity (Pettit, Shao, & Y akel, 2001; Liu, Kawai, & Berg, 2001) and limits nAChR-dependent cholinergic neurotransmission, the synthesizing analogs of various nicotinic compounds (Jensen et al., 2005), could be as potential curative targets for Alzheimer’s disease. One of these compounds is α4β2-containing nAChRs that are a target for the development of smoking cessation therapies (Salminen et al., 2004).

Varenicline is a recently developed nicotinic ligand that has recently been approved by the U.S. Food and Drug Administration for use as a smoking cessation therapy (Obach et al., 2006). In equilibrium binding assays, varenicline is selective for the α4β2 receptor compared with α3β4, α7 and muscle-like nAChRs; whereas in a functional assay, varenicline is a partial agonist at α4β2 receptors (Coe et al., 2005). In this study, we examined the efficacy of acute varenicline pretreatment on alleviation of β-amyloid-induced deficits in learning and memory using Y-maze, passive avoidance, and 8-arm radial maze (RAM) tests.

2. Methods

2.1. Animals

Adult male Wistar rats (Pasteur’s Institute, Tehran), weighing 250–300 g at the start of the experiment were housed three to four per cage in a temperature-controlled colony room under seasonal light/dark cycle. The animals were given free access to water and kept at 80–85% of their free feeding body weight throughout the experiment. This study was conducted in accordance with the policies stipulated in the Guide for the Care and Use of Laboratory Animals (NIH) and by the Research Council of Iran University of Medical Sciences (Tehran, Iran).

2.2. Experimental Procedure

Rats (n = 56) were randomly divided into the following groups: (1) Control (n = 8) (2) Sham operation (n = 10); (2) Varenicline treatment (2 µg/µl), Sham operation (n = 10); (3) Aβ injection (A-beta; n = 8); (4) Varenicline treatment (0.5 and 2 µg/µl) Aβ injection (n = 10). For stereotoxic surgery, rats were anesthetized with a combination of ketamine (Ratiopharm, Germany; 100 mg/kg, i.p.) and xylazine (Ratiopharm, Germany; 5 mg/kg, i.p.) and then placed in a stereotaxic apparatus (USA) (incisor bar _3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution and incised on the midline, and a burr hole was drilled through the skull. Animals in the Aβ group were bilaterally injected in the dorsal hippocampus at coordinates of -3.5 mm posterior to bregma, 2 mm lateral to sagittal suture, and 2.8 mm below dura, according to the stereotoxic atlas (Paxinos & Watson, 1986) with a solution containing 10µg aggregated Aβ(25–35) (5µg/µl, Sigma, USA) or 0.9% normal saline (sham-operated) of the same volume. To produce neurotoxicity, saline-diluted Aβ(25–35) was incubated at 37°C for 7 d to allow fibril formation. Varenicline (Sigma Chemicals, USA) was injected i.c.v at doses of 0.5 and 2 µg/µl at coordinates of -0.8 mm posterior to bregma, 1.4 mm lateral to bregma, and 4 mm below dura. All injections were done using a hamilton microsyringe. Varenicline was administered five minutes before intrahippocampal injection of Aβ25–35. The dosage was chosen according to the results of our pilot study. Varenicline was dissolved in 0.9% normal saline and diluted to the required volume with artificial CSF (aCSF) containing the following: 120 mM NaCl, 3 mM KCl, 1.15 mM CaCl2, 0.8 mM MgCl2, 27 mM NaHCO3, and 0.33 mM NaH2PO4; pH adjusted to 7.2 (Merck Chemical, Germany).

Behavioral tests were conducted two weeks after the surgery and were evaluated blind to the treatments by the observer.

2.3. Y-maze task

Spatial recognition memory was assessed by recording spontaneous alternation behavior in a single-session Y-maze on the 14th day post-surgery, as described elsewhere (Rasoolijazi, Joghataie, Rognani, & Nobakht, 2007). The maze was made of black Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The
arms converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was as follows: each rat, naive to the maze, was placed at the end of one arm and was allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually. Entry was considered to be complete when the base of the animal’s tail was entirely within the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The maximum number of possible spontaneous alternations was determined as the total number of arms entered - 2, and the percentage was calculated as the ratio of actual to possible alternations × 100

2.4. Single-Trial Passive Avoidance Test

The apparatus (40 cm long - 20 cm wide - 30 cm high) consisted of an illuminated chamber connected to a dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed in the apparatus for 15 min to habituate. On the third day, an acquisition trial was performed. Rats were placed individually in the illuminated chamber. After a habituation period (5 min), the guillotine door was lifted, and, after the rat had entered the dark chamber, the door was lowered and an inescapable scrambled single electric shock (1 mA, 1 s) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded, and all rats had ILs greater than 60 s and were included in the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between placement in the illuminated chamber and entry into the dark chamber was measured as step-through latency (STL, up to a maximum of 300 s). This test was conducted on 17–20 days after surgery.

2.5. 8-Armed Radial Maze Task

Spatial learning and memory were tested using a radial maze according to the paradigm described previously (Baluchnejadmojarad & Roghani, 2009). The apparatus consisted of a 50-cm-elevated (above the floor) eight-armed radial maze (RAM) made of black Plexiglas. The maze was placed in a sound-attenuated and dimly lit room. The 60-cm-long, 10-cm-wide, and 15-cm-high arms extended radially from a central octagonal starting platform (35 cm in diameter), and there was a recessed food cup at the end of each arm. In some of the arms, the cup contained a single small food pellet as a reinforcer. A plastic cylinder (30 cm in diameter, 20 cm high) was placed on the central platform, and a rat was placed inside this cylinder 15 s before the test. Following this interval, the rats were allowed to move freely and timing began. The RAM was surrounded by various extra-maze cues; their orientation relative to the maze was kept constant throughout the experiment. The maze was cleaned with diluted ethanol between trials. Prior to acquisition (i.e., before surgery), the rats were maintained on a restricted feeding schedule designed to keep their body weight at about 85% of the free-feeding level. The rats learned to visit each arm, eat the pellet, and not re-enter the arm that had been visited during the same test. Each entry into each arm with all four paws was scored during a period of 10 min. Behavioral observation was discontinued after 10 min, even if the animal did not finish the task. The number of correct choices or errors was used to assess the performance of the animal in each session. An error was defined as a re-entry into an already visited arm. Rats that made at least seven correct choices in each of three consecutive sessions were used in the subsequent behavioral experiments. Training was performed at 24-h intervals, and rats that fulfilled the above-mentioned criteria within two weeks were included in the study (37 of 45 eligible rats). Retention trials were performed once on the 16th day post-surgery.

2.6. Statistical Analysis

All results were expressed as mean ± S.E.M. The non-parametric Kruskal–Wallis test was used to analyze the behavioral tests, and if a difference was found to be significant, pair-wise comparison was done using the Mann–Whitney U-test. In all calculations, a difference at p < 0.05 was regarded as significant.

3. Results

There was no significant difference between control, sham-operated and varenicline-treated sham-operated groups, we only reported the results for sham-operated group.

3.1. Spatial Recognition Memory in Y-Maze

Fig. 1 shows the results for the performance of rats in Y-maze task, in which short-term spatial recognition memory performance as alternation behavior can be examined. In this respect, the alternation score of the Aβ injected rats was found to be significantly lower (56.1 ± 4.95%) compared to the sham-operated group (82.8 ± 9.01%) at the end of the study (P = 0.03). Meanwhile, varenicline-treated Aβ injected rats at a dose of 2 µg/µl showed a higher alternation score (91.9 ± 6.19%) as compared to Aβ group (P < 0.001). To assess compound-
Figure 1. Alternation behavior displayed in the Y-maze by rats. Sham, A-beta (bilateral hippocampal injection of Aβ(25–35); 10µg aggregated Aβ; 5µg/µl), Varenicline pretreatment (vare1, single dose 0.5 µg/µl; vare2, single dose 2 µg/µl). Values are means ± SEM
*P< 0.05 (vs. A-beta).

Figure 2. Number of total entrance in the Y-maze. Sham, A-beta (bilateral hippocampal injection of Aβ(25–35); 10µg aggregated Aβ; 5µg/µl), Varenicline pretreatment (vare1, single dose 0.5 µg/µl; vare2, single dose 2 µg/µl). Values are means ± SEM
*P< 0.05 (vs. sham), # P < 0.001 (vs. A-beta).
ing effect of locomotor activity on memory processes in experimental groups, total number of arms entered was considered as an index of locomotor activity. In this regard, there was no statistically significant difference between the Aβ injected rats (19.4±1.96) compared to the sham-operated group (14.7 ± 1.1). But, there was considerable increase in total number of arms entered in both doses of varincline treated Aβ injected rats compared to untreated Aβ injected group (P<0.05) (Fig. 2).

3.2. Passive Avoidance Test

Fig. 3 shows the performance of rats in passive avoidance paradigm as indicated by IL and STL. Regarding initial latency, there was no significant difference among the groups. In addition, Aβ injected rats developed a significant impairment in retention and recall in passive avoidance test (P < 0.05), as it is evident by a lower STL.Varenicline treatment at doses of 0.5 and 2 µg/µl did produce an improvement in this respect (P < 0.05).

3.3. RAM Task

Aβ injected rats showed a significant deficit in spatial cognition in the radial eight-arm maze task, as indicated by a lower number of correct choices (P < 0.001) and a higher number of errors (P < 0.001) compared to relevant data for the sham-operated group. Administration of Varenicline at doses of 0.5 and 2 µg/µl caused a significant increase in the number of correct choices (P < 0.0001) and significantly lowered the number of errors (P < 0.0001) (Fig. 4).

4. Discussion

Alzheimer’s disease is one of the most prominent type of dementia that its major pathological feature is the production of senile plaques in the brain particularly in the cortex and hippocampus(Brookmeyer, Johnson, & Ziegler-Graham, 2007). The main component of senile plaques is amyloid β protein (Aβ), which derives from the proteolysis of amyloid precursor protein (APP) and consists of 39–43 amino acids (Selkoe,1998). Experiments in vivo or in vitro have shown that while the full-length of Aβ molecule is neurotoxic (Deshpande, Mina, Glabe, & Busciglio, 2006) but Aβ25–35, a short synthetic fragment of Aβ has the same neurotoxicity as that natural full-length of Aβ molecule produced, and thought to be the active center of whole molecule of Aβ (Zamani & Allen, 2001; Loo et al., 1993; Pike et al., 1995).

In present study, first we evaluated the effect of intrahippocampal injection of Aβ(25–35) on learning and memory deficit in rat. Second, the influence of varincline, partial agonist of α4β2- containing nAChRs, pretreatment on learning and memory disturbance in amyloid β(25–35) rat model of Alzheimer’s disease was examined. The main findings were as follows: (1) compared with the sham-operated group, within 2 weeks the Aβ(25–35) -injected rats had a lower alternation score in the Y-maze task, impaired retention and recall in the passive avoidance test, and fewer correct choices and more errors in the RAM task. (2) varenicline administration significantly improved short-term spatial recognition memory in the Y-maze task, retention and recall aspects of learning and memory in the passive avoidance test and performance in RAM task.

It is clear that neuronal nicotinic cholinergic transmission is involved in neuronal survival and neuroprotection as well as in synaptic plasticity. Deficiency of neuronal nAChRs is increasingly associated with a number of disease states including Alzheimer’s disease (AD), Parkinson’s disease (PD), Lewy body disease (LBD), schizophrenia, autism, and attention deficit/hyperactivity disorder (ADHD) (Bourin, Ripoll, & Dailly, 2003; Pimlott et al., 2004; Forgacs & Bodis-Wollner, 2004; Court, Martin-Ruiz,Graham, & Perry, 2000; Nordberg, 2001; Perry, Smith, Court, & Perry, 1990; Todd, Lobos, Sun, & Neuman, 2003). Significant reduction in nAChRs levels have been shown in some brain regions of AD patients such as cerebral cortex and hippocampus (Kellar, Whitehouse, Martino-Barrows, Marcus, & Price, 1987). For example α4 subunit has been detected to be 80% lower in AD than that of age-matched normal ones(Perry, Martin-Ruiz, Lee, & Griffiths, 2000).

On the basis of "the amyloid cascade hypothesis", infusion of Aβ into the cerebral ventricles resulted in neuronal dysfunction, neurodegeneration and impaired learning and memory (Nabeshima & Nitta, 1994; Nitta, Itoh, Hasegawa, & Nabeshima, 1994). In addition, Aβ infusion decreased choline acetyltranserase activity in the cerebral cortex and hippocampus (Yamada, Tanaka, Senzaki, Kameyama, & Nabeshima, 1998) and activated glial cells, seen as increased immunoreactivity for glial fibrillary acidic protein (Nitta, Fukuta, Hasegawa, & Nabeshima, 1997). Furthermore, following infusion of Aβ in the hippocampus and cerebral cortex, marked reduction in nicotine- and/or KCl-induced release of acetylcholine as well as reduced dopamine release was observed in the striatum(Itoh et al., 1996).
Figure 3. Initial latency (IL) and step-through latency (STL) recorded in a single-trial passive avoidance test for rats. Sham, A-beta (bilateral hippocampal injection of Aβ(25–35), 10 µg aggregated Aβ, 5 µg/µl), Varenicline pretreatment (varenicline1, single dose 0.5 µg/µl; varenicline2, single dose 2 µg/µl). Values are means ± SEM. *P < 0.05 (vs. sham), # P < 0.05 (vs. A-beta), § P < 0.05 (vs. A-beta).

Figure 4. The effect of varenicline on the spatial cognition deficit induced by A-beta injection in rats, measured 2 weeks after treatment. Values are means ± SEM of the number of correct choices or the number of errors. Varenicline1, single dose 0.5 µg/µl; Varenicline2, single dose 2 µg/µl. *P < 0.001 (vs. sham), # P < 0.0001 (vs. A-beta), § P < 0.0001 (vs. A-beta).
Recent studies have established a relation between harmful effects of Aβ and nAChR-mediated synaptic plasticity. It has been reported that soluble Aβ1–40 has affinity to various subtypes of nAChRs. So that in AD, Aβ binds with high affinity to α7 nAChRs in the cortical region and hippocampus (Wang, Lee, Davis, & Shank, 2000). The complex of α7 nAChRs - Aβ accelerates intraneuronal τ phosphorylation. Also, nanomolar concentrations of Aβ1–40 or Aβ1–42 cause functional antagonism of both human and rat homomeric α7 receptors (Pettit et al., 2001; Liu et al., 2001). Moreover, the heteromeric α4β2 receptor is also a target of Aβ. It was found that Aβ bind to αβ2 receptors but with lower affinity. Using low nanomolar or micromolar (Lamb et al., 2005) concentrations of Aβ, functional antagonism of α4β2 receptors was observed in both transfected human SH-EP1 cells and Xenopus oocytes, while similar concentrations of Aβ elicited receptor activation and agonist potentiation in other studies (Fu & Jhamandas 2003). Aβ can interact with additional nAChR subtypes include αβ4, αβ2, αβ5β2, and αβ2. Furthermore, nicotine acts as an intensifier of memory in a model of Aβ1–40 – induced impairment of hippocampal LTP (long-term potentiation) (Warburton, Rusted, & Fowler, 1992; Abdulla, Calaminici, Stephenson, & Sinden, 1993). An in vivo study showed that epibatidine, a specific agonist of αβ2 nAChRs, can suppress the LTP, while its specific antagonist, DHBE, prevent Aβ(31–35)-induced LTP suppression (Wu, He, Guo, & Qi, 2008). On the other hand, α7 nAChRs also play a major role in Aβ-induced attenuation of glutamate release and LTP (Chen, Yamada, Nabeshima, & Sokabe, 2006). Pre and post-synaptic nAChRs play important role in synaptic plasticity in different region of brain containing the hippocampus. For instance, in hippocampal CA1 interneurons, presynaptic nAChRs activation causes GABA release at synapses (Lena & Changeux, 1997). Since this response is blocked by DHβE, suggesting that it is mediated by αβ2 nAChRs. In contrast, pre-synaptic α7 nAChRs improve the glutamatergic transmission and synaptic plasticity in different brain regions (Gray et al., 1996). Because changes in synaptic effectiveness between neurons, such as short-term potentiation (STP), LTP, and LTD (long-term depression), are generally considered as cellular basis of learning and memory thus it seems that nAChR has a main role in modulating of learning and memory (McKay et al., 2007).

Varenicline is a cytosine derivative that has been used as a nicotine replacement therapy. As mentioned, It has been characterized as a potent partial agonist at αβ2 receptors, a less potent high efficacy agonist at αβ4 receptors, a partial agonist at α7β2 and α6-containing receptors, and a potent full agonist at α7 receptors.

Some studies shows that varenicline cause amelioration of ethanol-induced learning deficits. Although its effect mechanism has not yet been clarified but since varenicline is both an αβ2 nAChR partial agonist and α7 nAChR full agonist; thus, it seems that either receptor subtype that exist in large numbers in the hippocampus (Marks, Whiteaker, & Collins, 2006) and amygdala (Addy, Nakajama, & Levin, 2003; Han et al., 2003), could mediate the effects of varenicline on improvement of learning (Logue et al., 1997). As a partial agonist, varenicline has high affinity for αβ2 receptors but has lower efficacy at these receptors compared to other nAChR agonists such as nicotine and acetylcholine (Coe et al., 2005; Mihalak, Carroll, & Luetje, 2006; Rollema et al., 2007a, 2007b). Some studies have indicated that the αβ2 nAChR underlies the reinforcement of learning by nicotine (Davis et al., 2007). Animals without the β2 subunit of this receptor do not show intensification of learning by nicotine (Davis & Gould, 2007; Wehner et al., 2004). Thus, evidence supports the possibility that varenicline may be acting at αβ2nAChRs to ameliorate learning deficits. Varenicline also binds to α7 receptors and, as a full agonist, has a high level of efficacy at these receptors (Mihalak, Carroll, & Luetje, 2006); however, varenicline has a much lower affinity for α7 receptors compared to αβ2 receptors (Coe et al., 2005; Rollema et al., 2007b). Although α7 nAChRs may be implicated in the effects of varenicline on learning, but because α7 knockout mice show a decreased sensitivity to the memory impairing effects of ethanol (Wehner et al., 2004), it suggests that a drug acting primarily as an α7 agonist may fail to reverse learning deficits. This, combined with the lower affinity of varenicline for α7 receptors, suggests that the αβ22 rather than α7 receptors are mediating the effects of varenicline on AD-induced deficits in learning memory. Indeed, it is necessary further research for explaining the mechanism by which varenicline exerts its effects on improvement of learning and memory.

In conclusion, our results suggest that varenicline pre-treatment could prevent Aβ25–35-induced impairment of short-term spatial recognition memory in a Y-maze and learning and memory in the passive avoidance and RAM test.
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References


