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Aβ-AGE AGGRAVATES COGNITIVE DEFICIT IN RATS VIA RAGE PATHWAY

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Abstract—β-Amyloid (Aβ) accumulation has been proved to be responsible for the pathogenesis of Alzheimer's disease (AD). However, it is not yet clear what makes Aβ accumulate and become toxic in the AD brains. Our previous studies demonstrated that glycated Aβ (Aβ-AGE) could be formed, and it exacerbated the authentic Aβ-mediated neurotoxicity *in vitro*, but we did not show the role of Aβ-AGE *in vivo* and the underlying mechanism. In the current study, we synthesized Aβ-AGE by incubating Aβ with methylglyoxal *in vitro*, and then stereotactically injected Aβ-AGE into lateral ventricle of Sprague–Dawley (SD) rats. We found that Aβ-AGE aggravated Aβ-induced cognitive impairment, which was characterized by higher speed of deterioration of long-term potentiation (LTP), more decrease of dendritic spines density and more down-regulation of synaptic proteins. We also observed the overexpression of receptor for advanced glycation endproducts receptor for AGEs (RAGE) and the activation of downstream molecular (GSK3, NF-κB, p38) in RAGE-mediated pathways. On the other hand, simultaneous application of RAGE antibody or GSK3 inhibitor LiCl reversed the cognitive decline amplified by Aβ-AGE. Our data revealed that *in vivo* the Aβ-AGE is more toxic than Aβ, and Aβ-AGE could lead to the aggravation of AD-like pathology though the RAGE pathway, suggesting that Aβ-AGE and RAGE may be new therapeutic targets for AD.

Key words: Alzheimer's disease, β-amyloid, glycation, receptor of advanced glycation endproducts, glycogen synthase kinase 3.

INTRODUCTION

Alzheimer disease (AD) is a neurodegenerative disorder involving severe cognitive decline (Yankner, 1996; LaFerla and Oddo, 2005). A hallmark of AD is the extracellular amyloid plaques formed by aggregated β-amyloid (Aβ), a 39–43 amimo acid peptide derived from the transmembrane amyloid precursor protein (APP). Numerous studies demonstrate that the Aβ-mediated neurotoxicity is responsible for the pathogenesis of AD (Glenner and Wong, 1984; Hsiao et al., 1996; Ramirez et al., 2012). A few papers have suggested that the glycated Aβ (Aβ-AGE) is a more pathogenic species than Aβ itself (Gasic-Milenkovic et al., 2003; Münch et al., 2003; Kuhla et al., 2004), however, how Aβ-AGE exerts exaggerated neurotoxicity *in vivo* needs to be further clarified.

The deregulation of glucose metabolism leads to the generation of the advanced glycation endproducts (AGEs) in AD brains. In this process, AGEs are formed by glycating amino groups of proteins with reduced sugars or dicarbonyl compounds, such as methylglyoxal and glyoxal, both intracellularly and extracellularly in a complex nonenzymatic multistep reaction (Yan et al., 1995; Srikanth et al., 2011). AGEs levels elevate more significantly in glucose metabolism and oxidative stress. The drastic disturbance of glucose metabolism and oxidative stress in the brain are striking features of AD (Markesbery, 1997; Perry et al., 1998; Lovell et al., 2001; Butterfield, 2002), which lead to the formation of numerous reactive carbonyl compounds. These carbonyl compounds can react with proteins to form AGEs. The association of AGEs with AD pathology has been known for some time. *In vitro*, AGEs modification seeds and augments amyloid misfolding (Smith et al., 1994; Vitek et al., 1994; Loske et al., 2000; Fawver et al., 2012). *In vivo*, studies demonstrated that AGEs were co-localized with pathological protein deposits such as Aβ plaques in AD (Yan et al., 1994; Smith et al., 1995; Choei et al., 2004; Krautwald and Munch, 2010). These evidences suggested that AGE-modified proteins are potentially involved in the pathology and etiopathogenesis of AD. However, the type of AGEs formed in AD and their effects on pathogenesis of AD remain to be elucidated.

Studied have reported that Aβ-AGE could contribute to the accumulation of Aβ in tube (Vitek et al., 1994; Fawver et al., 2012). Our previous research also showed that Aβ was a suitable substrate of non-enzyme glycation, and it could form Aβ-AGE both *in vitro* and *in vivo* (Li et al., 2013b). We have further demonstrated

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Abbreviations: Aβ, β-amyloid; Aβ-AGE, glycated Aβ; AD, Alzheimer's disease; AGEs, advanced glycation end-products; AP, anterior to posterior; APP, amyloid precursor protein; DV, dorsal to ventral; EDTA, ethylenediaminetetraacetic acid; EPSP, excitatory postsynaptic potential; GSK3, synthase kinase-3; HFS, high-frequency stimulation; LTP, long-term potentiation; MAPK, mitogen-activated protein kinases; ML, mid to lateral; NF-κB, nuclear factor κB; pAb, polyclonal antibody; PB, phosphate buffer; PCR, polymerase chain reaction; PS, population spike; PSD, postsynaptic density; RAGE, receptor for AGEs.

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that Aβ-AGE could exacerbate the Aβ-induced neurotoxicity *in vitro* (Li et al., 2013b), but role of Aβ-AGE and the possible mechanism are still not clear.

The extracellular AGEs affect the neurons via the receptor for AGEs (RAGE) (Neeper et al., 1992; Schmidt et al., 1992), which is a multiligand receptor in the immunoglobulin superfamily of cell surface molecules. This AGEs–RAGE interaction may activate receptor-mediated signal transduction pathways and perturb cell functions (Guimaraes et al., 2010). Recent studies have demonstrated that Aβ is another ligand of RAGE, and RAGE plays an important role in Aβ-mediated neurotoxicities (Yan et al., 1996; Takuma et al., 2009). When pathogenic Aβ accumulates in AD brain or transgenic animal models, RAGE expression increases in neurons, microglia, and cerebral vessels (Du Yan et al., 1997; Miller et al., 2008). Transgenic mice that overexpressed mutant human APP/Aβ and RAGE in neurons displayed early stage deficits of spatial learning/memory and neuropathologic changes (Arancio et al., 2004). Enhanced expression of RAGE in AD provides a mechanism for direct Aβ-induced neuronal toxicity through Aβ-RAGE interaction, suggesting that RAGE is an enhancer for Aβ-induced neuronal perturbation.

We have verified that RAGE pathway was implicated in the development of exacerbated neurotoxicity induced by Aβ-AGE in cultured hippocampal neurons (Li et al., 2013b), however, whether RAGE, as the co-RAGE and Aβ, was still involved in the performance of Aβ-AGE in animal needs to be further investigated. We hypothesized that infusion of Aβ-AGE synthesized in tube may aggravate the Aβ-induced cognitive impairment *in vivo*, and RAGE-mediated signaling pathway may be implicated in aggravated cognitive deficit induced by Aβ-AGE.

EXPERIMENTAL PROCEDURES

Antibodies and chemicals

Mouse monoclonal antibody (mAb) RAGE, synapsin I, synaptophysin, β-actin and DM1A against α-tubulin were from Millipore (Billerica, MA, USA). Rabbit polyclonal antibody (pAb) Glutamate receptor 2A (NR2A), Glutamate receptor 2B (NR2B), postsynaptic density 93 (PSD93), PSD95, and nuclear factor-κB p65 (NF-κB p65) were from Abcam (Cambridge, UK). pAb pS9-GSK3β (phosphorylated synthase kinase-3β at Ser9), GSK3β, p38, and p-p38 (phosphorylated p38) were from Cell Signaling Technology (Beverly, MA, USA). Human peptide Aβ1-42, methylglyoxal and LiCl (GSK3 inhibitor) were from Sigma (St. Louis, Mo, USA). Peroxidase conjugated goat anti-rabbit and goat anti-mouse secondary antibodies were from Pierce (Rockford, IL, USA). Rhodamine red-X- and Oregon Green 488-conjugated secondary antibodies were from Invitrogen (Carlsbad, CA, USA).

Animals

Sprague–Dawley (SD) rats (male, weight 280–320 g) were supplied by the Military Academy of Medical Sciences of the People's Liberation Army. All rats were kept under standard laboratory conditions: 12-h light and 12-h dark; lights on at 6:00 AM; temperature: 22 ± 2 °C; water and food *ad libitum*. All animal experiments were performed according to the "Policies on the Use of Animals and Humans in Neuroscience Research" revised and approved by the Society for Neuroscience in 1995.

Preparation of A-AGE

1 mg/mL Aβ1-42 was incubated with or without 0.5 mM MG in 0.1 M phosphate buffer (PB), pH 7.2, at 37 °C for 1 month under sterile conditions. After dialyzing against PB for 48 h to remove methylglyoxal, the prepared Aβ-AGE was sterilized by filtration and kept at −20 °C. The production of Aβ-AGE was identified with western blotting and fluorescence spectrophotometer measuring AGE-specific fluorescence at emission of 440 nm and excitation of 370 nm (PerkinElmer, Waltham, MA, USA).

Stereotaxic injection

The rats were anesthetized with 6% intraperitoneal chloral hydrate (6 mL/kg) and placed in a stereotaxic instrument. One hole was made for injection at coordinates −1.0 mm anterior to posterior (AP) bregma, 1.5 mm mid to lateral (ML), −3.8 mm dorsal to ventral (DV) dura according to the stereotaxic atlas of Paxinos and Watson (Paxinos, 1996). 10 μL of Aβ (5 μg), Aβ-AGE (5 μg) with RAGE antibody (Ab) (50 μg) or LiCl (4 mg) was injected into the left side of the lateral ventricle. Totally 80 rats were randomly divided into Aβ group (n = 20), Aβ + Ab group (n = 10), Aβ-AGE group (n = 20), Aβ-AGE + Ab group (n = 10), Aβ + LiCl group (n = 10), or Aβ-AGE + LiCl group (n = 10).

Morris water maze test

Morris water maze test was performed according to the procedure described previously (Li et al., 2012a). Morris water maze test was used to measure the spatial learning and memory of rats. The rats were trained to find a submerged platform by using a stationary array of cues outside the pool tub at 2 days after injection. The water was made opaque by using black ink for chiaroscuro. Acquisition training consisted of a total of 28 trials, given as 4 spaced trials a day for 6 consecutive days (from 2 to 7 days after injection). At 9 days after injection, the platform was removed and the same test was performed. A computerized tracking system was used to record the swimming trace and calculate the latency to the platform and the time spent in each quadrant.

LTP measurement

Long-term potentiation (LTP) recording was performed according to the method previously described (Zhu et al., 2007). Briefly, rats were anesthetized and placed on the stereotaxic instrument. The skull was exposed, and a small hole was made at the contralateral of previous injection. The stimulating electrode was placed