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Amyloid-β at sublethal level impairs BDNF-induced arc expression in cortical neurons

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Abstract

Alzheimer's disease (AD) is characterized by progressive memory loss and cognitive dysfunction that probably due to a deficit in synaptic plasticity. One member of neurotrophins, brain-derived neurotrophic factor (BDNF), is known to be involved in the hippocampal long-term potentiation (LTP), a cellular model for learning and memory. Moreover, activity-regulated cytoskeleton-associated gene (*Arc*), an immediate early gene, is found to be a downstream effector of the BDNF signaling cascade. Inhibition of Arc protein synthesis impairs both the maintenance of LTP and the consolidation of long-term memory. In addition, the formation of senile plaques is a pathological feature in AD and mainly consists of the deposition of amyloid-β (Aβ), a proteolytic product of amyloid precursor protein. Several studies concerning neurobehavioral performance have suggested that Aβ at sublethal levels interfere with the signaling cascades critical for synaptic plasticity and thus lead to the cognitive impairment in early stage of AD. Whether the BDNF-mediated Arc synthesis is impaired by sublethal Aβ in early AD is still unclear. Therefore, in the present study, primary cultures of neonatal rat cortical neurons were used to evaluate the effect of sublethal Aβ on the BDNF-induced Arc protein expression. Consistent with the literature, Arc, an indicator of synaptic plasticity, was induced by BDNF (25 ng/ml) in both dose- and time-dependent manners. After treating cultures with sublethal Aβ (5 μM), a significant suppression was observed on the level of BDNF-induced Arc protein expression. This result indicates that Aβ at sublethal level impairs the BDNF-mediated signaling in cortical neurons and thus underlies the deficits of synaptic plasticity occurred at the early stage of AD before significant neuronal loss.

Keywords: Synaptic plasticity; BDNF; Arc; Alzheimer's disease; Amyloid-β

Activity-dependent modification of synapses, also known as synaptic plasticity, underlies learning and memory [2,24]. It is well known that neurotrophins serve as important regulators of synaptic plasticity [31]. The most extensively studied member of neurotrophin family is brain-derived neurotrophic factor (BDNF). The signaling cascade initiated by BDNF leads to the modification of synaptic efficacy [25]. Previous studies have demonstrated that enhancing synaptic transmission or neuronal excitability induces the BDNF-mediated modification of synaptic function [5,14]. A typical example is the hippocampal long-term potentiation (LTP), evoked by high-frequency afferent stimulation and served as a cellular model for learning and memory [25,39]. Hippocampal LTP is divided into an early phase, dependent on modifications of existing proteins, and a late phase, requiring new synthesis of mRNA and protein [13,34]. Thus, late phase LTP is thought to correspond to some forms of long-term memory consolidation because of the requirement for de novo protein synthesis for both processes [7]. Furthermore, neurons are known to modify their synaptic strength through activating the expression of specific genes. An immediate early gene named activity-regulated cytoskeleton-associated gene (*Arc*) is found recently to be involved in BDNF-mediated modification of synapses [19,41]. Previous studies have found that *Arc* mRNA is rapidly transported to dendrites and translated [35]. Inhibition of Arc protein synthesis impairs both the maintenance of LTP and the consolidation of long-term memory [8]. In addition, a decline in Arc expression level was correlated with the occurrence of cognitive impairment during aging [1] and in transgenic mice carrying genes responsible for Alzheimer's disease (AD) [4].

AD, a neurodegenerative disorder of the human brain rendering the loss of memory and cognitive abilities, is characterized by the progressive formation of senile plaques [20]. The deposition

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of amyloid-β (Aβ), a proteolytic product of amyloid precursor protein, has been shown to be a key pathological feature in the formation of senile plaques [33]. However, in certain animal models, impaired behavioral performance or defective synaptic transmission preceded abundant amyloid plaque formation [11,12]. Corresponding results were also found in studies made on postmortem brains, which revealed that cognitive decline may precede the formation of plaques and neurodegenerative changes [17,22,27]. These observations provide a strong support for the suggestion that Aβ at sublethal levels interfere with the signaling cascades critical for neuronal function and thus lead to the cognitive impairment in early stage of AD. As aforementioned, BDNF-signaling cascades modulate synaptic plasticity that closely related to cognitive function and Arc is one of the downstream effectors, however, it is still unclear whether the BDNF-mediated Arc synthesis is impaired in AD. In the present study, primary cultures of neonatal rat cortical neurons were used. The expression of Arc protein was detected after treating cultures with sublethal Aβ followed by incubating cultures with BDNF to examine if the BDNF-mediated Arc synthesis would be prevented by sublethal Aβ treatment.

Primary cortical neurons were prepared from neonatal Sprague–Dawley rats [26]. In brief, whole cortex cut into 1 mm^3 pieces were digested with 0.25% trypsin/EDTA in Hanks' balanced salt solution (HBSS) without calcium or magnesium at 37 °C for 15 min. The pieces were then gently rinsed in HBSS, washed twice in plating medium (10% FBS in DMEM), and gently triturated in plating medium through a fire-polished Pasteur pipette. Cell suspension was centrifuged at 800 rpm for 5 min. Pellets were resuspended and then approximately $4–5 \times 10^5$ cells/cm^2 were plated on poly-D-lysine pre-coated plastic dishes. Cultures were maintained at 37 °C in a 5% CO_2 incubator. After 24 h, the medium was replaced with DMEM containing 2% B27 supplement (Gibco), 500 μM Glutamax (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cytosine arabinoside at 5 μM (Sigma) was also added to inhibit the proliferation of non-neuronal cells. All experiments were performed on cells cultured for 7–8 days.

Fibrillar $Aβ_{1–42}$ (Biosource) with a final concentration at 100 μM was prepared in PBS. The solution was incubated at 37 °C for 96 h, and was centrifuged at 14,000 × *g* for 10 min. The pellet was collected and dissolved to 100 μM as a stock solution for all assays.

For measuring the cell viability, cells were plated onto poly-D-lysine-coated 24-well plates. These cells were then treated with culture media free from $Aβ_{1–42}$ or containing 5 or 10 μM fibrillar $Aβ_{1–42}$ for 24 or 48 h. The cell viability was assessed by using a cell proliferation kit according to the manufacturer's instructions (Roche). The 3-(4,5-dimethylthiaxol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 5 mg/ml in PBS) with a final concentration of 0.5 mg/ml was added into cultures and incubated for 4 h at 37 °C. Subsequently, solubilization buffer (10% SDS in 0.01 N HCl, 200 μl) was added and incubated at 37 °C overnight. Finally, the medium was removed to a 96-well plate and quantified at 550–630 nm on a Dynex MRX microplate reader. Cell viability was determined from three to four wells for each condition and normalized to parallel controls. Each well was treated as a single observation. Data were obtained from at least three separate experiments with $n \geq 12$.

Protein sample was prepared from cortical neurons homogenized in sodium dodecyl sulfate (SDS) sample buffer, and the proteins are resolved by 10% Bis-Tris gel (Invitrogen) and transferred to polyvinylidene difluoride (PVDF) membrane. Membranes were incubated at room temperature in Tris-buffered saline (TBS, 0.05 M Tris in 0.9% NaCl, pH 7.4) containing 5% nonfat milk for 60 min to block the nonspecific binding. Following incubation with the primary antibodies specific for each protein (mouse anti-Arc monoclonal IgG, 1:200, Santa Cruz; mouse anti-actin monoclonal IgG, 1:10000, Chemicon), the blots were washed in TBS containing 0.1% Tween20 (TTBS) and then incubated with the horseradish peroxidase-conjugated goat secondary antibodies (1:2000, Chemicon). Blots were then washed three times with TTBS. Immunolabeling was detected by enhanced chemiluminescence (Santa Cruz) according to the recommended conditions. Immunoreactivity was quantified using densitometric analysis.

Fig. 1A showed the expression of Arc protein in cortical neurons after exposing to BDNF with various concentrations (0–50 ng/ml) for 2 h. The results of western blot analysis revealed an obvious concentration-dependent increase in Arc protein expression. Since BDNF at 25 ng/ml is sufficient to induce a strong expression of Arc protein, the following experiments were performed with this concentration.

Fig. 1B illustrated the temporal pattern of Arc protein expression following BDNF treatment. Cortical neurons were treated with BDNF at 25 ng/ml for various periods (0–240 min). The results of western blot analysis showed that the expression of Arc protein was significantly increased after exposing to BDNF for longer than 60 min. Corresponded to the result shown in Fig. 1A, a 2-h exposure of BDNF at 25 ng/ml resulted in a robust increase of Arc protein expression that lasted until the end of 4-h exposure. Thus, the exposure duration of BDNF was set at 2 h for the following experiments.

To determine the sublethal concentration of fibrillar $Aβ_{1–42}$, rat cortical neurons cultured for 7 days were treated with culture media free from $Aβ_{1–42}$ (served as control) or containing 5 μM or 10 μM fibrillar $Aβ_{1–42}$ for 24 h or 48 h. Then the cell viability was assessed by MTT assay. Significant cytotoxicity was observed only in cultures treated with 10 μM fibrillar $Aβ_{1–42}$ for 48 h (Fig. 2). No significant effect on cell viability could be found after exposure of 5 μM fibrillar $Aβ_{1–42}$ for 24 h and 48 h. Thus, 5 μM was used as the sublethal concentration for fibrillar $Aβ_{1–42}$ in the further experiments.

In order to examine the effect of $Aβ_{1–42}$ treatment on the plasticity-related BDNF-mediated signaling pathways, the expression of Arc protein that was induced by BDNF was analyzed after exposing to $Aβ_{1–42}$ at sublethal level. Cortical neurons cultured for 8 days were treated with 5 μM $Aβ_{1–42}$ for 3 h and 6 h followed by incubated with BDNF (25 ng/ml) for 2 h. The results showed that after pretreatment of $Aβ_{1–42}$, the BDNF-induced Arc protein expression was decreased significantly in a time-dependent manner (Fig. 3A). Exposure of $Aβ_{1–42}$ (5 μM) alone for various durations (3–24 h) represented no effect on the expression of Arc protein (Fig. 3B).