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Neurobiology of Aging 31 (2010) 1743–1757

NEUROBIOLOGY OF AGING

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Differential cerebral deposition of IDE and NEP in sporadic and familial Alzheimer's disease

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Received 23 October 2007; received in revised form 23 July 2008; accepted 26 September 2008
Available online 18 November 2008

Abstract

Alzheimer's disease (AD) is characterized by amyloid β (Aβ) accumulation in the brain and is classified as familial early-onset (FAD) or sporadic late-onset (SAD). Evidences suggest that deficits in the brain expression of insulin degrading enzyme (IDE) and neprilysin (NEP), both proteases involved in amyloid degradation, may promote Aβ deposition in SAD. We studied by immunohistochemistry IDE and NEP cortical expression in SAD and FAD samples carrying the E280A presenilin-1 missense mutation. We showed that IDE, a soluble peptidase, is linked with aggregated Aβ40 isoform while NEP, a membrane-bound protease, negatively correlates with amyloid angiopathy and its expression in the senile plaques is independent of aggregated amyloid and restricted to SAD cases. NEP, but not IDE, is over-expressed in dystrophic neurites, both proteases are immunoreactive in activated astrocytes but not in microglia and IDE was the only one detected in astrocytes of white matter from FAD cases. Collectively, our results support the notion that gross conformational changes involved in the modification from "natively folded-active" to "aggregated-inactive" IDE and NEP may be a relevant pathogenic mechanism in SAD.

Keywords: Alzheimer's disease; Amyloid β; Insulin degrading enzyme; Neprilysin; Human brain; Presenilin; Astrocytes

1. Introduction

Alzheimer's disease (AD) is the most prevalent aging-associated brain amyloidoses clinically characterized by dementia and classified as genetic, which is responsible of a rare disease known as familial early-onset AD (FAD), or sporadic late-onset AD (SAD), the most common form of dementia in subjects over 65 years old (Maurer and Hoyer, 2006). FAD is an inherited autosomal dominant disorder with mutations in the genes encoding amyloid precursor protein (APP) or presenilin 1 or 2 (PSEN1, PSEN2) (Haass and De Strooper, 1999). APP is a single-pass transmembrane protein that is sequentially cleaved by the β-secretase (BACE) – a membrane spanning aspartic protease – to generate a membrane-bound C-terminal fragment which is further

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0197-4580/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2008.09.016

processed by the γ-secretase complex, in a PSEN-dependent manner, to generate amyloid β (Aβ) of 40–42 residues (De Strooper et al., 1998; Vassar et al., 1999; Wolfe et al., 1999). In the context of the amyloid hypothesis (Selkoe, 2000), pathogenic mutations in APP induce over-expression of Aβ42 or a mutated isoform of Aβ40 with increased amyloidogenic properties while mutations in PSEN1/2 proteins alter the normal proteolytic processing of APP resulting in the increase of brain soluble Aβ42/Aβ40 peptides ratio (Scheuner et al., 1996). Histopathological features of SAD and FAD include the abundance of cortical and hippocampal deposits of Aβ42 and Aβ40 and of neurofibrillary tangles (NFT) composed of abnormally phosphorylated tau protein. Parenchymal deposition of fibrillar Aβ develops in a progressive and age-related manner to senile plaques, associated to dystrophic neurites and glial reaction (Dewachter et al., 2000; Poirier, 2005; Shibata et al., 2000; Jellinger, 2002). Furthermore, both types of AD show amyloid angiopathy with the predominance of Aβ40 in SAD and Aβ42 in the FAD brain microvasculature, respectively (Lemere et al., 1996; Roher et al., 1993). In addition, severe cerebellar and white matter pathology is observed in FAD subjects (Lemere et al., 1996) and it has been suggested that the over-representation of Aβ42 in FAD cases produces – as compared to SAD – its earlier deposition and a higher percentage of brain area occupied by amyloid plaques. Mechanisms involved in the development of FAD may be explained by increments on local concentration of Aβ42 peptide, while in SAD, causes are still unknown and strongly influenced by genetic and environmental factors such as the apolipoprotein E genotype (Roses, 1997), chronic trauma or cholesterol metabolism (Hartman et al., 2002; Hartmann, 2001; Pappolla et al., 2003). Recent publications support the idea that vascular lesions or defects in blood–brain barrier transport may be pathogenic factors in the formation of the cortical amyloid deposits around or close to capillaries, arterioles and venules (Cullen et al., 2006; Jeynes and Provias, 2006). In addition, it has been suggested that in SAD cases an inefficient Aβ degradation by brain proteases may contribute to Aβ accretion and its further accumulation (Perez et al., 2000). Within the brain, the metabolism of Aβ is mainly regulated by the activity of at least by two major peptidases: neprilysin (NEP) and insulin-degrading enzyme (IDE) as it was demonstrated in knock-out animals for NEP and IDE, respectively (Iwata et al., 2001; Farris et al., 2003). The over-expression of IDE or NEP in the transgenic mice over-expressing the human mutated APP gene (K670N, M671L) responsible for FAD in a Swedish pedigree prevents the amyloid plaque pathology and the early death (Leissring et al., 2003) suggesting that promotion of Aβ degradation may be a therapeutic target for AD. NEP is a ubiquitous and highly conserved membrane-bound zinc metalloprotease (Turner et al., 2004) expressed in the human brain at relatively low amounts and mainly in the tunica media of cerebrocortical blood vessels and in pyramidal neurons. Endogenous IDE is expressed in the human brain by cortical and subcortical neurons, it was described within the cytoplasm of the three major components of the vascular wall: endothelial cells, pericytes and smooth muscle cells (Morelli et al., 2004) and it is considered a major soluble protease involved in the degradation of Aβ in the brain.

Experimental evidences suggest that impairments on Aβ-degrading proteases activity in the AD brain may be caused by at least 3 physiologic events: (1) decrements of mRNA expression in vulnerable areas of the brain susceptible for Aβ deposition, such as cortex and hippocampus (Cook et al., 2003; Yakoshima et al., 2001) or decreased protein levels or activity in the brain cortex (Perez et al., 2000; Zhao et al., 2007; Yakoshima et al., 2001), or in brain microvessels (Miners et al., 2006); (2) the presence of post-translational modifications, such as oxidation (Caccamo et al., 2005; Wang et al., 2003) and (3) the deposition of the enzymes, understood as a progressive accumulation of the proteases in the diseased brain with the consequent a loss of its native structure and of functionality. In this context, immunohistochemistry of SAD brains showed clusters of NEP-positive dystrophic neurites partially localized with senile plaques (Akiyama et al., 2001), a limited co-localization of deposits of IDE to amyloid plaques and blood vessels (Bernstein et al., 1999) and a differential deposition of IDE and NEP in vessels form subject with cerebral amyloid angiopathy, while IDE is over-expressed (Morelli et al., 2004) NEP is down-regulated (Miners et al., 2006). Together these results support the concept that both IDE and NEP expression and activity in the human brain participate in a differential manner in the pathogenesis of AD.

Moreover, the overall ageing-related down regulation of Aβ-degrading proteases was corroborated for NEP (Apelt et al., 2003) but not for IDE (Leal et al., 2006) in transgenic Tg2576 mice brains in which the relevant feature for both proteases was an astroglial up regulation in the vicinity of Aβ plaques without cortical deposition. In summary, the concept is that in SAD brain "age-dependent" accumulation of Aβ promotes inhibition of IDE and NEP expression and/or activity while in Tg2576 mice the effect of the "progressive" accumulation of Aβ on IDE and NEP does not affect the functionality of the endogenous enzymes and noteworthy may promote their expression.

Here we studied by immunohistochemistry the pattern of expression of IDE and NEP in AD brains with different etiologies, namely SAD and FAD carrying the E280A PSEN1 missense mutation and characterized by "accelerated" Aβ deposition, to test if differences in the rate of Aβ deposition in the human brain may impact on the expression and conformational state of Aβ-degrading proteases with potential impact in the progression of the disease.

2. Methods

2.1. Human tissues from AD and control subjects

Brain samples from late-onset SAD ($n = 8$) and control (CTL, $n = 11$) cases were obtained, under the approval of the