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 IPSCs (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.

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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. 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, histaminergic, and GABAergic 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, neuro-modulatory and neuroendocrine actions. IGF-1 and its receptor are highly concentrated in the hippocampus, an area severely affected in AD. Recently, IGF-1 has gained increasing attention for the pathogenesis of age-related neurodegenerative diseases. AD patients show changes in insulin and IGF-1 levels, which may protect hippocampal neurons against the toxicity of Aβ. Additionally, IGF-1 may be a key factor in regulating the clearance of Aβ from the brain. 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. 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...
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 P-83 micropipette puller (Narishige). The patch-pipette solution contained (in mM): 140 KCl, 10 HEPES, 2 MgCl\(_2\), 2 Na\(_2\)ATP, 1 CaCl\(_2\), pH 7.3. The typical resistance of glass electrodes was 3–5 M\( \Omega \) when filled with intracellular pipette solution. The range of the whole-cell series resistance is 10–15 M\( \Omega \). Data were collected with an Axopatch 200B amplifier (Axon Instruments). The rise and decay kinetics of sIPSCs and mIPSCs were similar for the control and A\(_{25–35}\) treatment. However, treatment with A\(_{25–35}\) for 24 h, the frequency of sIPSCs decreased by 47.5%. The average value of frequency of the normal group (\( n = 10 \)) was 3.37 ± 0.33 Hz.

When the neurons were pretreated with 10 ng/ml IGF-1 for 24 h prior to exposure to A\(_{25–35}\), some inhibitory firings were saved and the frequency of sIPSCs was 2.95 ± 0.36 Hz (\( n = 10 \)). There were no significant differences between the control and IGF-1 pretreated group (Fig. 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 A\(_{25–35}\)-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 to inhibit the growth of glia cells. After incubation with 25–35 for 24 h, GABA positive cells were 28.99%. After incubation with 4 \( \mu \)M A\(_{25–35}\) for 24 h, the frequency of sIPSCs decreased to 2.08 ± 0.45 Hz (\( n = 12 \)), and the mean amplitude did not change. A\(_{25–35}\) treatment had no significant effects on both the frequency and mean amplitude of mIPSCs (Fig. 2).

The range of the whole-cell series resistance is 10–15 M\( \Omega \) and 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 to inhibit the growth of glia cells. After incubation with 25–35 for 24 h, GABA positive cells were 28.99%. After incubation with 4 \( \mu \)M A\(_{25–35}\) for 24 h, the frequency of sIPSCs decreased to 2.08 ± 0.45 Hz (\( n = 12 \)), and the mean amplitude did not change. A\(_{25–35}\) treatment had no significant effects on both the frequency and mean amplitude of mIPSCs (Fig. 2).

After approximately 20 h, the medium was replaced by high glucose DMEM containing 10% fetal bovine serum, 5% serum-free Neurobasal medium containing B27 supplement and 0.5 mM \( \alpha \)-aspartate and 0.5 mM \( \alpha \)-aspartate glutamine to inhibit the growth of glia cells. After incubation with 25–35 for 24 h, the frequency of sIPSCs 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 \( \mu \)M A\(_{25–35}\) for 24 h, the frequency decreased to 1.77 ± 0.43 Hz (\( n = 11 \)). The similar results were observed with A\(_J^I–42\). After incubation with 250 nm A\(_J^I–42\) for 24 h, the frequency of sIPSCs decreased to 2.08 ± 0.45 Hz (\( n = 12 \)), and the mean amplitude did not change. A\(_J^I–35\) treatment had no significant effects on both the frequency and mean amplitude of mIPSCs (Fig. 2).

The rise and decay kinetics of sIPSCs and mIPSCs were similar for the control and A\(_{25–35}\)-treated hippocampal neurons, and there are no significant differences between the two groups. The details are in Table 1.
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

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Aβ25–35-treated group</th>
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<tbody>
<tr>
<td><strong>sIPSCs</strong></td>
<td></td>
<td></td>
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<tr>
<td>Frequency (Hz)</td>
<td>3.37 ± 0.33</td>
<td>1.77 ± 0.43*</td>
</tr>
<tr>
<td>Mean peak amplitude (pA)</td>
<td>−1020.45 ± 126.4</td>
<td>−1225.09 ± 373.7</td>
</tr>
<tr>
<td>Time to peak (ms)</td>
<td>5.01 ± 0.28</td>
<td>4.71 ± 0.20</td>
</tr>
<tr>
<td>Rise time constant (ms)</td>
<td>4.55 ± 0.75</td>
<td>3.41 ± 0.24</td>
</tr>
<tr>
<td>Decay time constant (ms)</td>
<td>39.99 ± 3.32</td>
<td>35.69 ± 1.99</td>
</tr>
<tr>
<td><strong>mIPSCs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>0.046 ± 0.005</td>
<td>0.056 ± 0.009</td>
</tr>
<tr>
<td>Mean peak amplitude (pA)</td>
<td>−57.76 ± 3.30</td>
<td>−55.04 ± 3.10</td>
</tr>
<tr>
<td>Time to peak (ms)</td>
<td>5.40 ± 0.25</td>
<td>4.65 ± 0.47</td>
</tr>
<tr>
<td>Rise time constant (ms)</td>
<td>1.37 ± 0.22</td>
<td>1.31 ± 0.20</td>
</tr>
<tr>
<td>Decay time constant (ms)</td>
<td>18.64 ± 2.02</td>
<td>16.99 ± 1.96</td>
</tr>
</tbody>
</table>

* 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, immuno- 
cytochemical staining for GABA was performed to identify 
inhibitory neurons. We did not observe a decrease in the ra-
tio of GABAergic neurons in proportion to the total num-
ber 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 neurotoxic-
ty of Aβ is related to the overactivation of glutamatergic 
transmission and excitotoxicity, and that blockade of gluta-
mate receptors prevents Aβ-induced cell death. Combined 
with our findings that Aβ disinhibited hippocampal neurons, 
it appears that Aβ destabilizes the homeostasis between ex-
citatory and inhibitory amino transmitter, and therefore, 
hances 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 neu-
rons 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 clar-
tified. Our experiments demonstrated the novel finding that 
the application of IGF-1 may enhance the inhibitory synap-
tic transmission and may be effective in to 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 spe-
cific regulation on postsynaptic GABA receptor. Pretreatment 
with IGF-1 could block Aβ-induced disinhibition. These re-
sults imply that application of IGF-1 may be a promising 
strategy for treatment to AD.

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References

hydroxyindole-3-acetic acid depolarized in hippocampus but not in sub-

[2] T.N. Bohar, Y.X. Li, I.T. Tran, W. Ma, D. Aman, C. Scott, J.L. Barker, GABA stimulates chemotaxis and chemokinesis of amphi-
ontic cortical neurons via calcium-dependent mechanisms, J. Neu-

[3] E. Carro, I. Torres-Aleman, The role of insulin and insulin-like growth factor I in the molecular and cellular mechanisms under-
lying the pathology of Alzheimer’s disease, Eur. J. Pharmacol. 490 

Serum insulin-like growth factor I regulates brain amyloid-beta lev-

[5] E. Cherubin, J.L. Gaara, Y. Ben-Ari, GABA: an excitatory trans-
515–519.

Insulin-like growth factor I receptor binding in brains of 
Alzheimer’s and alcoholic patients, J. Neurochem. 58 (1992) 
1205–1210.

[7] A.J. CrossRef, Serotonin in Alzheimer-type dementia and other dement-
415–417).

and rescues hippocampal neurons against beta-amyloid- and human 
4772–4777.

[9] S. Dore, S. Kar, W. Rowe, R. Quinton, Distribution and levels of 
[125I]IGF-I, [125I]IGF-II and [125I]insulin receptor binding sites in 
the hippocampus of aged memory-impaired and -impaired rats, 

[10] M.S. Eross, A. McCaffrey, G. Brewer, Electrophysiology of em-
byronic, adult and aged rat hippocampal neurons in serum-free cul-


[12] L. Gasparini, W.J. Netzer, P. Gerhard, H. Xu, Does insulin dys-
ofunction play a role in Alzheimer’s disease? Trends Pharmacol. 

Mello, S.T. Ferreira, Taurine presents the neurotoxicity of beta-
amyloid and glutamate receptor agonists: activation of GABA re-
teceptors and possible implications for Alzheimer’s disease and other 

Abeta(1–40)-or Abeta(1–42)-induced cell death and their rescue fac-

Laakso, P.J. Reikkinen, A pre-mortem study of noradrenergic, sero-
tonergic and GABAergic neurons in Alzheimer’s disease, J. Neurol. 

[16] K.J. Reinikainen, L. Paljarvi, M. Huuskonen, H. Soininen, M. 
Raizada, Insulin-like growth factor I receptor binding in brains of 

[17] M. Roos, M. Iversen, Non-cholinergic neurotransmitter abnormal-

[18] C. Schneider, D. Risser, E. Kitzmuller, H. Neufeld, S. Sanders, 
Serum insulin-like growth factor I in the molecular and cellular mechanisms under-
lying the pathology of Alzheimer’s disease, Eur. J. Pharmacol. 490 


415–417).

Figure 1. Insulin-like growth factor I in Alzheimer’s disease: noradrenaline, 5-hydroxytryptamine and 5-
hydroxyindole-3-acetic acid depolarized in hippocampus but not in sub-