Amyloid β-protein differentially affects NMDA receptor- and GABA$_A$ receptor-mediated currents in rat hippocampal CA1 neurons

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Abstract

Although the aggregated amyloid β-protein (Aβ) in senile plaques is one of the key neuropathological features of Alzheimer's disease (AD), soluble forms of Aβ also interfere with synaptic plasticity at the early stage of AD. The suppressive action of acute application of Aβ on hippocampal long-term potentiation (LTP) has been reported widely, whereas the mechanism underlying the effects of Aβ is still mostly unknown. The present study, using the whole-cell patch clamp technique, investigated the effects of Aβ fragments (Aβ$_{25-35}$ and Aβ$_{31-35}$) on the LTP induction-related postsynaptic ligand-gated channel currents in isolated hippocampal CA1 neurons. The results showed a rapid but opposite action of both peptides on excitatory and inhibitory receptor currents. Glutamate application-induced currents were suppressed by Aβ$_{25-35}$ in a dose-dependent manner, and further N-methyl-D-aspartate (NMDA) receptor-mediated currents were selectively inhibited. In contrast, pretreatment with Aβ fragments potentiated γ-aminobutyric acid (GABA)-induced whole-cell currents. As a control, Aβ$_{35-31}$, the reversed sequence of Aβ$_{31-35}$, showed no effect on the currents induced by glutamate, NMDA or GABA. These results may partly explain the impaired effects of Aβ on hippocampal LTP, and suggest that the functional down-regulation of NMDA receptors and up-regulation of GABA$_A$ receptors may play an important role in remodeling the hippocampal synaptic plasticity in early AD.
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1. Introduction

The earliest clinical symptoms of Alzheimer's disease (AD) include loss of short-term memory and subtle cognitive impairments that, over several years, progress to severe global dementia. It seems likely that the impaired synaptic plasticity and associated memory dysfunction experienced during the early stages of AD can be caused by amyloid β (Aβ) peptide-derived diffusible ligands acting upon particular neural targets. Due to the fact that the first clinical manifestations of AD are memory loss and cognitive impairment, alteration of synaptic function in the brain is likely to be involved [1] long before a significant cell loss is manifested.

Hippocampal long-term potentiation (LTP) is a neuronal model of activity-dependent synaptic plasticity, in which postsynaptic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors have been thought to be some key elements in LTP induction and maintenance [2,3]. The soluble full-length Aβ peptide 1–42 (Aβ$_{1-42}$) and some of its fragments, such as Aβ fragments 25–35 or 31–35 (Aβ$_{25-35}$ or Aβ$_{31-35}$) have been shown to inhibit LTP in the hippocampus [4–7]. There is considerable evidence to suggest that Aβ and its

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peptide fragments influence cellular homeostasis and neuronal signaling through modulation of ion channel function [8]. However, the mechanism of Aβ-induced LTP impairment is still mostly uncertain and even controversial. For example, Chen et al. [4] found that the suppression of LTP by Aβ was involved in the NMDA receptors, in which $A\beta_{1-42}$ reduced the amplitude of NMDA receptor-mediated synaptic currents in dentate granule cells. In contrast, Wu et al. reported that application of $A\beta_{1-40}$ by extracellular perfusion (200 nM) or intracellularly via the recording pipette (100 nM) resulted in a gradual enhancement of the NMDA receptor-mediated synaptic currents in dentate gyrus cells [9]. Meanwhile, Raymond et al. [6] concluded that NMDA receptor regulation by Aβ did not account for its inhibition of LTP in the rat hippocampus. In addition, it is still unclear whether Aβ-induced LTP impairment is involved in the regulation of GABA receptors, though Aβ may increase neuronal excitability by inhibiting GABA-induced Cl^- current in the neurons of the mammalian central nervous system [10].

Another important issue is the active center of Aβ. The neurotoxicity of $A\beta_{25-35}$ has been widely documented [5,11,12], and the sequence 25–35 has been accepted as an active center of Aβ accordingly, though it is still short of enough proof regarding its modulating effects on glutamate and the GABA receptor/channel. Interestingly, a much shorter Aβ fragment, $A\beta_{31-35}$, could also induce neuronal apoptosis [13] and suppress both large conductance of Ca^{2+}-activated potassium (BK) channels [14] and delay rectifying the potassium channel (I_k) [15]. After the substitution of isoleucine-31, glycine-33, or methionine-35 in Aβ with other amino acids, the full-length Aβ associated oxidative stress and neurotoxicity were totally abolished [16]. Similar to $A\beta_{25-35}$, $A\beta_{31-35}$ also suppressed the induction of LTP *in vitro* [7] and *in vivo* [17], according to our previous reports. Whether $A\beta_{31-35}$ directly affects the postsynaptic NMDA/GABA receptors is still an open question.

From all experimental evidence mentioned above, we are very interested in the exact effects of both $A\beta_{25-35}$ and $A\beta_{31-35}$ on the excitatory and inhibitory postsynaptic responses. With another intention, we used $A\beta_{31-35}$ in our experiments to further prove the hypothesis we proposed previously that the sequence of 31–35 in Aβ might be a shorter active center in the full length of the Aβ molecule. Therefore, in the present study, we investigated the effects of acute application of Aβ fragments ($A\beta_{25-35}$ and $A\beta_{31-35}$) on Glu, NMDA and GABA receptor-mediated whole-cell currents in isolated hippocampal neurons by the patch clamp technique.

2. Materials and methods

2.1. Isolation of the hippocampal neurons

Experiments were carried out on hippocampal CA1 pyramidal cells of Wistar rats aged 7–14 days (supplied by the Research Animal Center of Shanxi Medical University, China), and all efforts were made to minimize the number of animals used and their suffering. Single neurons were dissociated from the hippocampus of rats of both sexes using a method similar to that described by Kay and Wong [18]. Briefly, during ether anesthesia, the brain was rapidly removed and placed in oxygenated ice-cold artificial cerebral spinal fluid (ACSF) with a lower concentration of calcium; hippocampal tissue was dissected out and sliced at a thickness of 400–600 μm, then the brain slices were transferred into ACSF bubbled with 95% O_2 and 5% CO_2. The hippocampal CA1 area was microdissected and cut into 2–4 pieces when needed, then treated with incubation solution containing pronase E (1 mg/ml) for 30 min at 33 °C. The digestion medium was oxygenated during the treatment with enzyme. After digestion, tissue pieces were washed three times with enzyme-free incubation solution. Then, the pieces were dispersed by trituration with a graded series of Pasteur pipettes. Three drops of cell suspension were added onto a coverslip. After settling down on the bottom of the recording chamber, the neurons with large pyramidal-shaped cell bodies, thick apical dendritic stumps, bright and smooth appearance were selected for membrane current recordings. The hippocampal CA1 neurons isolated with this method can usually maintain a good appearance and normal electrical activity up to 6 h.

2.2. Solutions

The following solutions (in mM) were used for the experimental procedure: (1) ACSF: NaCl 126, KCl 5, $MgSO_4$ 2, $CaCl_2$ 2, glucose 10, $NaHCO_3$ 25, NaH_2PO_4 1.5 (pH 7.4 adjusted with NaOH); (2) ACSF with a lower concentration of calcium: NaCl 126, KCl 5, $MgSO_4$ 2, $CaCl_2$ 0.2, glucose 10, $NaHCO_3$ 25, NaH_2PO_4 1.5 (pH 7.4 adjusted with NaOH); (3) incubation solution: NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 25, HEPES 10 (pH 7.4 adjusted with NaOH); (4) bath solution (for NMDA-induced current): NaCl 128, glucose 30, HEPES 25, KCl 5, $CaCl_2$ 2, CNQX 10 μM, glycine 20 μM (pH 7.3 adjusted with NaOH); (5) pipette solution 1 (for glutamate- and NMDA-induced currents): K^+-gluconate 125, KCl 10, EGTA 2, HEPES 10, Tris–phosphocreatine 10, MgATP 4, Na_2GTP 0.5 (pH 7.3 adjusted with KOH); (6) pipette solution 2 (for GABA-induced currents): KCl 135, Tris–phosphocreatine 10, EGTA 2, HEPES 10, MgATP 4, Na_2GTP 0.5 (pH 7.3 adjusted with KOH).

2.3. Electrical measurement

Recording was made in a whole-cell voltage-clamp configuration [19] at room temperature (25 ± 1 °C). Pipettes of borosilicate glass were pulled by using a two-step vertical puller (PP-830) and had resistances between 5 and 10 MΩ when filled with recording solution (pipette solution). After neurons were settled down on the bottom and just before recording, 0.2 μM tetrodotoxin was added into the incubation solution (or NMDA bath solution when NMDA-