Glutamate Exacerbates Amyloid $\beta$1 – 42-Induced Impairment of Long-Term Potentiation in Rat Hippocampal Slices

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ABSTRACT—Amyloid $\beta$ (A$\beta$) is the principal constituent of senile plaques in Alzheimer’s disease patients. We investigated whether A$\beta$ and glutamate affect long-term potentiation (LTP) in rat hippocampal slices. Pretreatment with 1 $\mu$M A$\beta$1 – 42 alone for 3 h slightly inhibited LTP; however, the potentiation was maintained for 60 min. Although the impairment was not observed by pretreatment with 30 $\mu$M glutamate alone for 3 h, pretreatment with A$\beta$1 – 42 and glutamate impaired LTP significantly. These results raise the possibility that neurotoxicity of A$\beta$ is exacerbated by the enhancement of susceptibility to excitatory amino acids.

Keywords: Amyloid $\beta$, Long-term potentiation, Glutamate

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by a slowly progressive cognitive decline. In the early stages, neuropathological features are associated with synaptic dysfunction, formation of neurofibrillary tangles and senile plaques, followed by loss of neurons (1). Amyloid $\beta$ protein (A$\beta$) is a 40 – 42 amino acid peptide that is the principal constituent of senile plaques. The potential role of A$\beta$ as a neurotoxic agent has been demonstrated in vitro (2), in amyloid precursor protein-transgenic mice (3) and A$\beta$-infused rats (4), supporting the hypothesis that A$\beta$ is involved in the pathogenesis of AD.

Glutamate plays a key role in excitatory synaptic transmission of the brain, and it is massively released from neurons by various insults such as hypoxia. Neurotoxicity of excitatory amino acids is involved in disruption of calcium homeostasis followed by sustained elevations of intracellular calcium (5). Several groups have reported that A$\beta$ increases the vulnerability of neurons to excitotoxicity via calcium entry from N-methyl-D-aspartate (NMDA) receptors in vitro (6). We also demonstrated that co-injection of A$\beta$ with ibotenic acid, a NMDA-receptor selective agonist, induces synergistic loss of rat hippocampal neurons in vivo (7).

Long-term potentiation (LTP) in the hippocampus is one form of synaptic plasticity and is thought to be a cellular mechanism underlying learning and memory (8). LTP is induced by high-frequency stimulation (HFS), and requires activation of NMDA-type glutamate receptors and the consequent calcium entry into the postsynaptic spines, at least in the Schaffer collateral-CA1 pyramidal cell synapses. These reports prompted us to investigate whether A$\beta$ and/or glutamate impair LTP or not. Considering that the deposition of A$\beta$ is believed to occur in AD patients over a period of 20 years, a suitable approach appears to be not acute application of A$\beta$ but chronic treatment or pretreatment with A$\beta$. Based on this idea, we selected the approach of pretreatment and examined LTP in rat hippocampal slices that were pretreated with A$\beta$ and/or glutamate.

A$\beta$1 – 42 (HCl salt) was purchased from AnaSpec Inc. (San Jose, CA, USA). A$\beta$1 – 42 at a concentration of 500 $\mu$M was dissolved in 2 mM HCl and stored in small aliquots at $-20^\circ$C. To generate fibrils, A$\beta$1 – 42 was diluted to 100 $\mu$M in water, NaCl and HEPES (final concentrations: 150 mM NaCl, 0.4 mM HCl and 20 mM HEPES) and then incubated at room temperature for 1 – 2 days (9). The peptide was vortexed and then diluted to desired final concentrations in artificial cerebrospinal fluid (ACSF) immediately before pretreatment in each experiment. ACSF had the following composition: 127 mM NaCl, 1.6 mM KCl, 1.24 mM KH$_2$PO$_4$, 1.3 mM MgSO$_4$, 2.4 mM CaCl$_2$, 26 mM NaHCO$_3$ and 10 mM glucose.

Transverse hippocampal slices, 400-$\mu$m-thick, were prepared from 3 – 4-week-old male Wistar rats. Slices were maintained in oxygenated (95% O$_2$ – 5% CO$_2$) ACSF at 30°C for at least 1 h prior to pretreatment with drugs.

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After the recovery period, slices were transferred to a small chamber filled with the same ACSF containing Aβ1–42 and/or glutamate. Control slices were also transferred to the same chamber filled with only ACSF. After pretreatment, the slices were then transferred to a recording chamber in which the slices were continuously perfused with warmed (30°C) and oxygenated ACSF at a rate of 1.5–2.0 ml/min. To thoroughly remove the drug-containing ACSF, the slices were perfused for at least 30 min before recording. Recording of field excitatory postsynaptic potentials (fEPSPs) from the stratum radiatum of CA1 region was carried out as described in our previous report (10). The degree of paired-pulse facilitation (PPF) was initially determined at an interpulse interval of 50 ms.

In the control slices, application of HFS produced a robust potentiation of fEPSP, which lasted for 60 min and reached up to a 160% level (Fig. 1, open circles). Next, hippocampal slices were pretreated with 1 μM Aβ1–42 for 3 h. This concentration and application period did not induce any cell death in the primary hippocampal neurons (our unpublished data). In Aβ1–42-pretreated slices, slight decline of fEPSPs was observed for 40–60 min; however, the potentiation was maintained for 60 min (Fig. 1, closed circles). The effect of higher concentration (20 μM) was also investigated, but the basal response was unstable (data not shown). We tested the effect of pretreatment with 30 μM glutamate alone, at which concentration the frequency of spontaneous excitatory postsynaptic currents did not change compared to control slices (our unpublished data). The pretreatment did not show any effect on LTP for 60 min (Fig. 1, open triangles).

To examine whether Aβ1–42 and glutamate further impairs LTP, hippocampal slices were pretreated with 1 μM Aβ1–42 in the presence of 30 μM glutamate. The same HFS was delivered at time 0, but fEPSPs gradually declined and returned to the baseline level in Aβ1–42 and glutamate-pretreated slices (Fig. 2: A and B, closed circles). Comparison of the slices showed that the magnitude of potentiation 0–60 min after HFS was significantly smaller in Aβ1–42-pretreated slices than in control slices (Fig. 2C). Moreover, in slices pretreated with Aβ1–42 and glutamate, the decrease in the fEPSPs’ magnitude was significant compared to the other three groups.

**Fig. 1.** Effect of pretreatment with Aβ1–42 or glutamate alone on LTP. The time course of LTP in control slices (open circles, n = 5), slices pretreated with 1 μM Aβ1–42 alone for 3 h (closed circles, n = 6) and slices pretreated with 30 μM glutamate alone for 3 h (open triangles, n = 5). HFS (100 pulses at 100 Hz) was applied at time 0 min. LTP was plotted as a percentage of the baseline fEPSP slope. All data in this study are expressed as means ± S.E.M.

**Fig. 2.** Effect of pretreatment with 1 μM Aβ1–42 and 30 μM glutamate on LTP. A: Typical fEPSPs recorded in the stratum radiatum of the CA1 area by stimulating the Schaffer collaterals. The fEPSPs immediately before and 60 min after HFS are imposed at the right. Test stimulation was delivered at the time indicated by arrowheads. B: The time course of LTP in control slices (open circles, n = 5) and slices pretreated with Aβ1–42 and glutamate for 3 h (closed circles, n = 5). C: Summary of the effect of pretreatment of Aβ1–42 and/or glutamate on LTP. The average percentage of the fEPSP slope 0–60 min after HFS was calculated for each slice. *P < 0.05, **P < 0.01 vs control slices; ***P < 0.01 vs Aβ1–42-pretreated slices; Tukey’s test, n = 5–10.
The change in basal synaptic response without HFS was also investigated. The baseline response did not change over a 60-min period in the Aβ1–42 and glutamate-pretreated slices (Fig. 3, closed circles), indicating that the impairment of LTP is not attributable to a decrease in synaptic response during measurement. In addition, there was no apparent difference between the control slices and slices pretreated with Aβ1–42 and glutamate in the waveform of fEPSP (Fig. 2A); in the size of maximal fEPSP (Fig. 2A); in the size of maximal fEPSP (2.43 ± 0.15 mV, n = 10 vs 2.30 ± 0.17 mV, n = 10); or in PPF (138.24 ± 5.0%, n = 10 vs 135.9 ± 3.9%, n = 10). These results suggest that the basal properties of synaptic transmission did not change by the pretreatment, and it is unlikely that decrease in presynaptic function resulted in the impairment of LTP.

We have demonstrated for the first time that pretreatment with Aβ1–42 and glutamate further impairs LTP than that with Aβ1–42 alone in rat hippocampal slices. This finding is consistent with previous reports that Aβ and ibotenic acid synergistically disrupt the acquisition of spatial learning (11) and induce loss of rat hippocampal neurons (7). These data support the notion that neurotoxicity of Aβ is exacerbated by the enhancement of susceptibility to excitatory amino acids.

The mechanisms by which Aβ and glutamate contribute to the impairment of LTP remain to be elucidated. Pretreatment with Aβ and glutamate did not affect fEPSPs and PPF. Therefore, it is unlikely that the pretreatment influences the excitability of the postsynaptic neurons or the neurotransmitter release from presynaptic terminals. Because NMDA receptor-dependent, short-term (<30 min) potentiation (8) is significantly inhibited in Aβ and glutamate-pretreated slices, it raises the possibility that the NMDA receptor is functionally damaged by the pretreatment. Additionally, Kimura and Schubert (12) demonstrated that Aβ activates the tachykinin receptor in the presence of glutamate. The other plausible explanation for the impairment of LTP might be substantially enhanced intracellular calcium entry (6) followed by excessive generation of reactive oxygen species (13). These results allow us to assume that glutamate exacerbates the toxicity of Aβ on hippocampal neurons during pretreatment, in part, by elevation of intracellular calcium followed by several sequential events.

Aβ exists in various states during the process in which it assembles from monomer to fibril. To the best of our knowledge, the states of Aβ are now classified into the following four forms: monomer, oligomer, protofibril and fibril (14). Aβ1–42 prepared in this study was shown to have a high proportion of fibrils (9). Recently, Lambert et al. (15) reported that pretreatment with Aβ-derived diffusible ligands (ADDLs) inhibited hippocampal LTP in vitro. ADDLs comprise only oligomers and thus are a protofibril- and fibril-free preparation with a diffusible profile. Therefore, the molecular states of Aβ should be noted in each study, and it is also necessary to clarify whether the mechanisms of Aβ’s cytotoxicity differ among its molecular states or not. Although there are few reports about the impairment of LTP by pretreatment with Aβ so far (15), the model of pretreatment with Aβ is now available. Their and our experimental protocols are important because a fast screening tool for Aβ inhibitors is indispensable for the development of new therapeutic drugs for AD.

In conclusion, we have demonstrated that glutamate exacerbates Aβ1–42-induced impairment of LTP in rat hippocampal slices. This result supports the concept that Aβ acts as a potent neurotoxin, especially under the condition in which excitatory amino acids are abundant in extracellular space. Elucidation of the cascade induced by Aβ and excitatory amino acids would contribute to further understanding of the molecular mechanisms underlying Aβ-induced neurodegeneration in AD.

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