Neurochemistry International 76 (2014) 114–121

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Histone deacetylase mediates the decrease in drebrin cluster density induced by amyloid beta oligomers

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ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 13 June 2014
Accepted 14 July 2014
Available online 21 July 2014

Keywords:
Drebrin
Histone acetylation
Histone deacetylase
Dendritic spine
Aβ-derived diffusible ligands
Alzheimer's disease

ABSTRACT

Dendritic spine defects are found in a number of cognitive disorders, including Alzheimer's disease (AD). Amyloid beta (Aβ) toxicity is mediated not only by the fibrillar form of the protein, but also by the soluble oligomers (Aβ-derived diffusible ligands, ADDLs). Drebrin is an actin-binding protein that is located at mature dendritic spines. Because drebrin expression is decreased in AD brains and in cultured neurons exposed to Aβ, it is thought that drebrin is closely associated with cognitive functions. Recent studies show that histone deacetylase (HDAC) activity is elevated in the AD mouse model, and that memory impairments in these animals can be ameliorated by HDAC inhibitors. In addition, spine loss and memory impairment in HDAC2 over-expressing mice are ameliorated by chronic HDAC inhibitor treatment. Therefore, we hypothesized that the regulation of histone acetylation/deacetylation is critical to synaptic functioning. In this study, we examined the relationship between HDAC activity and synaptic defects induced by ADDLs using an HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA). We show that ADDLs reduce the cluster density of drebrin along dendrites without reducing drebrin expression. SAHA markedly increased the acetylation of histone proteins, and it simultaneously attenuated the ADDL-induced decrease in drebrin cluster density. In comparison, SAHA treatment did not affect the density of drebrin clusters or dendritic protrusions in control neurons. Therefore, SAHA likely inhibits ADDL-induced drebrin loss from dendritic spines by stabilizing drebrin in these structures, rather than by increasing drebrin clusters or dendritic protrusions. Taken together, our findings suggest that HDAC is involved in ADDL-induced synaptic defects, and that the regulation of histone acetylation plays an important role in modulating actin cytoskeletal dynamics in dendritic spines under cellular stress conditions, such as ADDL exposure.

1. Introduction

Neuronal activity is known to regulate a complex program of gene expression underlying the structural and functional plasticity of the brain (Flavell and Greenberg, 2008). Chromatin remodeling through histone-tail acetylation is emerging as a fundamental mechanism of gene regulation (Kurdistani and Grunstein, 2003; Goldberg et al., 2007). Histone deacetylases (HDACs) are a class of enzymes that remove acetyl groups from lysine amino acids on a histone, allowing the histones to wrap the DNA more tightly and downregulate gene expression (for review, see Gräff and Tsai, 2013).

Recently, HDAC inhibitors were shown to improve memory and cognitive function in a mouse model of Alzheimer's disease (AD) (Francis et al., 2009; Kilgore et al., 2010). In addition, the reduction in synapse number and the learning impairment in HDAC2 over-expressing mice are ameliorated by chronic treatment with an HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA; vorinostat) (Guan et al., 2009). Furthermore, depression-like behavior induced by chronic mild stress in BALB/c mice is suppressed by chronic administration of SAHA (Uchida et al., 2010).

Various studies indicate that the severity of memory impairment in AD correlates with levels of amyloid beta (Aβ) oligomer, also known as Aβ-derived diffusible ligands (ADDLs) (Lue et al., 1999; Shankar et al., 2007). In addition, it was recently reported that Aβ levels are increased in the brains of patients with

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http://dx.doi.org/10.1016/j.neuint.2014.07.005
0197-0186/

depression (Kumar et al., 2011). These findings suggest that Aβ is involved in the pathogenesis of neuropsychiatric disorder.

Several studies have shown that expression of drebrin, which stabilizes actin filaments in the dendritic spine (Shirao and González-Billault, 2013), is decreased in AD brains (Harigaya et al., 1996; Counts et al., 2012). Furthermore, it has been reported that a reduction in expression of drebrin A, a neuron-specific isoform, underlies the impairment in activity-dependent glutamate receptor trafficking in an AD animal model (Lee and Aoki, 2012).

Aβ decreases the expression of drebrin both *in vitro* (Zhao et al., 2006; Lacor et al., 2007) and *in vivo* (Harigaya et al., 1996; Counts et al., 2012). Because drebrin is known to be involved in the morphogenesis and maintenance of dendritic spines (Hayashi and Shirao, 1999; Takahashi et al., 2003), the reduction in drebrin expression induced by ADDLs could potentially cause AD pathology, including dendritic spine structural abnormalities and a decrease in spine density (Knafo et al., 2009), resulting in cognitive decline. Histone deacetylation and Aβ-induced drebrin loss may be major factors underlying the pathogenesis of various neuropsychiatric disorders. In this study, we examined whether ADDL-induced changes in drebrin distribution can be attenuated by HDAC inhibition.

2. Materials and methods

2.1. Antibodies

The primary antibodies used in this study include mouse monoclonal antibodies against drebrin (clone M2F6, hybridoma supernatant) (Shirao and Obata, 1985), $A\beta_{1-16}$ (clone 6E10, Covance Inc., Princeton, NJ, USA) and β-actin (clone AC-15; Sigma–Aldrich, St. Louis, MO, USA); rabbit polyclonal antibodies against synapsin I (Merck Millipore, Billerica, MA, USA) and acetyl-histone H3 protein (Lys9/14) (Cell Signaling Technology, Inc., Danvers, MA, USA); and a rabbit monoclonal antibody against histone H3 protein (Cell Signaling Technology, Inc.). The secondary antibodies used for immunocytochemistry were fluorescein-5-isothiocyanate-conjugated goat anti-mouse IgG (MP Biomedicals, LLC-Cappel Products, Santa Ana, CA, USA) and Cy5-conjugated goat anti-rabbit IgG antibodies (Jackson ImmunoResearch, West Grove, PA, USA). The secondary antibodies used for Western blot analysis were horseradish peroxidase (HRP)-conjugated sheep anti-mouse IgG and HRP-conjugated donkey anti-rabbit IgG antibodies (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, UK).

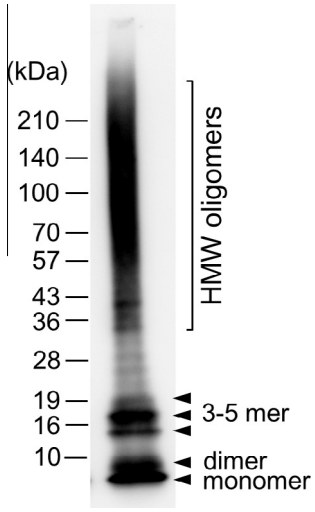

Fig. 1. Western blot analysis of ADDLs. Closed arrowheads show the monomeric to pentameric forms. The broad smear represents high molecular weight (HMW) oligomers (indicated by the bracket).

2.2. Preparation of Aβ-derived diffusible ligands (ADDLs)

$A\beta_{1-42}$ peptide (human sequence) was purchased from Peptide Institute, Inc. (Osaka, Japan). Aβ-derived diffusible ligands (ADDLs) were prepared in accordance with a previously published method, with slight modification (Lambert et al., 2001; Fa et al., 2010). Briefly, $A\beta_{1-42}$ was dissolved in hexafluoro-2-propanol and aliquoted into microcentrifuge tubes. Hexafluoro-2-propanol was completely removed using a SpeedVac centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA) at room temperature until a clear peptide film was observed at the bottom of the vials. An aliquot of $A\beta_{1-42}$ was dissolved in anhydrous dimethyl sulfoxide (DMSO) to 5 mM and sonicated in a water bath for 10 min to

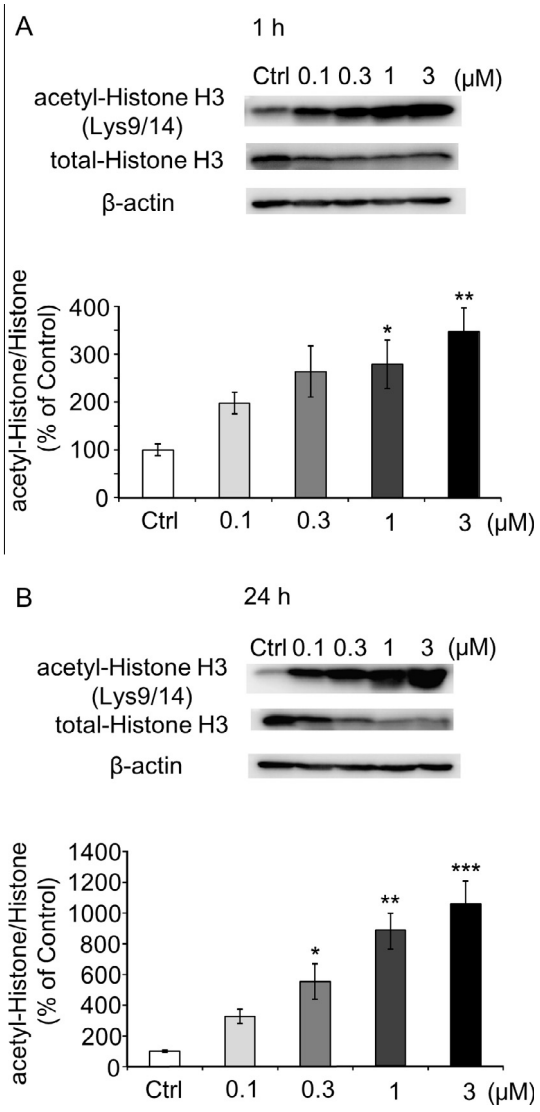

Fig. 2. Effect of SAHA on the acetylation of histone H3 protein assessed with Western blot analysis. Cultured neurons were treated with various dosages of SAHA (0.1, 0.3, 1, 3 μM) for 1 h (A) and 24 h (B). Top panels show typical Western blots of histone H3 protein acetylated at Lys9 and 14. Middle panels show typical Western blots of total histone H3 protein. Bottom panels show β-actin as loading control. Western blots were quantified with NIH ImageJ software after standardizing the ratio of acetyl/total histone H3 protein. Data are presented as means ± SEM, $n = 3$ experiments; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control, ANOVA, followed by Dunnett's post hoc test.