

Zinc-induced aggregation of A β (10–21) potentiates its action on voltage-gated potassium channel

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Abstract

Zinc may play an important role in the pathogenesis of Alzheimer's disease (AD) through influencing the conformation and neurotoxicity of amyloid β -proteins (A β). Zn²⁺ induces rapid aggregation of synthetic or endogenous A β in a pH-dependent fashion. Here we show for the first time that Zn²⁺-induced aggregation of A β (10–21) potentiates its action on outward potassium currents in hippocampal CA1 pyramidal neurons. Using the whole-cell voltage-clamp technique, we showed that A β (10–21) blocked the fast-inactivating outward potassium current (I_A) in a concentration- and aggregation-dependent manner, but with no effect on the delayed rectifier potassium current (I_K). Both the unaggregated and aggregated forms of A β (10–21) significantly shifted the activation curve and the inactivation curve of I_A to more negative potentials. But the aggregated form has more effects than the unaggregated form. These data indicated that aggregation of amyloid fragments by zinc ions is required in order to obtain full modulatory effects on potassium channel currents. © 2006 Elsevier Inc. All rights reserved.

Keywords: Alzheimer's disease; β -Amyloid; Hippocampus; Patch-clamp; Potassium channel

Alzheimer's disease (AD) is one of the most common diseases of modern society. Affecting 10% of the world's population, this progressive neurodegenerative disorder causes untold human suffering and consumes more than 100 billion US \$ per year in health-care costs [1]. The main symptoms for AD are the presence of senile plaques in the brain and intraneuronal neurofibrillary tangles [2]. The major components of brain amyloid plaques are peptides, 39–42 residues long, termed amyloid β -proteins (A β). A β is present in AD brain in both soluble and insoluble forms [3] and interacts with a variety of materials. The extent of deposition of insoluble amyloid in AD correlates with the degree of neuronal damage, cognitive impairment, and memory loss [4]. Factors that influence the aggregation state of A β in vivo include pH [5], peptide concentration [6], incubation time [7], and the presence of certain metal ions [8–10]. Zinc and copper ions have been implicated in

both A β aggregation and neurotoxicity. High concentrations of zinc have been found in neurons in regions of the brain that are vulnerable in AD, such as cortex and the hippocampus. Up to 300 μ M Zn²⁺ is released into the extracellular space during neurotransmission in the hippocampus [10], which may be sufficient to induce aggregation of A β .

We propose that A β aggregation by Zn²⁺ may be a response to its neurotoxicity. It has shown that the C-terminal amino acids are crucial for A β aggregation in aqueous solution. This C-terminal region contains only hydrophobic amino acids and would be expected to increase both β -sheet and aggregation propensity by increasing hydrophobicity. The side chains within this region would not be expected to be involved in metal coordination due to the absence of charged residues [10]. Metal-induced aggregation of A β appears to be initiated by metal coordination with charged residues within the amino acid region 6–28 of A β [11]. While the histidine residues at positions 6, 13, and 14 of full-length A β (1–42) have been

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implicated in metal binding, 13 and 14 appear to be most critical. In this paper, a shortened variant, A β (10–21), was studied to expand our previous work [12]. This peptide maintains the overall amphiphilic distribution of amino acids, polar N-terminal and nonpolar C-terminal residues that exist in A β (1–42), found to be critical for the self-assembly of A β (10–35) [13], and has been studied as a model for zinc-induced aggregation of A β [12,13].

Increasing evidences suggest that voltage-gated potassium channels may play a crucial role in neurodegenerative process. Two studies have shown that A β can cause apoptotic cell death via an increase in the delayed rectifier K⁺ channel current [14,15]. In contrast to these two studies, Chen [16] and Ye et al. [17] reported that A β inhibit specially A-type K⁺ current but not other outward or inward rectifying K⁺ channels. All peptides used in the earlier studies were aggregated by higher temperatures and longer incubation times and no experiments with the Zn²⁺-induced aggregated form of A β were carried out. Since zinc may accumulate in AD hippocampus with aging and influence the conformation and neurotoxicity of A β , we investigated the potential effects of Zn²⁺-induced aggregated form of A β (10–21) on outward potassium channels in hippocampal CA1 pyramidal neurons.

Methods

Hippocampal slice preparation. Hippocampal CA1 neurons were acutely isolated by enzymatic digestion and mechanical dispersion from 7-day-old Wistar rats as previously described [18]. In brief, hippocampal CA1 region was cut into 300–500 μ m thick slices and incubated for 30–40 min at 32 °C in artificial cerebrospinal solution (ACS) containing (in mM): NaCl 124, KCl 5, KH₂PO₄ 1.2, MgSO₄ 1.3, CaCl₂ 2.4, glucose 10, and NaHCO₃ 26, pH 7.4, and successively transferred into ACS containing 0.5 mg/ml protease (Sigma) and digested at 32 °C for 35 min. Throughout the entire procedure the media were continuously saturated with a 95% O₂–5% CO₂ gas mixture to maintain a pH of 7.4. After digestion, the tissue pieces were washed 3–4 times with ACS and neurons were triturated through a series of fire-polished glass pipettes with opening diameter from 0.1 to 0.5 mm. The cell suspension was transferred into a 35 mm culture dish, filled with 1.5 ml external solution containing (in mM): NaCl 150, KCl 5, MgCl₂ 1.1, CaCl₂ 2.6, glucose 10, and Hepes 10, pH 7.4. Twenty minutes later, the neurons were attached to the bottom of the culture dish and were ready for experiments. The pyramidal neurons with a diameter of 15–30 μ m were identified by their characteristic bright pyramid-shaped soma under a phase contrast microscope and two or three short-branched dendrites and a long axon. Neurons with bright and smooth appearance and no visible organelles were selected for recording [19]. All experiments conformed to local and international guidelines on ethical use of animals and all efforts were made to minimize the number of animal used and suffering.

Potassium currents' recording technique. Whole-cell currents were recorded with Axopatch 200B patch-clamp amplifier (Axon Instrument, USA), filtered at 2 kHz, digitized at 5 kHz, and stored in a PC 486 computer using digidata 1200B interface (Axon Instrument, USA) and pCLAMP version 6.0.4 software (Axon Instrument, USA). The patch electrodes (BJ-40, diameter 1.5 \pm 0.1 mm, Beijing) were pulled in two steps by a microelectrode puller (PP-830, Narrishage, Japan) and had tip resistance of 5–8 M Ω when filled with pipette solution containing (in mM): KCl 65, KOH 5, KF 80, MgCl₂ 2, Hepes 10, EGTA 10, and Na₂ATP 2, pH 7.3. Liquid junction potential between the pipette solution and external solution was corrected after the pipette tipped into the external solution. After forming a conventional "gigaseal", the membrane was

ruptured with a gentle suction to obtain the whole-cell voltage-clamp configuration. To minimize the duration of capacitive current, membrane capacitance and series resistance were compensated after membrane rupture. Total outward potassium current (I_{TOTAL}) stimulated with 150 ms depolarizing pulses from –50 mV to +60 mV in 10 mV steps following a hyperpolarizing prepulse of 400 ms to –110 mV. Delayed rectifier potassium current (I_K) was elicited by a similar protocol in which a 50 ms interval at –50 mV was inserted stimulated after the prepulse. Fast-inactivating outward potassium current (I_A) was obtained by subtracting current traces of I_K from those of I_{TOTAL} . To record potassium current, 1 μ M tetrodotoxin and 0.2 mM CdCl₂ were added to external solution before the electrophysiological recording. All experiments were carried out at room temperature (22–25 °C) within 6 h after isolation of rat hippocampus.

Analysis of electrophysiological recording. Current recording was analyzed using the pCLAMP CLAMPFIT procedures (Axon Instrument, USA) following leak subtraction using a P/5 subtraction protocol. Further analyses were performed using Microsoft Excel 97 and Origin 5.0 (Microcal Software, USA). All data are given as means \pm SD. Student's *t*-test was used to determine the significance of differences between the means, with *P*-values of less than 0.05 considered significant.

Application of amyloid β protein. A β (10–21) was purchased from Chinese Peptide Company (China). Stock peptide was stored as powder at –70 °C. Stock solution of A β (10–21) was prepared by dissolving the peptides at a concentration of 1 mM in deoxygenated, deionized water, aliquoted to 100 μ l, and stored at –20 °C until needed. The concentration of peptide was determined from the UV absorption intensity of tyrosine ($\epsilon_{275} = 1410 \text{ M}^{-1} \text{ cm}^{-1}$) at pH 7.4 [20]. In most experiments, unaggregated A β (10–21) was added to bath solution for external application. Peptide actions were measured only after steady-state conditions were reached, which were judged by the amplitudes and time courses of currents remaining constant with further perfusion of peptide. To test the effects of peptide aggregation on neuronal response, samples were prepared from peptide stock solution by dilution with 20 mM sodium phosphate buffer (pH 7.4) containing 150 mM NaCl. For metal-induced aggregation experiments, Zn²⁺ was added from a 0.1 M metal stock solution. The final A β (10–21) concentration was 100 μ M, containing 100 μ M or 200 μ M Zn²⁺. Samples were incubated for 24 h (5% CO₂, 37 °C), after which insoluble aggregates were removed by centrifugation at 13,000g for 10 min. The ratio of A β (10–21) concentrations, as determined by fluorescamine assay, of the supernatant relative to total peptide was used to calculate the levels of aggregation [10]. Control experiments were performed in parallel without the addition of Zn²⁺ and/or centrifugation.

Results

Separation of I_A and I_K

Step depolarization to potential from –50 mV to +60 mV (10 mV steps) following a hyperpolarizing prepulse of 400 ms to –110 mV clearly activated two components of outward current (Fig. 1A). First, a rapidly activating and inactivating current, sensitive to 4-aminopyridine, referred to as I_A ; and then a delayed rectifier current, sensitive to tetraethylammonium, named as I_K . To study the effects of lithium chloride on I_A and I_K , respectively, a signal subtraction procedure was used to separate I_K from the I_{TOTAL} . Previous studies have demonstrated that I_A undergoes steady-state inactivation at holding potential positive than –50 mV, therefore, I_K could be activated nearly uncontaminated with I_A by holding at –50 mV and stepping to more positive potentials. With this protocol the I_K was activated slowly to a plateau with minimal time-dependent inactivation (Fig. 1B). Subtraction of

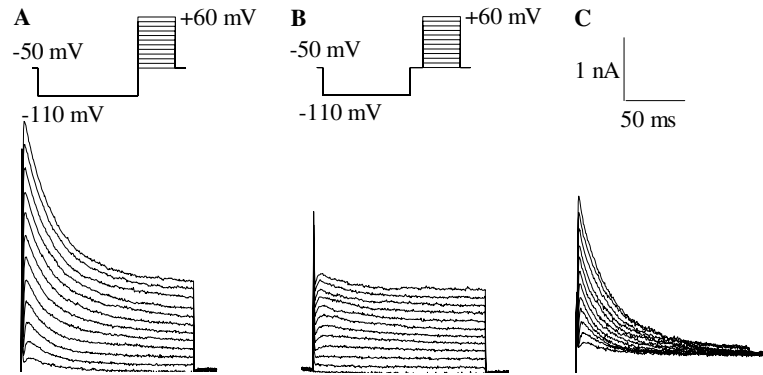


Fig. 1. Outward potassium current families in a hippocampal pyramidal neuron. (A) Total outward potassium current stimulated with 150 ms depolarizing pulses from -50 mV to $+60$ mV in 10 mV steps following a hyperpolarizing prepulse of 400 ms to -110 mV (inset). (B) Delayed rectifier potassium current (I_K) stimulated with similar protocol as in (A), except for a 50 ms interval at -50 mV, was inserted after the prepulse (inset). (C) Isolated fast-inactivating outward potassium current (I_A) by subtracting current traces of (B) from those of (A).

I_K from I_{TOTAL} revealed I_A (Fig. 1C). Currents at the end of the depolarizing pulse were referred to as I_K . The peak of the subtracted currents was referred to as I_A [21].

Effects of $A\beta$ (10–21) on I_A and I_K

Bath application of $A\beta$ (10–21) caused a decrease in the amplitude of I_A (Fig. 2). This decrease reversed upon the peptide washout (data not shown). However, the decrease in I_A developed slowly and 15 min of bath application was necessary before reaching a steady state, an effect which may be attributed to the slow penetration of $A\beta$ in the slice or into the cells. The effect of unaggregated $A\beta$ (10–21) on I_A was concentration-dependent (Fig. 3). At the concentration of 50 μ M, $A\beta$ (10–21) inhibited I_A by $36.92 \pm 1.18\%$ ($n = 6$, $P < 0.01$) at 60 mV when depolarized from -50 mV. At the concentration of 1 μ M or below there was no effect of $A\beta$ (10–21) on I_A . In the aggregated form, 10 μ M $A\beta$ (10–21) reduced I_A by $48.93 \pm 3.45\%$ ($n = 5$, $P < 0.01$) in the acute application, indicating that aggregation of $A\beta$ enhances its modulatory effects on potassium channel currents in hippocampal neurons. In contrast to their action on I_A , both unaggregated and aggregated $A\beta$ (10–21) had no effect on I_K amplitude. These results suggested that $A\beta$ (10–21) selectively blocked the I_A .

Fig. 4A shows current–voltage (I – V) curves of I_A generated by depolarizing steps from a holding potential of

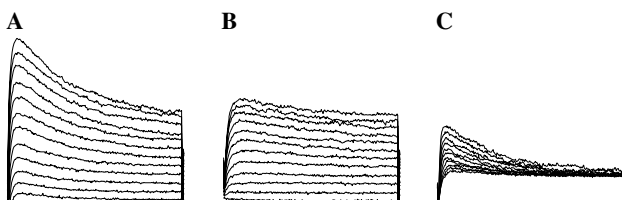


Fig. 2. Effects of $A\beta$ (10–21) on voltage-dependent potassium currents. Representative recording from a responding cell of total (A), delayed rectifier (B), and fast-inactivating (C) potassium currents after unaggregated $A\beta$ (10–21) (10 μ M) application. Pulse protocols and subtraction procedure were same as those in Fig. 1.

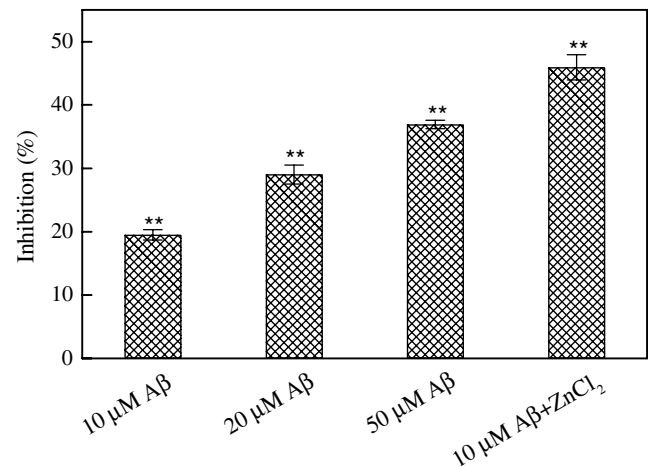


Fig. 3. $A\beta$ (10–21) decreased I_A in a concentration- and aggregation-dependent manner. The percentage inhibition was defined as $(I_{CONTROL} - I_{A\beta})/I_{CONTROL}$ and plotted as a function of $A\beta$ (10–21) concentration. Each point represents mean \pm SD ($n = 6$). ** $P < 0.01$.

-50 mV to $+60$ mV with a 10 mV increment. In the presence of 10 μ M $A\beta$ (10–21), the amplitude of I_A was significantly decreased at the test potentials between 10 mV and 60 mV ($n = 6$, $P < 0.01$). The percent inhibition of I_A upon $A\beta$ (10–21) application was not a function of the depolarizing potential (Fig. 4B), indicating that $A\beta$ (10–21) does not sense the electric field in the pore.

Effect of $A\beta$ (10–21) on the activation kinetics of I_A

The steady-state activation curves for I_A under control and after exposure to unaggregated or aggregated $A\beta$ (10–21) are shown in Fig. 5. I_A was converted into conductance by use of the equation $G = I/(V - V_K)$, where G is conductance, V is membrane potential, and V_K is reversal potential (calculated as -87 mV for the potassium concentrations used). With the least-squares fit procedure, the normalized conductance was well fitted with a Boltzmann equation: $G/G_{max} = 1/1 + \exp[-(V - V_h)/k]$, where G_{max} is the maximum conductance, V is membrane potential,

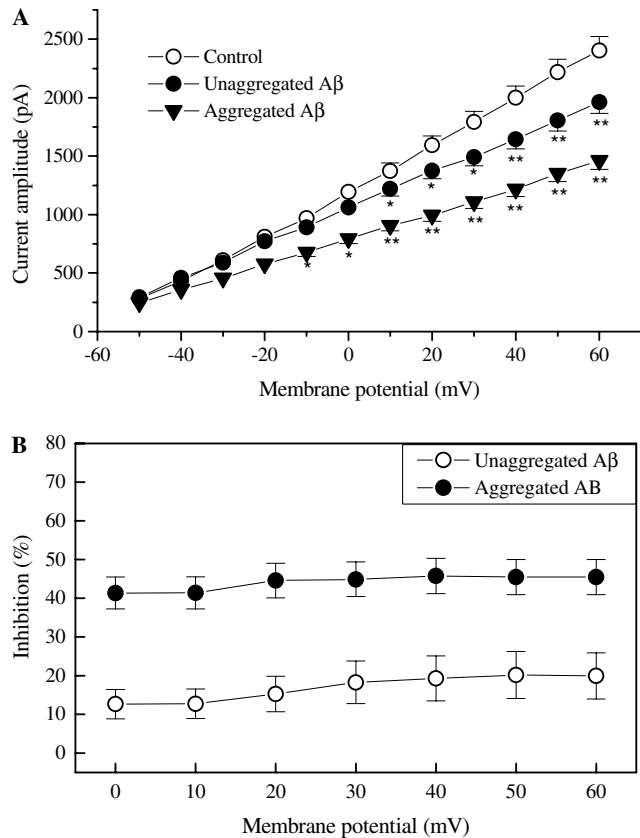


Fig. 4. Effects of A β (10–21) on current–voltage relationships of I_A . (A) Current–voltage relationships of I_A in the control (○), in the presence of 10 μ M unaggregated A β (10–21) (●) and aggregated A β (10–21) (▼). (B) Percentage inhibition in I_A caused by A β (10–21) application as a function of depolarizing potentials. Each point represents mean \pm SD ($n = 5$). * $P < 0.05$, ** $P < 0.01$ vs. control.

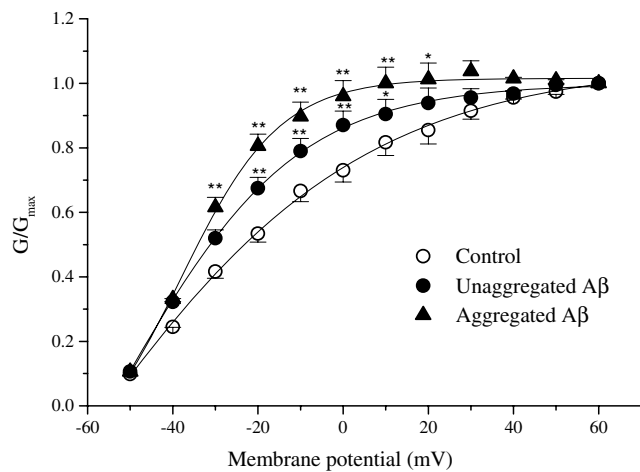


Fig. 5. Effects of A β (10–21) on the steady-state activation curves of I_A . The amplitudes of I_A are converted to conductance and are normalized by maximal conductance. The data were fitted with a Boltzmann equation: $G/G_{\max} = 1/[1 + \exp[-(V - V_h)/k]]$. Each point represents mean \pm SD ($n = 6$). * $P < 0.05$, ** $P < 0.01$ vs. control.

V_h is the voltage at which half-maximal effect is obtained, and k is the slope factor. During control conditions, average V_h was -20.31 ± 1.87 mV and k was 34.68 ± 4.66 mV.

After the addition of 10 μ M unaggregated A β (10–21), the activation curve was shifted to negative potentials: V_h was -30.09 ± 2.93 mV ($n = 6$, $P < 0.01$) and k was 20.49 ± 1.30 mV ($n = 6$, $P < 0.01$). Ten micromolar aggregated A β (10–21) shifted the activation curve to more negative potentials: V_h was -32.26 ± 2.05 mV ($n = 6$, $P < 0.01$) and k was 11.68 ± 0.92 mV ($n = 6$, $P < 0.01$).

Effect of A β (10–21) on the inactivation kinetics of I_A

The steady-state inactivation was examined by changing the prepulse potentials at levels between -120 and -10 mV (80 ms duration) before depolarization to a test pulse of $+50$ mV (duration of 120 ms). Selected I_A inactivation traces from a typical experiment are shown in Fig. 6A. The inactivation curves shown in Fig. 6B were obtained by plotting the normalized I_A against the prepulse potentials. The curves were well fitted with a Boltzmann equation: $I/I_{\max} = 1/[1 + \exp[(V - V_h)/k]]$, where V is the prepulse potential, V_h is potential where normalized I was reduced to one-half, and k is the slope factor. Before application of A β (10–21), the value of V_h was -55.13 ± 0.56 mV and k was 10.06 ± 0.51 mV. A β (10–21) (10 μ M) caused a negative shift of the inactivation curve along the potential

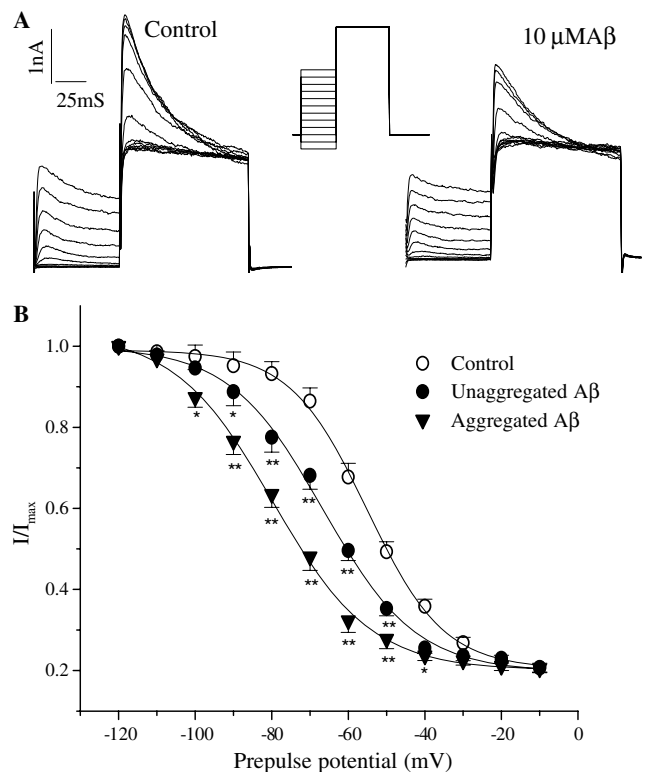


Fig. 6. Effects of A β (10–21) on the steady-state inactivation curves of I_A . (A) Inactivation current traces in control (left traces) and 10 μ M A β (10–21) treated (right traces) examined with a double-pulse protocol (inset). (B) Peak amplitudes for I_A currents were normalized and plotted versus prepulse potentials. The curves were fitted with a Boltzmann equation: $I/I_{\max} = 1/[1 + \exp[(V - V_h)/k]]$. Each point represents mean \pm SD ($n = 6$). * $P < 0.05$, ** $P < 0.01$ vs. control.

axis. The values of V_h for unaggregated and aggregated A β (10–21) were -66.63 ± 1.91 mV ($n = 6$) and -79.73 ± 0.86 mV ($n = 6$, $P < 0.01$), with k of 12.20 ± 0.89 mV ($n = 6$) and 12.89 ± 0.78 mV ($n = 6$, $P > 0.05$), respectively. A β (10–21)-induced shift of V_h was statistically significant but k remained unchanged.

Discussion

Although A β (1–42) and A β (1–39) are generally considered the appropriate models for use in AD, both of them are not suitable for the zinc-induced aggregation of A β since they can aggregate in the absence of Zn^{2+} . A β (25–35) is not to be involved in Zn^{2+} coordination due to the absence of the most important charged residues—histidine residues. Zn^{2+} -induced aggregation of A β appears to be initiated by Zn^{2+} coordination with histidine residues at positions 13 and 14 of full-length A β (1–42). It has been shown that Zn^{2+} binds to the N_τ atom of the histidine imidazole ring and the peptide aggregates through intermolecular His (N_τ)- Zn^{2+} -His (N_τ) bridge [20]. For the above reasons, we choose A β (10–21) as a model for use in Zn^{2+} -induced aggregation of A β . The value of A β (10–21) as an in vitro model has been strengthened by recent works [12,13] and indeed formed amyloid in the presence of Zn^{2+} . In the present experiment, A β (10–21) showed no β -sheet structure in aqueous solution and did not aggregate significantly in the absence of Zn^{2+} . Incubation of A β (10–21) at concentrations of 10 and 100 μ M at pH 7.4 for 1 week showed less than 10% aggregation at both concentrations (8.1% for 10 μ M, 9.8% for 100 μ M). This is consistent with previous results where C-terminal amino acids have been shown to be crucial for A β aggregation in aqueous solution. In the presence of Zn^{2+} , A β (10–21) aggregated rapidly through intermolecular His13 (N_τ)- Zn^{2+} -His14 (N_τ) bridge [12]. Both the unaggregated and aggregated forms of A β (10–21) modulate potassium channel activity, but the aggregated form has great effects: (1) aggregated A β (10–21) had a larger block effect on I_A than unaggregated A β (10–21); (2) aggregated form shifted the activation curve of I_A to more negative potentials; (3) aggregated form shifted the inactivation curve of I_A to more negative potentials. Our experiment is consistent with Ye et al. [17] who show that A β blocked potassium channel was aggregation dependent. However, we disagree with Good et al. [22] who show that K^+ current inhibition was independent of aggregation. A neurotoxic disruption of intracellular ion homeostasis may be the result of an action by A β on voltage-gated ion channel activity. Neurotoxicity appears to require A β to be in the form of β -sheet aggregates, although the degree of aggregation of the neurotoxic species is unknown. The hypothesis that aggregation of amyloid fragments is required in order to obtain full neurotoxic effects has been followed up and confirmed by many researchers [23–25]. The reason why some studies showed negative results was explained by the fact that the aggregated A β was induced by different factors.

Previous studies have shown that A β enhanced the I_K , but did not affect the I_A . These studies differed from ours in several important respects: first, there were differences in species (mouse vs. rat) and in cell types (cortical neurons vs. hippocampal neurons). Second, all peptides used in these studies were aggregated form and no experiments with unaggregated form were carried out. Third, no characterization was presented to indicate the actual assembly state of the A β . Finally, these studies did not reveal the immediate acute effect of A β interaction with neurons, which could then trigger subsequent changes in neuronal functioning.

Since a significant increase in zinc was found in hippocampus, area showing severe histopathologic alterations in AD [26], we propose that zinc may accumulate in brains with aging, and influence the conformation and neurotoxicity of A β . The pharmacological compounds, which could dissolve the aggregated A β , are possible candidate drugs for treatment of AD. Clioquinol [27] [*N,N,N,N*-tetrakis(2-pyridylmethyl)ethylenediamine] and bathocuproine [28] were reported to be effective in preventing the decline of skills of AD patients. Our results indicating the solubilization of aggregated A β by addition of these metal chelators may suggest mechanisms based on zinc-chelation therapy and a key to search for new drugs for the treatment of AD.

The mechanism by which A β (10–21) affect hippocampal potassium channels are not fully understood at this time. However, we hypothesize that there are several potential interpretations. One possibility is the electrostatic interactions between A β and potassium channel proteins. In our study, the fractional reduction in I_A upon A β (10–21) application was unaffected by changes in the depolarizing potential, implying that A β (10–21) does not sense the electric field in the pore, coming in the vicinity of the selectivity filter of potassium channel. Therefore, if A β (10–21) blocks via binding to channel proteins, it likely binds to an external site rather than inserting into the pore. Furthermore, the inhibition of I_A was obvious only at potentials more positive than 0 mV. These effects could be attributed to electrostatic interaction of A β (10–21) with the membrane [29]. Electrostatic interactions between basic residues Lys¹⁶ in the A β (10–21) with acidic residues in the potassium channel mouth have been proposed to be crucial for the affinity. Another possible mechanism might involve changes in membrane fluidity. Previous studies have indicated that membrane fluidity is altered in central and peripheral cell systems in AD [30]. A β can decrease fluidity in rat and mouse brain membranes as well as in membranes of human lymphocytes in a concentration-dependent manner [31]. It is suggested that the small changes of membrane fluidity seen in the presence of unaggregated form of A β may be sufficient to alter properties and functions of membrane-associated ionic channels. At aggregated form, A β seem to have more pronounced effects on membrane fluidity, probably by disrupting membrane integrity, and directly leading to its neurotoxic properties. A third possibility is A β was directly incorporated into neuronal membranes and that

polymerized A β formed nonselective ion channels. Evidence from a number of laboratories has demonstrated that A β peptides can form ion channels in lipid bilayers, liposomes, neurons, and endothelial cells [32,33]. But we did not detect an increase in nonselective cation conductance in neurons exposed to A β (10–21). It should be remembered that in vivo it would take a long time to accumulate enough A β peptide to form ion channel. The channel formation could not happen in our experiment in acute application of A β . Measurements of cellular properties after long-term incubation of neurons with A β are under way.

Voltage-gated potassium channels are essential to the function of all excitable cells and have been suggested to govern the discharge pattern of action potentials. The I_A is of particular importance in contributing to hippocampal dendritic membrane excitability and synaptic plasticity since there is a linear increase in its density with distance from soma to distal dendrites in hippocampal CA1 pyramidal neurons [16]. I_A and I_K contribute to membrane hyperpolarization and their inhibition should broaden the duration of the action potential [34,35]. This broadening will cause secondary changes in a number of other voltage- and Ca^{2+} -dependent currents, which also influence the firing rate and Ca^{2+} influx [17]. Very recently it has been shown that inhibition of I_A resulting from deposition of A β in hippocampal neurons causes a sustained increase in dendritic Ca^{2+} influx and lead to loss of Ca^{2+} homeostasis [16]. In addition, A β increase cell excitability and cause a rise in intracellular Ca^{2+} in hippocampal neurons [36] and that K^+ channel openers protect hippocampal neurons against oxidative injury and A β toxicity, presumably because of their ability to hyperpolarize the plasma membrane and reduce Ca^{2+} influx [37]. The level of intracellular Ca^{2+} and its dynamic regulation are critical for long-term synaptic plasticity, however, a sustained increase in intracellular Ca^{2+} can result in neuronal degeneration and cell death [38]. Therefore, potassium channel inhibition may be one of the earliest events in A β -induced neurotoxicity. It needs further studies to determine if this attenuation of specific potassium current may be responsible for the initiation of A β -induced neuronal dysfunction or cell death.

We also noticed that the maximum inhibition of A β (10–21) on I_A only reached about 50% even at aggregated form. Because voltage-activated potassium channels in rat pyramidal neurons are composed of various potassium channel subtypes such as Kv1, Kv2, Kv3, and Kv4, A β (10–21) is probably selective for some fast-inactivating potassium channel subtypes in hippocampal neurons. Additional studies will also be needed to understand the channel selectivity of A β (10–21).

In conclusion, our work demonstrates that a shortened peptide, from the beta amyloid sequence (10–21), blocks a fast-inactivating potassium current in rat hippocampal neurons. The effect of A β (10–21) on the potassium current has been potentiated by zinc, a widely known crosslinker. Our findings suggest that A β (1–42) is not the only toxic

form of A β . Although A β peptides terminating in position 39–42 are the major forms produced, it is possible that very low levels of previously undetected shorter/longer forms of A β may also exist. If they do exist, they could also be neurotoxic especially an additional insult, such as certain metal ions, is present.

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