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Short communication

Unique pharmacological property of ISRIB in inhibition of Aβ-induced neuronal cell death

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ABSTRACT

A pharmacological approach to ameliorate Alzheimer's disease (AD) has not yet been established. In the present study, we investigated the pharmacological characteristics of the recently identified memory-enhancing compound, ISRIB for the amelioration of AD. ISRIB potently attenuated amyloid β-induced neuronal cell death at concentrations of 12.5–25 nM, but did not inhibit amyloid β production in the HEK293T cell line expressing the amyloid precursor protein (APP). These results suggest that ISRIB possesses the unique pharmacological property of attenuating amyloid β-induced neuronal cell death without affecting amyloid β production.

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that is accompanied by cognitive decline and memory loss with age. The accumulation of β amyloid has been reported in the senile plaques of AD brains and the β amyloid hypothesis has been proposed for the pathogenesis of AD (1). β amyloid is produced through amyloid precursor protein (APP) processing, which is mediated by β and γ secretases. Therefore, pharmacological strategies targeting these secretases have been extensively studied in an attempt to ameliorate AD. However, these approaches have not yet succeeded and, thus, novel strategies are still required.

Emerging evidence has indicated the possible involvement of endoplasmic reticulum (ER) stress in the pathophysiology of AD (2, 3). The ER is an organelle involved in protein folding. Various stressors that perturb ER function increase the accumulation of unfolded proteins and cause ER stress (4). Cells activate three major branches of unfolded protein responses (UPR) when exposed to ER stress, i.e.: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK) branches of UPR (5). Increasing evidence suggests that pharmacological modulators of ER stress represent an attractive strategy for the treatment of AD (2, 3). The PERK-eukaryotic translation initiation factor α (eIF2α) branch of UPR was previously reported to be activated in the AD brain (6), and the attenuation of the PERK branch was found to alleviate plasticity and memory deficits in AD (7). A modulator of the integrative stress response (ISR), named ISRIB has recently been identified (8), and reverses the effects of eIF2α phosphorylation by regulating eIF2B (9, 10). However, it currently remains unknown whether ISRIB ameliorates neuronal cell death in AD.

2. Materials and methods

2.1. Materials

The human amyloid β_{1-42} and amyloid β_{25-35} peptides were obtained from Wako Pure Chemical Ltd. (Japan). ISRIB was obtained from Cayman Chemical (Ann Arbor, MI). Neurobasal medium and B-27 supplement were obtained from Life Technologies (Waltham, MA).

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2.2. Cell culture

The PC12 neuronal cell line was cultured in RPMI medium containing 10% horse serum and 5% fetal calf serum. The HEK293T cell line was cultured in DMEM medium containing 10% fetal calf serum, 0.29 mg/ml L-glutamine, 100 units/ml of penicillin G, and 100 μg/ml of streptomycin (Nacalai Tesque, Japan). These cells were maintained at 37 °C in 5% CO_2/95% air.

2.3. Measurement of amyloid β-induced cell death

Prior to being stimulated, PC12 cells were plated on poly-D-lysine-coated 24-well plates and maintained in neurobasal medium containing 2% B-27 supplement and 0.5 mM L-glutamine. After culturing for 3 days, medium was replaced with antioxidant-free neurobasal medium and stimulated with an amyloid β peptide. The amyloid β_{1-42} peptide was dissolved in 0.02% NH_4OH at 200 μM, pre-incubated on ice for 30 min, and then added to the cultured medium. The final concentration of NH_4OH in the cultured medium was 0.002%. We analyzed amyloid β peptide-induced cell death using a lactate dehydrogenase (LDH) assay. LDH leakage into the cultured medium was assessed using a cytotoxicity detection kit (Roche Molecular Biochemical) according to the manufacturer's protocol.

2.4. Measurement of amyloid β42 protein levels by ELISA

The pCAX FLAG APP plasmid (a gift from Drs. Dennis Selkoe and Tracy Young-Pearse, Addgene plasmid #30154) (11) was transfected into HEK293T cells using a standard calcium precipitation protocol. The amount of the amyloid β peptide released into the cultured medium was then measured using ELISA kit (Millipore, Billerica, MA).

2.5. Western blotting analysis

Cells were washed with ice-cold PBS and lysed in buffer containing 10 mM HEPES-NaOH (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF, and 1% NP-40 for 20 min. The lysates were centrifuged at 15,000 rpm at 4 °C for 20 min, and the supernatants were collected and boiled with Laemmli buffer for 3 min. Samples were fractionated by SDS-PAGE, and transferred at 4 °C to nitrocellulose membranes. The membranes were incubated with anti-ATF4 (Cell signaling, Danvers, MA), anti-APP (eBioscience, San Diego, CA), and anti-GAPDH (Acris, Germany) antibodies followed by an anti-horseradish peroxidase-linked antibody. Peroxidase was detected by chemiluminescence using an ECL system.

2.6. Statistical analysis

Results are expressed as the means ± S.E. Statistical analyses were performed using Dunnett's test or a paired *t*-test.

3. Results

3.1. ISRIB protected amyloid β-induced neuronal cell death

Amyloid β peptides accumulate in AD brains and cause neuronal cell death (12, 13). In order to determine whether ISRIB exerts neuroprotective effects on AD neurons, we treated PC12 neuronal cells with ISRIB and analyzed amyloid β_{1-42}-induced cell death. PC12 neuronal cells were treated with ISRIB and the amyloid β_{1-42} peptide for 48 h and cell death was then analyzed. As shown in Fig. 1A, a two-fold increase was observed in cell death by the

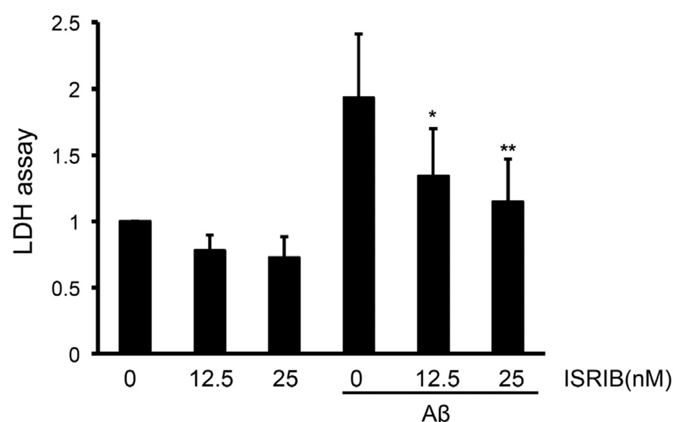

Fig. 1. ISRIB protected against amyloid β-induced neuronal cell death. PC12 neuronal cells were treated with the amyloid β_{1-42} peptide (Aβ) and ISRIB and cell viability were analyzed by measuring LDH. ISRIB dose-dependently (12.5–25 nM) attenuated neuronal cell death. $n = 4–7$, $^{*}p < 0.05$, $^{**}p < 0.01$ Dunnett's test.

amyloid β_{1-42} peptide treatment and amyloid β_{1-42} peptide-induced cell death was significantly attenuated by ISRIB. The effective dosage of ISRIB was in the order of nM (12.5–25 nM) (Fig. 1). ISRIB has been shown to reverse the effects of eIF2α phosphorylation by regulating eIF2B (9, 10), and inhibits the eIF2α-induced expression of activating transcription factor 4 (ATF4) (8).

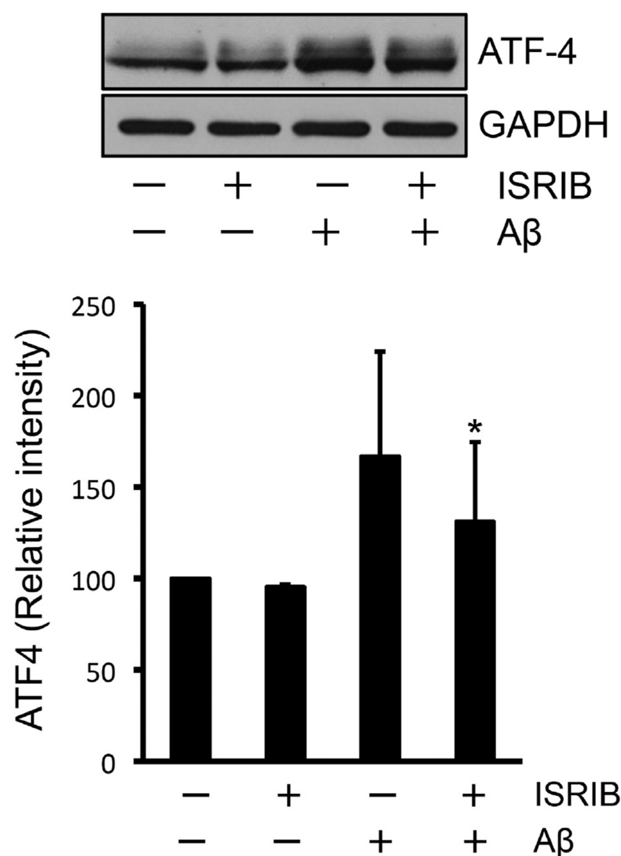

Fig. 2. ISRIB attenuated amyloid β-induced ATF4 expression. The amyloid β_{25-35} peptide (Aβ) was dissolved in H_2O and pre-incubated for 4 days at 37 °C. Aβ (40 μM) was simultaneously added with ISRIB (50 nM) for 4 h and ATF4 levels were analyzed by Western blotting. ISRIB significantly attenuated Aβ-induced ATF4 expression. $n = 4$, $^{*}p < 0.05$ paired *t*-test.

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