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The inhibitory and facilitatory actions of amyloid-β peptides on nicotinic ACh receptors and AMPA receptors

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Abstract

The present study investigated the effects of amyloid-β peptides on nicotinic ACh receptors (*Torpedo*, α4β2, and α7 receptors) and AMPA receptors expressed in *Xenopus* oocytes by monitoring whole-cell membrane currents. Ten-minutes treatment with amyloid-β_{1-42} (1 μM) inhibited *Torpedo* ACh receptor currents, reaching 53% of original levels 30 min after treatment. Amyloid-β_{1-40} inhibited the currents in a dose-dependent manner (0.1–10 μM) during treatment, gradually reversing after treatment. Amyloid-β_{1-40} and amyloid-β_{1-42} (0.1 μM) depressed α4β2 receptor currents to each 69% and 62% of original levels at 10-min treatment and lesser depression was obtained with α7 receptors. Amyloid-β_{1-42} (0.1 μM) did not significantly inhibit AMPA receptor currents, but amyloid-β_{1-40} (0.1 μM) potentiated the currents to 145–191% of original levels. Amyloid-β peptides, thus, exert their diverse actions on nicotinic ACh receptors and AMPA receptors, and the inhibitory actions on nicotinic ACh receptors may account for the deterioration of learning and memory in Alzheimer's disease. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Amyloid-β peptide; Nicotinic ACh receptor; *Torpedo* ACh receptor; α4β2 Receptor; α7 Receptor; AMPA receptor; *Xenopus* oocyte; Voltage-clamp

Of particular interest are studies focused upon dementia associated with neurodegenerative diseases, such as Alzheimer's disease. Lines of evidence have pointed to the disruption of the cholinergic systems in Alzheimer's disease since before [1–3]; however, it has been not fully understood why the disruption of the cholinergic systems impairs learning and memory. In explanation of this, we earlier found that nicotinic acetylcholine (ACh) receptors, which as well as muscarinic ACh receptors form the cholinergic systems, are required for the expression of long-term potentiation (LTP), a cellular model of learning and memory, i.e., nicotinic ACh receptors are downstream of *N*-methyl-D-aspartate (NMDA) receptor signal in the LTP pathway [4]. Dysfunction or afunction of nicotinic ACh receptors, therefore, would result in the deficits of learning and memory. The question to address is as to what factors are endowed with the perturbation of nicotinic ACh receptor functions in Alzheimer's disease. Then, we hypothesized that amyloid-β peptides, which play a crucial role in Alzheimer's disease [5–7], might inhibit nicotinic ACh receptor activities.

To obtain evidence for this, the present study investigated the effects of amyloid-β_{1-40} and amyloid-β_{1-42} on *Torpedo* ACh receptors, the brain type nicotinic ACh receptors, α4β2 and α7 receptors, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, composed of the GluR1, GluR2, and GluR3 subunits, expressed in *Xenopus* oocytes, by monitoring whole-cell membrane currents. The results of the present study demonstrate that amyloid-β peptides exert their inhibitory and facilitatory actions on nicotinic ACh receptors and AMPA receptors.

Materials and methods

In vitro transcription and translation in Xenopus oocytes. mRNAs coding for the *Torpedo* ACh receptors α, β, γ, and δ subunit; the rat

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neuronal nicotinic ACh receptors α4β2 and α7 subunit; and the AMPA receptors GluR1, GluR2, and GluR3 subunit were synthesized by in vitro transcription, as described previously [8]. *Xenopus* oocytes were manually separated from the ovary and injected with the α, β, γ, and δ subunit mRNAs; the α4 and β2 subunit mRNAs; the α7 subunit mRNAs; or the GluR1, GluR2, and GluR3 subunit mRNAs.

Two-electrode voltage-clamp recording. Oocytes were transferred to a recording chamber 2–7 days after injection and continuously superfused at room temperature (20–22 °C) in standard frog Ringer's solution (in mM: 115 NaCl, 2 KCl, 1.8 $CaCl_2$, and 5 HEPES, pH 7.0). Two-electrode voltage-clamp was made to oocytes, and ACh (100 μM) or kainate (100 μM), an agonist of AMPA receptor, was bath-applied to oocytes in the presence and absence of amyloid-β peptides. Whole-cell membrane currents were recorded using a GeneClamp 500 amplifier (Axon Instruments) and analyzed by using a pClamp software (Axon Instruments, version 6.0.3).

Results

Effects of amyloid-β peptides on Torpedo ACh receptor responses

We initially examined the effect of amyloid-β peptides on *Torpedo* ACh receptors. Amyloid-β_{1-40} depressed ACh-evoked currents to 85 ± 6% of original levels for 0.1 μM, 64 ± 8% for 1 μM, and 59 ± 10% for 10 μM at 10-min treatment, reversing to 98 ± 38%, 69 ± 8%, and 76 ± 12%, respectively, at 30-min washing-out of the peptide (Fig. 1A). A transient potentiation was found after treatment with 0.1 μM, but it was not significant (Fig. 1A). Amyloid-β_{1-42} gradually depressed the currents, reaching 73 ± 13% of original levels for 0.1 μM, 53 ± 6% for 1 μM, and 73 ± 8% for 10 μM 30 min after 10-min treatment (Fig. 1B). It is indicated from these results that amyloid-β peptides inhibit nicotinic ACh receptor activities, but in a different manner between amyloid-β_{1-40} and amyloid-β_{1-42}.

Effects of amyloid-β peptides on responses of α4β2 and α7 receptors

Of neuronal nicotinic ACh receptors, α4β2 and α7 receptors, are dominantly expressed in the brain [9]. We next, therefore, examined the effects of amyloid-β peptides on those receptors. Amyloid-β_{1-40} and amyloid-β_{1-42} at a concentration of 0.1 μM depressed α4β2 receptor currents to 69 ± 6% and 62 ± 6% of original levels, respectively, at 10-min treatment, but instead the following washing-out of the peptides potentiated the currents (113 ± 14% of original levels for amyloid-β_{1-40} and 107 ± 11% for amyloid-β_{1-42} 20 min after treatment, not significant) (Fig. 2A). For α7 receptors, amyloid-β_{1-40} and amyloid-β_{1-42} (each 0.1 μM) gradually depressed ACh-gated channel currents, reaching 82 ± 8% and 92 ± 3% of original levels, respectively, 20 min after 10-min treatment (Fig. 2B). These results indicate that amyloid-β peptides inhibit ACh receptor activities for the neuron type as well.

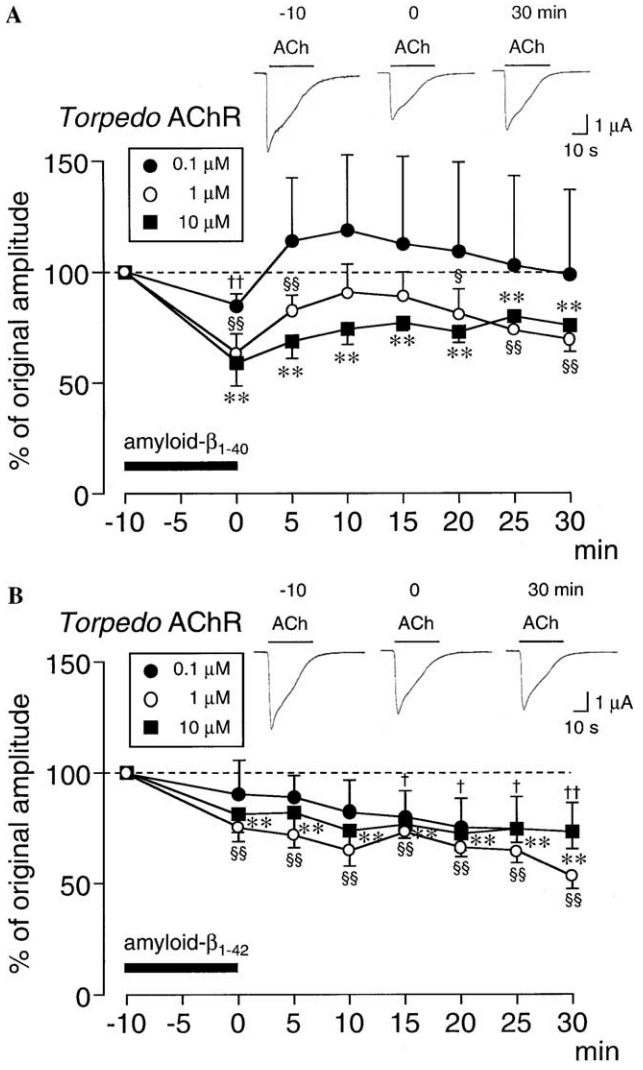

Fig. 1. Effects of amyloid-β peptides on *Torpedo* ACh receptors. Two-electrode voltage-clamp was made to oocytes expressing *Torpedo* ACh receptors. ACh (100 μM) was applied to a single oocyte for 30 s before and after 10-min treatment with amyloid-β_{1-40} (A) and amyloid-β_{1-42} (B) at concentrations as indicated. The currents illustrated were recorded −10, 0, and 30 min. In the graph, each data point represents the mean percentage of the original amplitude (−10 min) from 5 oocytes and the SD is indicated by the bars. $^{\S}P < 0.1$, $^{**,\S\S,\dagger\dagger}P < 0.01$, Student's *t* test.

Effects of amyloid-β peptides on AMPA receptor responses

AMPA receptors mediate a large part of excitatory synaptic transmission and LTP expression in the hippocampus [10]. If amyloid-β peptides inhibit AMPA receptor activities, then the action could also account for the deterioration of learning and memory in Alzheimer's disease. This prompted a final set of experiments to examine the effects of amyloid-β peptides on AMPA receptors. Amyloid-β_{1-42} (0.1 μM) depressed AMPA receptor currents, but not significantly (Fig. 3).

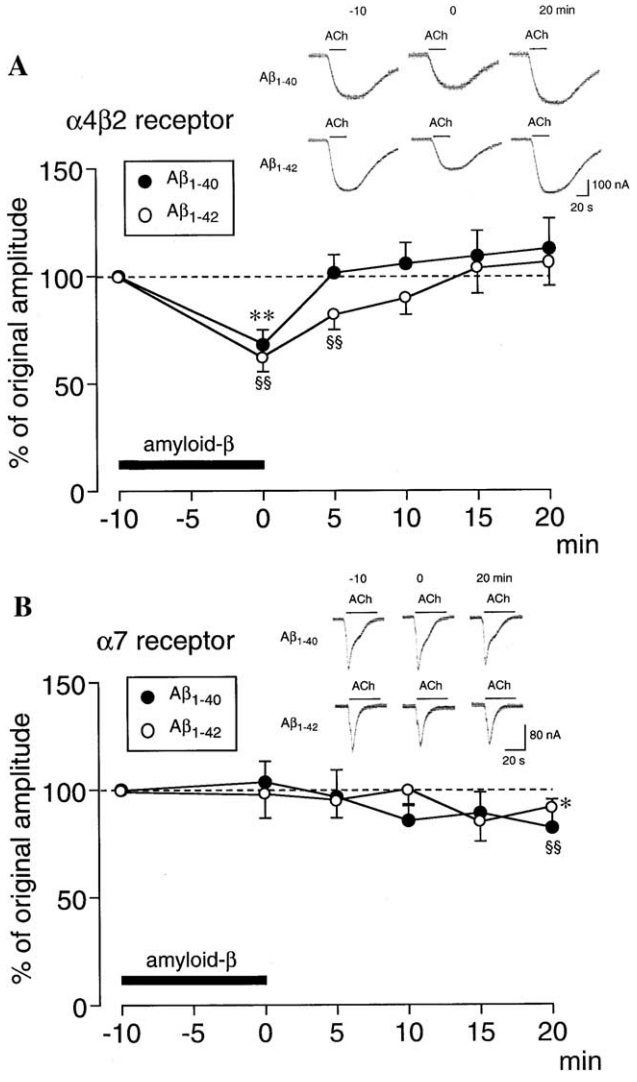

Fig. 2. Effects of amyloid-β peptides on α4β2 (A) and α7 receptors (B). ACh (100 μM) was applied to a single oocyte for 30 s before and after 10-min treatment with amyloid-β_{1-40} and amyloid-β_{1-42} at a concentration of 0.1 μM. The currents illustrated were recorded −10, 0, and 20 min. In the graph, each data point represents the mean percentage of the original amplitude (−10 min) from 5 oocytes and the SD is indicated by the bars. $^{*}P < 0.1$, $^{**,\S\S}P < 0.01$, Student's t test.

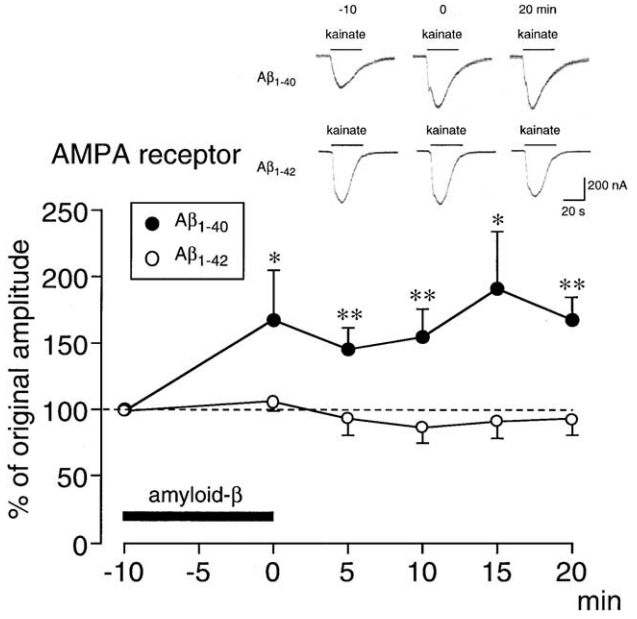

Fig. 3. Effects of amyloid-β peptides on AMPA receptors. Kainate (100 μM) was applied to an oocyte expressing the GluR1, GluR2, and GluR3 subunits for 30 s before and after 10-min treatment with amyloid-β_{1-40} and amyloid-β_{1-42} at a concentration of 0.1 μM. The currents illustrated were recorded −10, 0, and 20 min. In the graph, each data point represents the mean percentage of the original amplitude (−10 min) from 5 oocytes and the SD is indicated by the bars. $^{*}P < 0.1$, $^{**}P < 0.01$, Student's t test.

Unexpectedly, amyloid-β_{1-40} (0.1 μM) potentiated AMPA receptor currents to 145–191% of original levels, the effect being evident 20 min after 10-min treatment (Fig. 3). The effects of amyloid-β peptides on AMPA receptor responses, thus, do not interpret learning and memory disturbance in Alzheimer's disease.

Discussions

Amyloid-β peptides, likely a major factor for Alzheimer's disease [5–7], reveal neurotoxic effects by their aggregation and deposit. Severe atrophy in the fronto-temporal lobe, i.e., neuronal cell death, is found in the progressive stage of Alzheimer's disease. The neurotoxic effects of amyloid-β peptides could cause neuronal cell death in the disease. Dementia, however, occurs from the earlier stage of Alzheimer's disease. This suggests that amyloid-β peptides have a role in the deterioration of the cognitive functions except for the neurotoxic actions.

Nicotinic ACh receptors in the brain are preferentially localized on presynaptic terminals and stimulate neurotransmitter release [9,11]. In our earlier studies, LTP is expressed by activating presynaptic nicotinic ACh receptors in an NMDA receptor-independent manner [4], and the agent targeting nicotinic ACh receptors is capable of inducing an 'LTP'-like long-lasting facilitation of hippocampal neurotransmission [8]. These findings provide evidence that nicotinic ACh receptors are closely related to the cognitive function. Inhibiting nicotinic ACh receptor activity, therefore, would impair learning and memory. Studies have shown that amyloid-β_{1-42} binds selectively to neuronal nicotinic ACh receptors with high affinity and inhibits nicotinic ACh receptor responses in brain tissues, hippocampal slices, and cultured hippocampal neurons [12–15]. In the present study, amyloid-β peptides depressed activities of the nicotinic ACh receptors, *Torpedo* ACh receptor, α4β2 receptor, and α7 receptor, but

to a different extent and in a different manner among the receptors, suggesting that nicotinic ACh receptors have a common binding site for amyloid-β peptides. Of amyloid-β peptides, amyloid-β_{1-42} is thought to be a key factor for the pathogenesis of Alzheimer's disease. Interestingly, amyloid-β_{1-40} inhibited responses of the neuronal nicotinic ACh receptors, α4β2 receptor and α7 receptor, to an extent similar to that induced by amyloid-β_{1-42}, suggesting that amyloid-β_{1-40} also bears a part in the deterioration of the cognitive function. The inhibitory action of amyloid-β peptides on nicotinic ACh receptors, thus, may cause dementia in Alzheimer's disease.

Amyloid-β_{1-42} did not significantly depressed AMPA receptor responses, but otherwise amyloid-β_{1-40} significantly and persistently potentiated AMPA receptor responses. If this is true, amyloid-β_{1-40} should affect LTP expression; the peptides could serve as a cognition-enhancer. Indeed, amyloid-β_{1--40} is shown to enhance an early phase of LTP in rat hippocampal slices [16]. In contrast, a study shows that amyloid-β_{1-40} and amyloid-β_{1-42} inhibit the duration of potentiation of synaptic transmission after high frequency stimulation, to induce LTP, without affecting basal synaptic transmission in the intact rat hippocampus [17]. The effect of amyloid-β peptides on LTP, thus, is under discussion. It is presently unknown about the effects of amyloid-β peptides on LTP as mediated via AMPA receptors and their role in Alzheimer's disease. The results here, however, suggest that AMPA receptors also have a specific binding site for amyloid-β peptides.

In conclusion, the results of the present study demonstrate that both of amyloid-β_{1-40} and amyloid-β_{1-42} inhibit nicotinic ACh receptor responses in a different manner, but that otherwise, amyloid-β_{1-40} potentiates AMPA receptor responses. The inhibitory action of amyloid-β peptides on nicotinic ACh receptors may represent the mechanism underlying the impairment of learning and memory in Alzheimer's disease.

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