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Effect of Amyloid β-Peptide on Passive Avoidance Learning in Rats: A Behavioral Study

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Background: Alzheimer's disease (AD) is the most common form of dementia that leads to neurotoxicity. Amyloid β-peptide (Aβ) has a pivotal role in the pathogenesis of AD.
Objectives: Given the contradictory results of Aβ (25-35) on the memory, in the present study we have examined the effect of Aβ - induced memory impairment.
Materials and Methods: Wistar male rats received an intrahippocampal (IHP) injection of Aβ (25-35).The learning function in the rats was examined by the passive avoidance task.
Results: The results showed that Aβ (25-35) significantly impaired both step-through latency and time in dark compartment in the passive avoidance task.
Conclusions: These data suggest that single bilateral microinjection of Aβ (25-35) could impair memory and can be used as an AD model in Wistar rats.

*Keywords:*Alzheimer's disease; Amyloid β-peptide; Learning; Rats

1. Background

Alzheimer's disease (AD) is the most common cause of dementia, and it is estimated to affect approximately 36 million people worldwide (1). AD is characterized by the occurrence of neurofibrillary tangles and senile plaques in the cortex, hippocampus, basal forebrain and amygdala (2). Neurofibrillary tangles are intracellular fibrillar aggregates of the tau microtubule-associated protein, which is hyperphosphorylated and oxidized. Senile plaques consist of insoluble fibrillar amyloid β-peptide (Aβ). The Aβ is formed after sequential cleavage of the amyloid precursor protein, and is then secreted to the extracellular space. It inhibits hippocampal long-term potentiation (LTP) and disrupts synaptic plasticity (3, 4). In addition, Aβ induces the elevation of reactive oxygen species (ROS) levels in neurons, leading to apoptotic neuronal death in the rat and mouse models (5, 6).

The hippocampus is an essential structure that is highly involved in cognition and psychological function. There is evidence, in rat models, that this structure is rapidly and extremely affected by an injection of the Aβ fragment (Aβ 25-35) in rat (7). There are several tests used to evaluate learning and memory functions in animal models. The passive avoidance learning (PAL) is believed to be based on contextual memory, which is associated with the place and the event of "being given the electric shock in the dark box". Because the hippocampus plays an important role in contextual memory, injuries of the hippocampal region decrease the performance of PAL (8).

2. Objectives

The accumulation of Aβ (25-35) leads to toxicity of the hippocampus and it is therefore involved in the navigation of the passive avoidance tasks. However, there are contradictory results concerning the role of Aβ (25-35) on the memory. The aim of this study was to evaluate the effect of Aβ (25-35) on memory in the passive avoidance task.

3. Materials and Methods

3.1. Materials

The Aβ (25- 35) (Sigma-Aldrich Corp., St. Louis, MO, USA) was solubilized in sterile water at a concentration of 1 μg/μL and stored at –20°C.

3.2. Animals

Twenty-one adult male Wistar rats (Pasteur Institute of

Iran, Teheran, IR Iran), weighing 250-300 g, were kept in a standard animal facility (21 ± 2 °C, relative humidity of 50 ± 5%, 12-hours light/dark cycle, food and water ad libitum). All animal experiments were carried out according to the Veterinary Ethics Committee of the Hamadan University of Medical Sciences, Hamadan, Iran. The animals were randomly divided into the following groups (seven individuals per group): intact control group, which remained undisrupted; the sham-operated group; and the Aβ (25-35) group received bilateral intrahippocampal (IHP) injections of Aβ (25-35) (9).

3.3. Intrahippocampal Injection of Aβ 25- 35

The rats were anesthetized intraperitoneally with ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic instrument (Stoelting, Wood Dale, IL, USA). The scalp was incised and drilled at an appropriate location to allow the insertion of a Hamilton microsyringe (Hamilton, Reno, NV, USA). Coordinates for the dentate gyrus were chosen based on Paxinos and Watson (10) atlas of rat brain (posterior -3.6 mm; lateral ± 2.3 mm; dorsal 3mm). The Aβ solution (5 μL) was bilaterally injected into the region at a rate of 1 μL/2 min. Sham operated rats received vehicle solution.

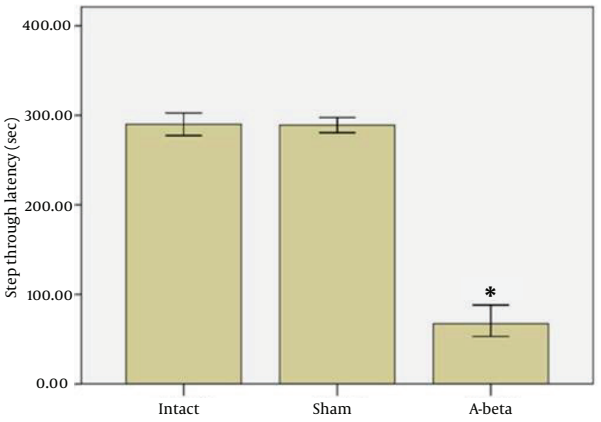
3.4. Inhibitory Avoidance Apparatus (Shuttle box)

The passive avoidance test was started 2 weeks after the Aβ injection using a step-through inhibitory avoidance apparatus. It consisted of two boxes of the same size (20 × 20 × 30 cm). There was a guillotine door in the middle of a dividing wall. The walls and floor of one compartment consisted of white opaque resin and the other one was dark. Intermittent electric shocks (50 Hz, 3 seconds, 1.5 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator. Each animal was gently placed in the white compartment and after 5 seconds the guillotine door was opened and the animal was allowed to enter the dark module (11). Once the animal entered with all four paws to the next chamber, the guillotine door was closed and the rat was immediately withdrawn from the compartment. This trial was repeated after 2 minutes. As in the acquisition trial, when the animal entered the dark (shock) compartment the door was closed, and a foot shock (50 Hz, 1.5 mA, 3 seconds) was immediately delivered to the grid floor of the dark room. After 20 seconds, the rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials; if the rat did not enter the dark compartment during 300 seconds, a successful acquisition of inhibitory avoidance response was recorded. Otherwise, when the rat entered the dark compartment (before 300 seconds) a second time, the door was closed and the animal received the shock again. Twenty-four hours later, each rat was again placed in the light chamber (retention trial) and after 5 seconds the door was opened and the latency with which the animal entered the dark chamber (STL) and the total time spent in dark compartment (TDC) was recorded in the absence of electric foot shocks, as an indicator of inhibitory avoidance behavior.

3.5. Statistical Analysis

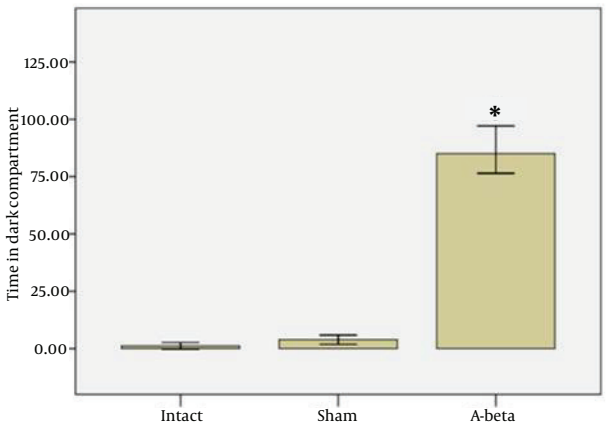
The data were expressed as Mean ± SEM and analyzed with the SPSS version 16.0 software (SPSS Inc., Chicago, ILL, USA). The statistical analyses were performed using one way analysis of variance (ANOVA) and the post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate. A $P < 0.05$ was considered statistically significant.

Figure 1. The Mean of the Step-Through Latency in the Passive Avoidance Task



The values were presented as mean ± SEM (* P<0.01 *vs.* intact and sham groups).

Figure 2. The Mean of the Time Spent in Dark Compartment in the Passive Avoidance Task



Vertical bars show SEM (*P<0.001 *vs.* intact and sham groups).

4. Results

4.1. Effect of Aβ (25-35) on Step Trough Latency in Passive Avoidance Task

In the acquisition trial, we found no difference between the intact, sham and Aβ-treated groups in the STL (data not shown). However, the injection of Aβ (25-35) reduced the STL in the retention trial compared to intact and sham-operated groups ($P < 0.01$, Figure 1).

4.2. Effect of Aβ (25-35) on Time in Dark Compartment in Passive Avoidance Task

As shown in Figure 2, Aβ-treated rats spent more TDC-compared to the other groups ($P < 0.001$, Figure 2). There was no significant difference between the groups in the acquisition trial.

5. Discussion

Alzheimer's disease is the most common form of dementia that gradually worsens over time and affects memory and behavior. Deposition of Aβ in the brain has a key role in the pathological features of AD, which slowly destroys neurons and impairs learning and memory (4, 12). The results of this study showed that IHP injection of Aβ (25-35) caused learning disturbances in the passive avoidance task within 2 weeks. Consistent with our results, Yamaguchi and Kawashima demonstrated that intracerebroventricular (ICV) injections of Aβ (25-35) in the rat induced impairment in the passive avoidance and radial-arm maze tasks (7). In addition, Maurice et al. reported that ICV injection of Aβ (25-35) can disrupt the learning in the Y-maze, passive avoidance and water maze tasks (13). Similarly, it has been reported that bilateral injection of Aβ (25-35) in rat nucleus basalis induced learning deficits in passive avoidance tasks (14). The deposition of β-amyloid protein in the brain is related to the impairment of learning and cholinergic neuronal degeneration (7). The key brain region involved in navigation in the passive avoidance task includes the hippocampus (15, 16). Several lines of evidence suggest that the Aβ inserts into the neuronal membrane bilayer and generates oxygen-dependent free radicals, which causes the lipid peroxidation and protein oxidation (17, 18). Oxidative stress disrupts the blood brain barrier that leads to the passage of toxic substances in the brain, ultimately resulting in the progression of various neurodegenerative diseases. In conclusion, our results showed that the single IHP administration of Aβ (25-35) induced deficits in passive avoidance learning and can be used as an animal model for AD.

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Author's Contributions

Ali Nikkhah, Fatemeh Ghahremanitamadon and Somayeh Zargooshnia performed all of the experiments. Siamak Shahidi undertook the statistical analysis. Sara Soleimani Asl managed the literature searches and wrote the first draft of the manuscript. All authors contributed to and have approved the final version of the manuscript.

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