Studies of the protective properties of the NMDA - receptor antagonist memantine on the viability of neurons in rat hippocampal culture when modeling excitotoxicity and Alzheimer's Disease

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects memory, thinking, and behavior (Fig. 1). There are currently no drugs that could reduce the pathological effects of this disease, but there may be symptomatic relief that can alleviate the disease. NMDA receptors play a crucial role in both synaptic plasticity and transmission.

Excessive stimulation of glutamate receptors, mainly NMDA - type, causes intense entry of calcium ions into cells, being the early key step in glutamate-induced excitotoxicity, resulting in many neurological diseases, including AD. In particular, memantine, NMDA -receptor antagonists block the receptor and reduce calcium influx into the neuron, thus blocking the activation of the toxic intracellular events. The purpose of this study was to find out whether memantine, the noncompetitive NMDA - receptor antagonist, can protect hippocampal culture neurons from NMDA - and amyloid Aβ1–42 - induced neurotoxicity.

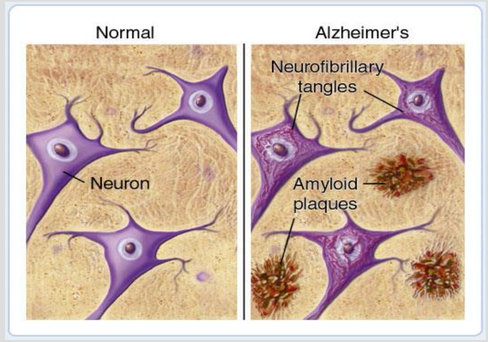

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Fig.1. Schematic representation of hippocampal tissue in normal (left) and in Alzheimer's disease (right)

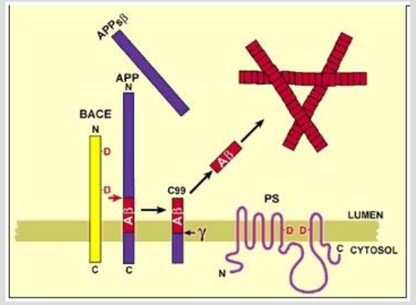

Fig. 2. Amyloid Precursor Protein (APP) is first cut by the protease BACE1 to create APPs b and membrane bound C99. g-secretase makes a second cut within the transmembrane region of C99 releasing Ab.

Methods

The studies were performed carried out by 24-h-long culturing of hippocampal cells in the presence of 2 μM amyloid β1–42 (βA) (Fig. 2). We used combined staining of cells by an indicator of viable and apoptotic cells, Hoechst 33258, and an indicator of dead cells, propidium iodide. The viability of neurons was determined by counting them using confocal laser scanning microscopy, comparing cell fluorescence under control conditions, and after 24 hours of incubation with reagents (NMDA, memantine, 50 μM, Sigma-Aldrich, USA). Cells were counted from 4 independent experiments. In each experiment, >200 cells were examined in 5 random fields for each condition. Differences among means were assessed by one-way ANOVA followed by Tukey's post hoc test. The value of P<0.05 was considered statistically significant.

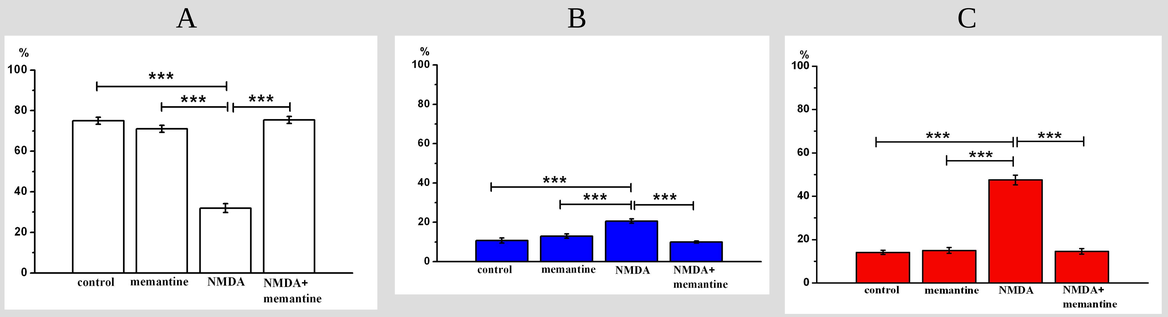

Fig. 3. Diagrams of the mean values of the relative numbers of cytologically intact cells (A), cells with signs of apoptosis (B), and necrosis (C) in the culture of rat hippocampal neurons after incubation with NMDA (10 μM) and memantine (50 μM). The statistical significance of differences was estimated using a Student's t-test. *** P<0,001, n= 5.

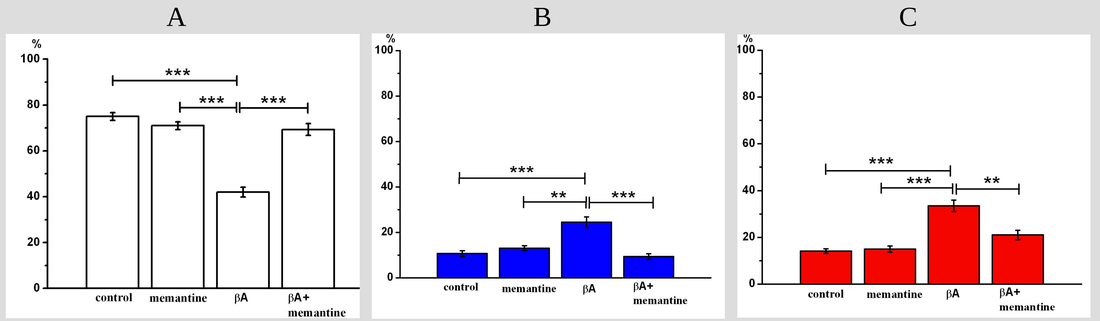

Fig. 4. Diagrams of the mean values of the relative numbers of cytologically intact cells (A), cells with signs of apoptosis (B), and necrosis (C) in neurons after incubation with Aβ1–42 (2 μM) and memantine (50 μM) . ** P< 0,01, *** P< 0,001, n= 5.

Results and conclusions

We found that 24-hour incubation of rat hippocampal culture neurons with NMDA or amyloid Aβ1–42 caused more than a twofold increase in the number of cells with signs of apoptosis and/or necrosis compared to the control (Fig.3, 4).

Obtained data indicated a decrease in cell viability under excitotoxicity conditions induced by NMDA or amyloid Aβ1-42 administration during AD modeling. With that, the joint application of memantine and NMDA or joint application of memantine and amyloid Aβ1–42 increased the number of living cells and decreased the number of apoptotic and necrotic neurons the hippocampal cultures (Fig.3, 4). Thus, the memantine application increased the percentage of viable cells, reducing the number of cells that died from apoptosis and/or necrosis.

We concluded that memantine could act as a neuroprotective agent against neuronal degeneration mediated by overactivation of NMDA - receptors and amyloid Aβ1–42. Despite limiting our experiments to cell culture only, our results show the opportunity of memantine use to reduce the neuronal loss caused by NMDA - and amyloid Aβ1–42 - induced neurotoxicity seen in patients with AD.