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A role of insulin-like growth factor 1 in β amyloid-induced disinhibition of hippocampal neurons

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Abstract

In the present study we investigated the effects of β amyloid (Aβ) on inhibitory synaptic transmission in the cultured hippocampal neurons using whole-cell patch-clamp recordings and immunocytochemistry, and examined the role of insulin-like growth factor 1 (IGF-1). Incubation with 4 μM Aβ25–35 for 24 h significantly decreased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs), but had no effect on the mean amplitude. Pretreatment with 10 ng/ml IGF-1 for 24 h prior to Aβ25–35 exposure blocked Aβ-induced disinhibition of hippocampal neurons. The frequency and mean amplitude of miniature IPSC (mIPSCs) were not significantly affected by Aβ. The rise and decay kinetics of sIPSCs and mIPSCs were similar for the control and Aβ25–35-treated hippocampal neurons. Immunocytochemistry showed no changes in the ratio of γ-aminobutyric acid (GABA) positive cells subsequent to treatment with Aβ, or IGF-1. Together these data suggest that Aβ-induced the disinhibition in cultured hippocampal neurons, whereas IGF-1 could block this effect.

Keywords: Insulin-like growth factor 1; β Amyloid; Inhibitory postsynaptic current; Whole-cell patch-clamp; γ-Aminobutyric acid

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most frequent cause of cognitive deficit in the aged. Insoluble fibril deposits of Aβ is the major component of senile plaques and plays an important role in the neurodegenerative process [11,16,20]. It is well established that the cholinergic and glutamatergic systems are adversely affected in the progressive course of AD. However, other neurotransmitter systems, such as serotonergic [1,7,15], histaminergic [18], and GABAergic [17] systems are less well studied. Previous studies have focused on the effects of Aβ on excitatory synaptic transmission but little is known how Aβ influences inhibitory synaptic transmission. Therefore, to elucidate the effects of Aβ on this important pathway, we recorded sIPSCs and miniature IPSCs using whole-cell patch-clamp in cultured rat hippocampal neurons, and counted the numbers of GABA positive cells using immunocytochemical staining.

IGF-1 is a pleiotropic factor with structural and functional homologies to IGF-2 and insulin. IGF-1 has important functions in the brain, including metabolic, neurotrophic, neuromodulatory and neuroendocrine actions [19]. IGF-1 and its receptor are highly concentrated in the hippocampus, an area severely affected in AD [6,9]. Recently, IGF-1 has gained increasing attention for the pathogenesis of age-related neurodegenerative diseases [12]. AD patients show changes in insulin and IGF-1 levels, which may protect hippocampal neurons against the toxicity of Aβ [8]. Additionally, IGF-1 may be a key factor in regulating the clearance of Aβ from the brain [4]. Based on these previous reports, we investigated the effects of IGF-1 in the regulation Aβ-induced changes of inhibitory synaptic transmission.

Hippocampal neuron cultures were prepared as described previously [10]. Briefly, pregnant Wistar rats were anesthetized with pentobarbital and the E18–19 embryos delivered by cesarean section. The hippocampi were dissected and incubated with 0.25% Trypsin–EDTA for 15 min at 37 °C and mechanically dissociated. The resulting single cell

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suspension was diluted at a density of 1×10^5 cells/ml in high glucose DMEM containing 10% fetal bovine serum, 5% equine serum and 2 mM L-glutamine, then plated in 35 mm-cell plates coated with poly-D-lysine (20 μg/ml). Cells were incubated at 37 °C in a humidified incubator with 5% CO_2. After approximately 20 h, the medium was replaced by serum-free Neurobasal medium containing B27 supplement and 0.5 mM L-glutamine to inhibit the growth of glia cells. Every 3–4 days half of the media was replaced and the cultures were used for experiments on 10–14 days after plating. Aβ25–35 (Sigma) was dissolved in sterile distilled water at a concentration of 2 mM as a stock solution and incubated at 37 °C for 96 h before use. To determine the effects of Aβ, we incubated 4 μM Aβ25–35 with cultured hippocampal neurons for 24 h and measured electrophysiological and immunocytochemical changes. In order to explore the action of IGF-1 on these effects, cultures were pretreated with 10 ng/ml IGF-1 (PeproTech) for 24 h prior to exposure to Aβ25–35. Data were expressed as the mean ± S.E.M. Statistical significance was determined as $p < 0.05$ by one-way ANOVA (SPSS, Chicago, IL). In all cases, *n* refers to the number of neurons studied from multiple dissections and data pooled.

The whole-cell patch-clamp technique was used to record current. The patch electrodes of thick-walled boro-silicate glass (VWR Scientific) were pulled on a PP-83 micropipette puller (Narishige). The patch-pipette solution contained (in mM): 140 KCl, 10 HEPES, 10 EGTA, 2 $MgCl_2$, 2 Na_2ATP, 1 $CaCl_2$, pH 7.3. The typical resistance of glass electrodes was 3–5 MΩ when filled with intracellular pipette solution. The range of the whole-cell series resistance is 10–15 MΩ. Data were collected with an Axopatch 200B amplifier (Axon Instruments) and acquired and analyzed using pCLAMP 9 (Axon Instruments). During experiments, culture dishes were rinsed and perfused with extracellular solution containing (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 Glucose, 10 HEPES, pH 7.4. Synaptically spontaneous IPSCs were isolated by the application of 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50 μM aminophosphonobutyrate (APV), antagonist to the excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methy-D-aspartate (NMDA) receptors, respectively. mIPSCs were recorded using 1 μM TTX added to the extracellular solution. sIPSCs and mIPSCs were recorded without synaptic stimulation at a holding potential of −70 mV from cultured hippocampal neurons for at least 5 min. The currents of sIPSCs were completely abolished after using 50 μM bicuculline, the antagonist of the inhibitory $GABA_A$ receptor, indicating that they were mediated by $GABA_A$ receptors. Cells were chosen for recording based on their morphology and density of surrounding cells. Relatively isolated or spherically shaped cells were avoided.

Aβ25–35 treatment for 24 h significantly decreased the frequency of sIPSCs in cultured hippocampal neurons, but had little influence on the mean amplitude of sIPSCs (Fig. 1). Quantitative analysis of all neurons recorded indicated that sIPSCs frequency decreased by 47.5%. The average value of frequency of the normal group ($n = 10$) was 3.37 ± 0.33 Hz. However, after treatment with 4 μM Aβ25–35 for 24 h, the frequency decreased to 1.77 ± 0.43 Hz ($n = 11$). The similar results were observed with Aβ1–42. After incubation with 250 nM Aβ1–42 for 24 h, the frequency of sIPSCs decreased to 2.08 ± 0.45 Hz ($n = 12$), and the mean amplitude did not change. Aβ25–35 treatment had no significant effects on both the frequency and mean amplitude of mIPSCs (Fig. 2). When the neurons were pretreated with 10 ng/ml IGF-1 for 24 h prior to exposure to Aβ25–35, some inhibitory firings were saved and the frequency of sIPSCs was 2.95 ± 0.36 Hz ($n = 10$). There were no significant difference between the control and IGF-1 pretreated group (Fig. 1). Treatment with IGF-1 alone did not affect the frequency of sIPSCs. Therefore, it appears that Aβ25–35-induced synaptic disinhibition and this effect could be blocked by IGF-1.

Furthermore, the other postsynaptic properties of sIPSCs and mIPSCs including rise time constant, decay time constant and the time to peak were analyzed using Clampfit 9.0 (Axon Instruments). The rise and decay kinetics of sIPSCs and mIPSCs were similar for the control and Aβ25–35-treated hippocampal neurons, and there are no significant differences between the two groups. The details are in Table 1.

The numbers of GABAergic neurons was examined in cultured hippocampal neurons using immunocytochemistry. Hippocampal neurons were fixed with 4% paraformaldehyde for 30 min at room temperature, permeabilized with 0.1% Triton X-100 for 5 min, and blocked with 5% horse serum for 30 min at 37 °C. The cultures were incubated with rabbit anti-GABA primary antibody (Sigma, 1:1000) for 1 h at 37 °C and followed by TRITC-labeled secondary antibody (1:200) for 45 min at room temperature. Cells were examined using a fluorescence microscope with six to ten high magnification (400×) fields randomly selected for counting. Experiment was repeated three times.

Aβ25–35 treatment did not affect the ratio of GABA-positive cells (Fig. 3). In the control group, the percentage of GABA positive cells was 30.19%. After incubated with 4 μM of Aβ25–35 for 24 h, GABA positive cells were 28.99%. Pretreatment with IGF-1 prior to exposure to Aβ25–35 or treatment with IGF-1 alone did not change the ratio.

GABA mediates neuronal inhibition by binding to the GABA/benzodazepine receptor and opening an integral chloride channel. In addition, GABA functions as an excitatory modulator: it depolarizes the membrane of embryonic neurons and axons, exerts trophic, chemoatractant or chemokinetic influences that promote cell migration to form specific systems [2,5]. Aβ-induced lesions are involved in decreasing GABAergic transmission. It has been reported that GABA and the $GABA_A$ receptor agonists protect neurons against Aβ-induced neurotoxicity [13]. In the current study, Aβ significantly reduced the frequency of sIPSCs but did not affect the frequency of mIPSCs, indicating that the effects of Aβ on inhibitory synaptic transmission are action potential-related (i.e., TTX-sensitive). To further clarify the action site of Aβ-induced disinhibition in hippocampal

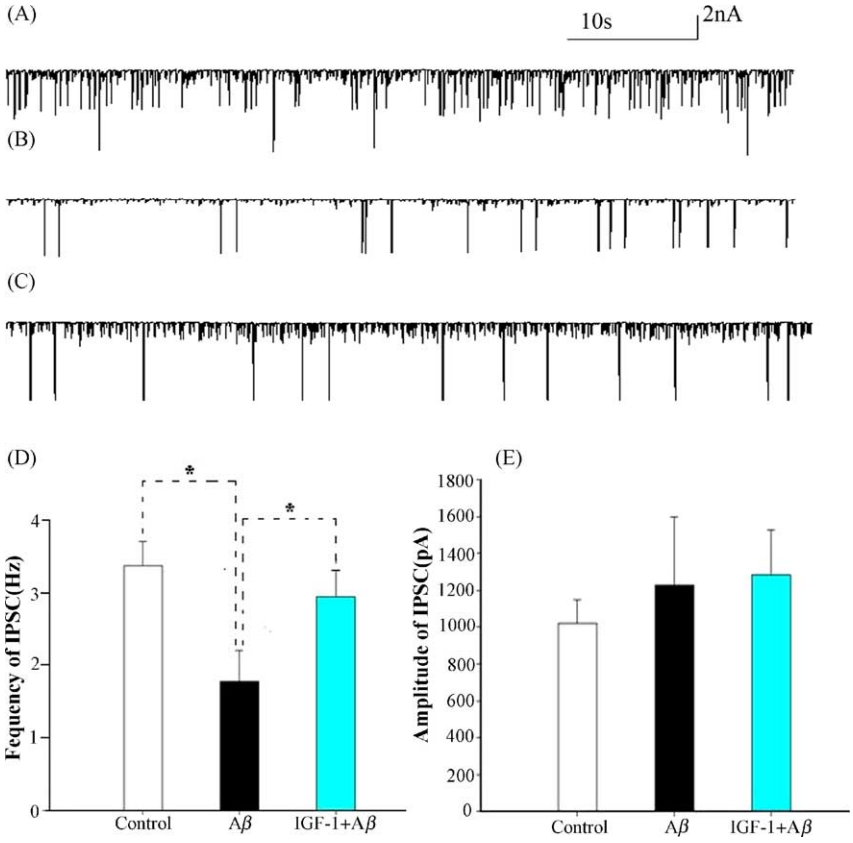

Fig. 1. Aβ25–35 decreased the frequency of sIPSCs, but had no effect on the mean amplitude. Pretreatment with IGF-1 blocked this effect. (A)–(C) Representative traces of sIPSCs from the normal cells (A), Aβ25–35-treated cells (B), and the cells pretreated with IGF-1 prior to Aβ25–35 incubation (C). (D) and (E) Bar graph of the frequency (D) and mean amplitude (E) of sIPSCs in control ($n = 10$), Aβ25–35-treated ($n = 11$), and IGF-1 pretreated ($n = 10$) groups. Data were expressed as mean ± S.E.M. Significant difference compared with the Aβ25–35-treated group (* $p < 0.05$).

neurons, we determined the amplitude and rise and decay kinetic properties of sIPSCs and mIPSCs which primarily depended on the number and activity of postsynaptic receptors. No difference was found in the mean amplitude, rise time and decay time constants of sIPSCs and mIPSCs. This may indicate that Aβ substantially decreased the numbers of GABAergic neurons, reduced the GABA of release at synapses from a subset of presynaptic inhibitory neurons, or altered the number of inhibitory neurons in functional synaptic contact between hippocampal neurons, but Aβ did not interact with postsynaptic GABA receptors. This suggests that Aβ may play an important role in the synaptic deficits between GABAergic neurons and pyramidal cells.

Table 1
Summary of quantitative data

	Control group		Aβ25–35-treated group	
	Mean ± S.E.M.	*n*	Mean ± S.E.M.	*n*
sIPSCs				
Frequency (Hz)	3.37 ± 0.33	10	1.77 ± 0.43*	11
Mean peak amplitude (pA)	−1020.45 ± 126.4	10	−1225.09 ± 373.7	11
Time to peak (ms)	5.01 ± 0.28	10	4.71 ± 0.20	13
Rise time constant (ms)	4.55 ± 0.75	10	3.41 ± 0.24	11
Decay time constant (ms)	39.99 ± 3.32	10	35.69 ± 1.99	11
mIPSCs				
Frequency (Hz)	0.046 ± 0.005	11	0.056 ± 0.009	10
Mean peak amplitude (pA)	−57.76 ± 3.30	11	−55.04 ± 3.10	10
Time to peak (ms)	5.40 ± 0.25	11	4.65 ± 0.47	10
Rise time constant (ms)	1.37 ± 0.22	11	1.31 ± 0.20	10
Decay time constant (ms)	18.64 ± 2.02	11	16.99 ± 1.96	8

* Compared with control group ($p < 0.05$).

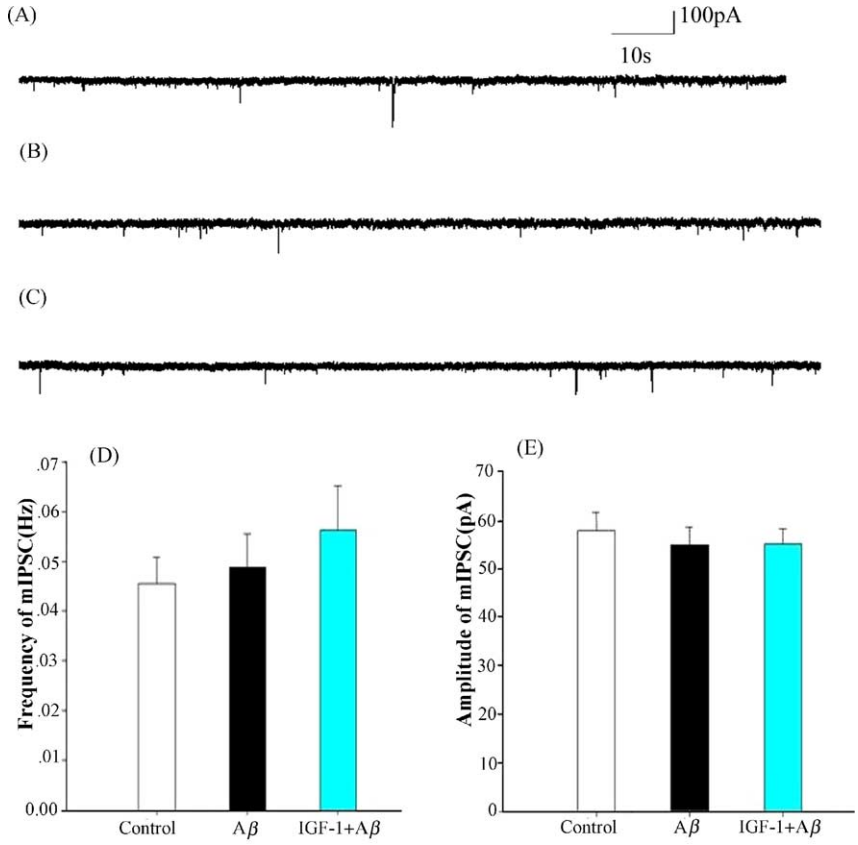

Fig. 2. No effects on the frequency and mean amplitude of mIPSCs. (A)–(C), Representative traces of mIPSCs from normal cells (A), Aβ25–35-treated cells (B), and cells pretreated with IGF-1 prior to Aβ25–35 incubation (C). (D) and (E) Bar graph of the frequency and mean amplitude of mIPSCs in control ($n = 11$), Aβ25–35-treated ($n = 10$), and IGF-1 pretreated ($n = 11$) groups. Data were expressed as mean ± S.E.M. There is no significant difference between the three groups.

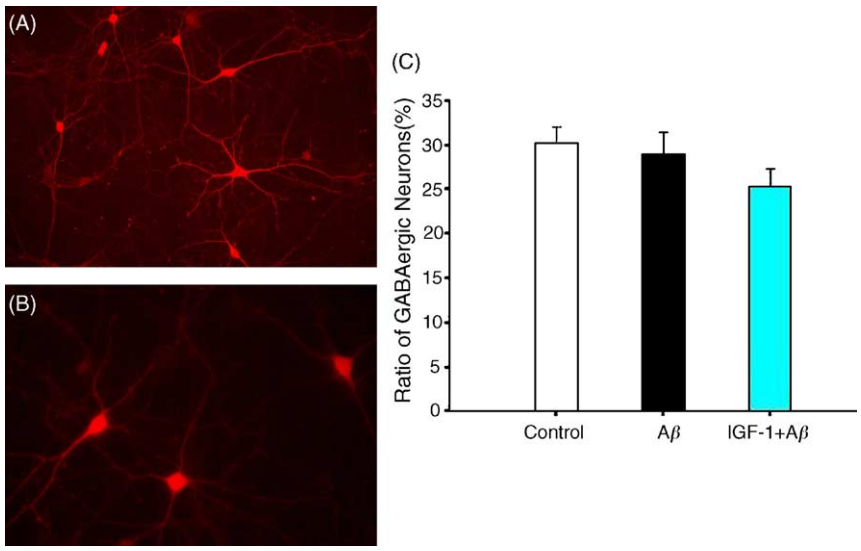

Fig. 3. No effects of Aβ25–35 and IGF-1 on the numbers of GABAergic neurons. (A) and (B) anti-GABA positive neurons (A, ×200; B, ×400). (C) Bar graph is representative for the ratio of the number of GABA positive neurons. Data were expressed as mean ± S.E.M. There is no significant difference between the groups.

To learn whether Aβ-induced disinhibition is due to the decrease of the numbers of GABAergic neurons, immunocytochemical staining for GABA was performed to identify inhibitory neurons. We did not observe a decrease in the ratio of GABAergic neurons in proportion to the total number of neurons. Therefore, Aβ does not induce the loss of GABAergic neurons selectively, but may reduce functional GABA transmitter or the functional synaptic contact between neurons. This may be a pathway by which Aβ disinhibited hippocampal neurons.

Previous studies have demonstrated that the neurotoxicity of Aβ is related to the overactivation of glutamatergic transmission and excitotoxicity, and that blockade of glutamate receptors prevents Aβ-induced cell death. Combined with our findings that Aβ disinhibited hippocampal neurons, it appears that Aβ destabilizes the homeostasis between excitatory and inhibitory amino transmission, and therefore, enhances excitotoxic insults. Aβ-induced disinhibition may be a mechanism to increase excitatory synaptic function in AD.

IGF-1 is a potent neuroprotective factor and can inhibit Aβ-induced cell death [14]. In addition, IGF-1 is found in lower amounts in AD patients and IGF-1 can regulate levels of phosphorylated tau, a major component of neurofibrillary tangles (NFT) [3]. In the present study, IGF-1-pretreated neurons maintained the frequency of sIPSCs with the presence of Aβ, which may be one of the protective mechanisms of IGF-1 against Aβ. However, whether this protection of IGF-1 depends on accelerating the clearance of Aβ or directly acting on remaining synaptic plasticity has not yet been clarified. Our experiments demonstrated the novel finding that the application of IGF-1 may enhance the inhibitory synaptic transmission and may be effective to in rescuing neurons from Aβ-induced injury.

We provided evidence demonstrating that Aβ significantly reduced the frequency of sIPSCs in hippocampal neurons. This effect may be mediated by a presynaptic modulation of the transmitter release, and was not due to the direct and specific regulation on postsynaptic GABA receptor. Pretreatment with IGF-1 could block Aβ-induced disinhibition. These results imply that application of IGF-1 may be a promising strategy for treatment to AD.

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