

Soluble Amyloid Precursor Protein Promote Activity-related Cytoskeletal-associated Protein synthesis in FAD mice of Alzheimer's Disease

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Abstract

Alzheimer's Disease is a common disease among elder people. However, there are few techniques that can treat AD directly and effectively. sAPP α protein and Arc protein are essential molecules for human normal brain activity. They contribute to the reduction of risk of AD and long term potentiation respectively. Recent studies also have proved that sAPP α can stimulate the synthesis of Arc protein. For these reason, the paper discusses the possible connection between those molecules in the brain of AD condition. Then, it provides designed hypothetical experiment for this research. Finally, the paper discusses the possible result, which turns to prove that sAPP α promote the synthesis of Arc protein in RT-PCR and immunoflorescence and may be seen as a potential for treating AD.

Keywords

Alzheimer's Disease; sAPP α ; Arc protein; Morris Water Maze.

1. Introduction

Alzheimer's Disease (AD) is a common degenerative disease in the nervous system, which is characterized by memory loss and decrease in cognitive abilities. Currently, there is no drug or any intervention used to treat AD successfully. A β is produced by the amyloidogenic processing of APP and the possibility of developing AD is increased. Soluble amyloid precursor protein-alpha (sAPP α) is a protein derived from amyloid precursor protein (APP) by α -secretase[1]. sAPP α decrease the level of A β and plaques by inhibiting BACE1 mediated β -secretase activity[2]. Therefore, sAPP α contributes to the reduction of risk of developing AD. Activity-related cytoskeletal-associated protein (Arc/Arg3.1), is an immediate early gene, has the ability of adjusting long-term potentiation (LTP), by moderating α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor localization [3] to interfere with memory. Arc mRNA is induced, transported into dendrites to carry out Arc protein synthesis in polysynaptic density/ NR2 complex[4]. In recent studies, it was discovered that glutamate receptors are involved in the mechanisms of the protective role of sAPP and the enhancement of Arc protein synthesis[3,5]. Moreover, multiple of lines of evidence demonstrates that sAPP α stimulates the synthesis of Arc in hippocampal neurons[3]. If there is a relationship between sAPP α and Arc protein, this may contribute to the treatment of AD and prevention of AD. Based on the importance of functioning of sAPP α in the production of Arc protein, we design the experiment for discovering the connection between sAPP α and Arc Protein. we hypothesize that under the condition of Alzheimer's Disease, sAPP α will still be functioning and enhance the synthesis of Arc expression. In the work, we insert sAPP α promoter into particular groups of mice, use Morris Water Maze to analyze and test the behavior of different groups of mice, and measure the level of Arc mRNA and Arc protein by RT-PCR in order to determine the effect of sAPP α on Arc protein synthesis.

2. Hypothetical experiment

2.1 Preperation

2.1.1 Drug

Tamoxifen can promote the functional recovery of chronic hypoperfusion white matter injury and the proliferation and differentiation of oligodendrocyte precursor cells. In this experiment, tamoxifen acts as a hippocampus promoter to stimulate the production of sAPP α .

2.1.2 Animals

FAD+ tamoxifen group: 10 FAD mice (age 6 weeks) were purchased. sAPP α gene of Human APP gene was inserted into the mice chromosomes in the brain. The tamoxifen drug was given to this group of mice, this drug acted as a hippocampus promoter of the synthesis of sAPP α , which increased the level of sAPP α in the hippocampus.

FAD group: 10 FAD mice (age 6 weeks) were purchased. The sAPP α gene was inserted. Without given the promoter, low level of sAPP α protein would appear.

WT+tamoxifen: 10 normal mice (age 6 weeks) were purchased. The sAPP α gene as part of Human APP gene was inserted into the chromosome. The tamoxifen drug was given to the positive control group. Therefore this group of mice contain a considerable amount of sAPP α .

WT group: 10 normal mice (age 6 weeks) were purchased. sAPP α gene was inserted as other group. Without given the promoter, low level of sAPP α protein would appear in this group.

During the experiment, the food and water were all available unless under some particular condition. Four groups of mice were from the same species and were kept in same living condition that has a regular 12h light/dark cycle and under a stable

temperature of 26°C. Two cages were provided for each group (5 mice per cage) and mice stayed in this condition for 7 days.

2.2 Behavior Analyzing using Morris Water Maze

A circular white tank (height:30cm; diameter:20cm) were prepared and filled with water of 26 °C, adding milk to make it opaque. Four different objects were pasted on

the curtain as external geographical clues to represent four different directions. During five days of acquired training, a transparent platform was placed 1cm under the water[6].

Training each experimental mouse each day at 10:00 am by placing them into the water from different direction and let them seek the platform for 60 seconds. If they fail to discover the platform in 60 seconds, guide them onto the platform and let them stay for 30 seconds. Dry them and Place them under warm condition of 30 °C. Removing the platform on the 6th day and releasing those groups of mice into the water from NE (the same direction) and record the swimming trails for 60 seconds. Counting the number of times mice have passed the platform and the time they spend in SW quadrant.

2.3 RT-PCR

5 Mice from each group were anesthetized. Their brain were removed and split into debris, extracted by first lysing cells, promoter and inhibitor of Arc mRNA. DNase and Proteinase K was used to denature DNA and protein. Arc mRNA was maintained by chaotropic agents by denaturing RNase. Using reverse transcription enzymes, purified RNA templates to form cDNA. The reverse chain reaction was first conducted by initial denaturation (94°C for 2 minutes). Then the amplification of the cDNA was performed by 50 cycles of denaturation (94°C for 30 seconds), annealing (65°C for 1 minutes) and extension (72°C for 1 minutes). The final extension lasted 6 minutes for 72°C.

2.4 Immunofluorescence

5 Mice from each group were anesthetized, the brain were removed and postfixed with 4% paraformaldehyde at 4 °C for 24 h and then dehydrated in sucrose at 4 °C for 48 h. Cutting the sample into slices, incubating them with the Arc antibody(C-7)and corresponding secondary antibodies.

Staining the nuclei of antibody for 10 minutes. The fluorescent microscope was used to detect Arc protein in synapses of neurons in hippocampus when Arc antibody bound to the Arc protein. Counting the number of Arc protein per square centimeter in each sample of hippocampus in each group.

3. Results

It was hypothesized that the behavior of these groups of mice will be presented as follow: the second group, mice showed declined memory and learning ability of mice and the percentage of swimming time in the SW quadrant is limited; the first group, which had FAD mice and mice were given tamoxifen drug did better than the second group; the fourth group, which were the normal mice, showed no cognitive impairment; the third group, mice presented the similar or a little better results as the fourth group. The Morris Water Maze justifies that FAD mice had developed AD and were capable to provide AD condition for the following experiment.

After measuring the Arc mRNA and Arc protein in hippocampus of each group of mice, the possible results were listed as follow: the level of Arc mRNA and Arc protein in the WT+tamoxifen group and WT group were higher the level of Arc protein in the FAD group is much lower. If the level of Arc mRNA and Arc protein of the first group is significantly higher than the result of the FAD group, the hypothesis we stated that in AD condition, the sAPP α still stimulates both transcription and translation, therefore, our hypothesis that sAPP α promote the synthesis of Arc protein is confirmed; however, if the result is similar to the FAD group, it demonstrates that synthesis of Arc protein is inhibited, our hypothesis is disproved.

4. Conclusion

Based on previous research of the relationship between sAPP α and Arc protein, we held a hypothesis that Alzheimer's Disease animal model, restoring the sAPP α was able to promote the synthesis of Arc protein. By conducting the experiment of Morris Water Maze, we analyzed the behavior of these groups of mice ensured that the AD condition in the brain had been created. In addition, the RT-PCR technique was used to determine the effect of Arc mRNA expression and measure the level of Arc mRNA. Furthermore, the immunofluorescent experiment allowed us to measure the level of Arc protein in hippocampus, therefore the regulating ability of sAPP α to Arc protein was determined. In the future, the experiment may worth continuing research in whether the increase in production of Arc will decrease the pathology of Alzheimer's Disease and whether the act of receptors in hippocampus will be altered during the Arc protein synthesis in AD. Those questions lead to a further research between the sAPP α and Arc protein in AD, and may contribute to drug development of AD, as well as promote a deeper study in this field.

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