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Memantine protects cholinergic and glutamatergic septal neurons from $A\beta_{1-40}$-induced toxicity

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Abstract

The medial septal region (medial septum and diagonal band of Broca, MS/DB) controls hippocampal excitability and synaptic plasticity. MS/DB cholinergic neurons degenerate early in Alzheimer's disease (AD). The presence of MS/DB glutamatergic neurons that project to the hippocampus and are vulnerable to Aβ suggests that excitotoxicity plays a role in AD septal degeneration and hippocampal dysfunction. To demonstrate the presence of excitotoxicity in Aβ-induced septal damage, we compared rats injected with $A\beta_{1-40}$ into the MS/DB with animals treated with memantine prior, during and after $A\beta_{1-40}$ injections. Controls were injected with phosphate buffered saline (PBS). MS/DB cholinergic, glutamatergic and GABAergic neurons were immunochemically identified. The number of MS/DB neurons was estimated using stereology. Our results show that memantine blocks $A\beta_{1-40}$-induced septal damage and suggest that excitotoxicity plays a role in basal forebrain neurodegeneration.

Keywords

Septum; excitotoxicity; Alzheimer's disease; acetylcholine; glutamate; GABA

Introduction

Alzheimer's disease (AD) is a progressive and devastating neurological disorder that leads to dementia and subsequent death. The basal forebrain, including the septum, is affected by AD with a severe reduction of cholinergic neurons [2, 8, 23]. The MS/DB region of the septum projects to the hippocampus. By controlling the excitability and synaptic plasticity of hippocampus, the MS/DB plays an important role in learning and memory [4]. The MS/DB

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Disclosure statement
The authors report no conflicts of interest.

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was previously thought to exclusively contain cholinergic and GABAergic neurons. However, our laboratory has characterized a third population of MS/DB neurons that uses glutamate as neurotransmitter and projects to the hippocampus [3]. Thus, glutamatergic neurons are well posed to play an important role in septo-hippocampal functions and their damage may contribute to AD brain dysfunction.

Medial septal cholinergic and glutamatergic neurons are vulnerable to both medial septal and hippocampal injections of amyloid β peptides (Aβ) [5, 6]. Thus, excitotoxicity triggered by Aβ-induced septal glutamatergic neuronal damage may contribute to both cholinergic and glutamatergic neuronal degeneration. Memantine an uncompetitive N-methyl- D-aspartate (NMDA) receptor antagonist improves both cognitive and behavioral symptoms of AD [7, 10, 14, 16, 21]. Our work is dedicated to determine whether excitotoxicity contributes to the Aβ-induced septal lesions. While $A\beta_{1–42}$ is the most fibrillogenic [26] Aβ form and a major component of the neuritic plaques, $A\beta_{1–40}$ is the most common Aβ variety in the brain [20]. Since plaques are not present in the basal forebrain until advanced AD stages, and degeneration of basal forebrain cholinergic neurons occurs earlier in this disorder, we decided to use $A\beta_{1–40}$ for this study. Our results show that memantine effectively protects against the damage produced by intraseptal administration of $A\beta_{1–40}$.

Materials and Methods

Twelve male Sprague Dawley Rats weighing 300–400g were divided into three groups. Two experimental groups were injected with 4μl $A\beta_{1–40}$ (Bachem) dissolved in distilled water [11, 22] concentration of 2μg/μl into the medial septal [5, 12] (coordinates: AP=−0.5, L=2, V= 6.5). One of the experimental groups was treated with a bolus of memantine hydrochloride (Sigma, St. Louis, MO) 20mg/kg in a volume of 1ml of saline solution subcutaneously injected 12 hours prior intraseptal injections of Aβ and the implantation of osmotic pumps (Alzet, 2ML2). Memantine was continuously delivered subcutaneously through the osmotic pumps for eight days (20mg/Kg/day) [8]. Control animals were injected with equal amounts of a PBS solution (4μl). One week after injection of $A\beta_{1–40}$ or PBS, animals were perfused intracardially with a fixative solution. Brains were removed, cryoprotected and 50μm slices prepared. Cholinergic neurons were labelled with goat anti-ChAT antibody (1:200, Chemicon), glutamatergic neurons with a mouse anti-glutamate antibody (1:2000, Immunostar) and GABAergic neurons with a mouse anti-GAD67 antibody (1:500, Chemicon). Slices were then incubated with their respective biotinylated secondary antibodies (1:200, Vector). Finally, slices were incubated with avidin-biotin complex (ABC) and neurons visualized using the chromogen 3-3′ diaminobenzidine. Two sections of each brain were incubated without the primary antibody to determine staining specificity. No immunostaining was observed in these cases.

Thioflavine S method was used to confirm the Aβ injection site and detection of fibrillary forms (Figure 1). A sample of the $A\beta_{1–40}$ was analyzed with Atomic Force Microscope (AFM) (Digital Instruments, Veeco, Santa Barbara, CA) to determine the $A\beta_{1–40}$ conformation. Aβ peptide adopted mostly a fibrillar form. The width of single fibrils was about 5nm with variable length. Some oligomers coexist with the fibrillar form (Figure 1). Neuronal numbers were estimated using stereological approaches (StereoInvestigator software, MicroBrightField, Williston, VT, USA) [24]. All numerical data were expressed as t value (t), means and standard error of the mean (SEM). Student-t-tests were used to assess statistically significant differences among neuronal populations. Differences were considered significant at $p<0.05$.

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Results

The number of MS/DB ChAT immunoreactive neurons was reduced from 10021± 664 in PBS injected animals to 6469±122 in $A\beta_{1-40}$ injected rats (t=6.134, df3),(p=0.009). The number of ChAT immunoreactive neurons in memantine/Aβ treated animals was similar to the one found for the control animals 9209±339 (p=0.25) (and significantly different from $A\beta_{1-40}$ treated animals 6469±122 (t=−11.879, df3), (p=0.001) (Figure 1 and 2). Thus, memantine treatment was able to protect against $A\beta_{1-40}$-induced toxicity (Figures 1 and 2). Similarly, $A\beta_{1-40}$ reduced the number of glutamate immunoreactive neurons from 19421±1216 to 14118±579 (t=4.671, df3),(p=.019). Here also, memantine treatment was able to protect against $A\beta_{1-40}$-induced toxicity and the number of glutamate immunoreactive neurons in memantine/$A\beta_{1-40}$ treated (Figure 1 and 2) animals was (20052±1118) and significantly different from $A\beta_{1-40}$ treated rats/no treatment 14118±579 (t=−3.522, df3),(p=.039). The number of glutamate immunoreactive neurons in memantine/Aβ treated animals was similar to the one found for the control animals 19421±1216 (p=0.75) (Figures 1 and 2). In contrast, $A\beta_{1-40}$ did not significantly reduce the number of GABAergic neurons and memantine treatment did not modify their numbers (Figures 1 and 2).

Discussion

The toxicity caused by excessive neuronal stimulation (excitotoxicity), with subsequent calcium entry into the neuron, may occur in any brain region containing NMDA receptors (or other calcium permeable glutamate receptors) [1] and axon terminals with capacity to release glutamate. Aβ administration disrupts neuron-glia signaling and glial glutamate uptake, increasing glutamate concentrations in the extracellular space that surround neurons. Via this mechanism, Aβ induces noxious glutamatergic stimulation of neurons. In fact, excessive activation of NMDA receptors has been postulated to play a critical role in AD neurodegeneration [9, 13]. Furthermore, Aβ increases the firing rates of MS/DB glutamatergic neurons by blocking specific K^+ conductances [15]. This network activity intensification may also activate excitotoxic mechanisms.

Memantine has been shown to protect neurons against Aβ-induced toxicity in several brain regions [10, 14, 17, 18]. This protective effect is thought to occur by selective blockade of the excitotoxicity associated with abnormal glutamatergic transmission, while allowing for the physiological transmission associated with normal neuronal functioning [19]. The presence of a major population of glutamatergic neurons in the MS/DB that participates in local circuits [3, 4, 25], suggests that excitotoxic mechanisms participate in the Aβ-induced damage of this basal forebrain region. Nevertheless, up to the present study, memantine effects on limited Aβ-induced septal lesions have not been investigated. Our work demonstrates that memantine protects against $A\beta_{1-40}$- induced MS/DB neuronal damage [5, 6] and that local excitotoxic mechanisms may significantly contribute to AD basal neurodegeneration. Neuronal network activity and intracellular calcium changes need to be investigated to determine the molecular mechanisms underlying the memantine protective effect.

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Highlights

- Neurons of the medial septum are affected by β Amyloid 1–40.
- Excitotoxicity contributes to septal degeneration in neurons.
- Memantine protects against β Amyloid 1–40.

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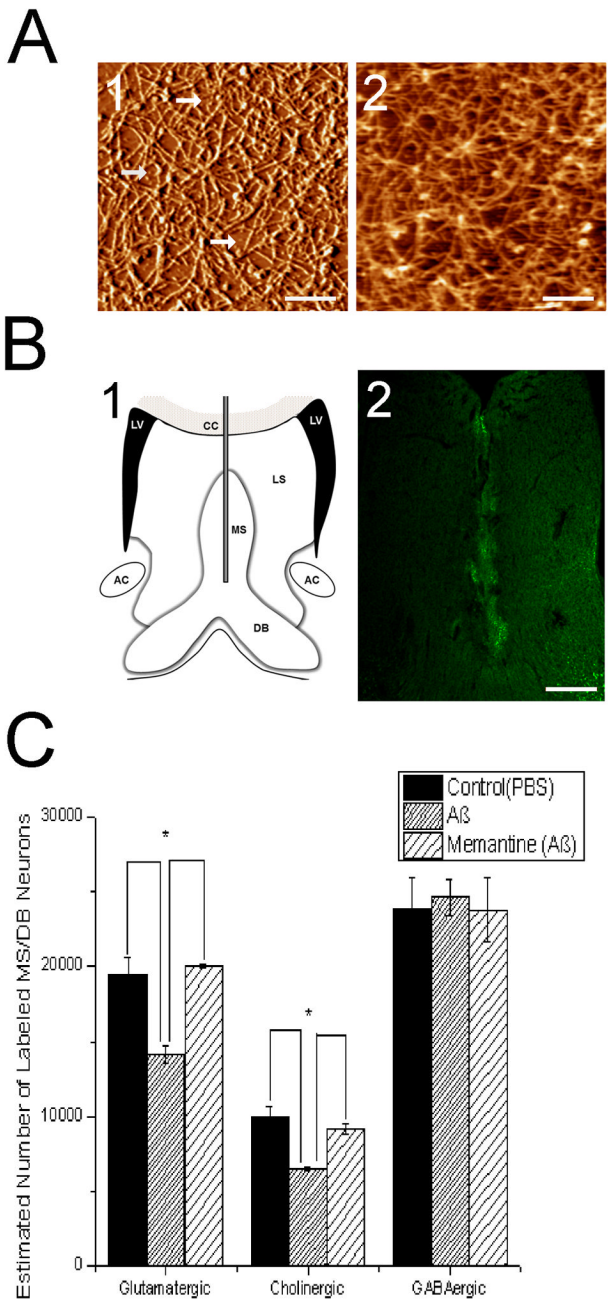

Figure 1.

(A-1) A deflection AFM image of $A\beta_{1–40}$ showing predominantly fibrillar form with some oligomers (white arrows). (A-2) A height AFM image of the $A\beta_{1–40}$ fibrils. The fibrillar form covers about 97% of the total surface (Scale bar: 550 nm). (B-1) Diagram depicting medial septum injection site. (B-2) Fluorescent image of Thioflavine S in medial septum verifying presence of Aβ 1–40 (Scale bar=100μm). (C)Graph comparing the estimated number of glutamatergic, cholinergic, and GABAergic labeled neurons in the MS/DB from control, $A\beta_{1–40}$ and Memantine + $A\beta_{1–40}$, rats. The graph shows $A\beta_{1–40}$ significantly reduced the medial septal glutamatergic and cholinergic neurons ($P<0.05$.) compared to PBS and memantine treated rats. In contrast, GABAergic neurons did not show significant alterations.

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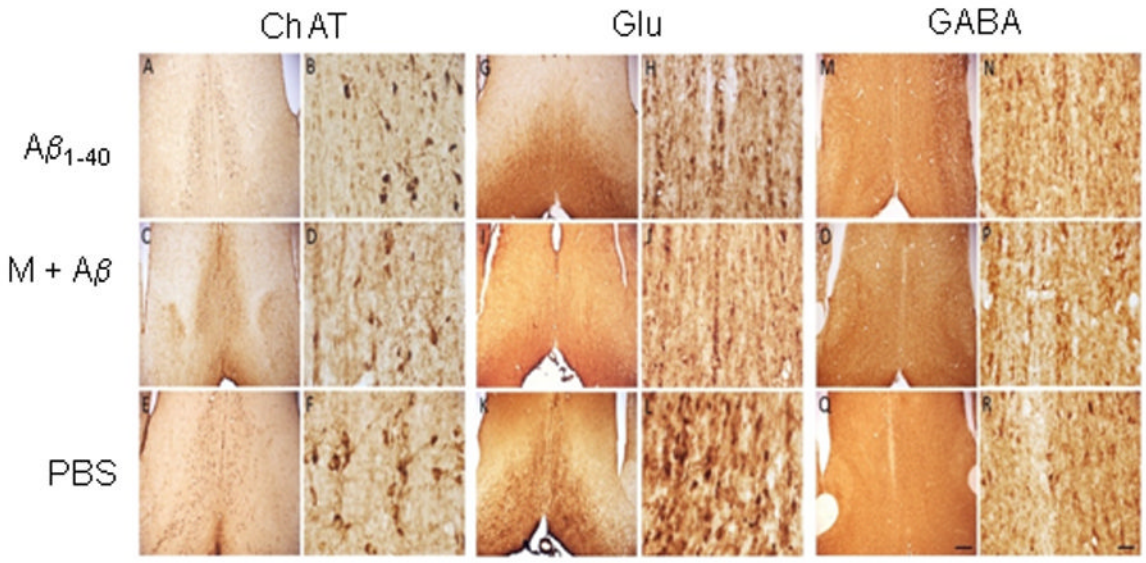

Figure 2.

Bright field photomicrographs of ChAT (A–F) Glutamate (G–L) and GAD67 (M–R) immunoreactive neurons in medial septum. Left columns (5X), right columns (40x) in $A\beta_{1–40}$+memantine group, $A\beta_{1–40}$, and PBS treated groups. In (D, J) notice a reduction in ChAT and Glutamate immunoreactive neurons in the $A\beta_{1–40}$ group. (P)GAD67 immunoreactivity shows no evident changes in $A\beta_{1–40}$injected group or memantine treated group. (Scale bar=50 μm in R).