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Age-related accumulation of Reelin in amyloid-like deposits

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Abstract

Accumulating evidence suggest that alterations in Reelin-mediated signaling may contribute to neuronal dysfunction associated with Alzheimer's disease (AD), the most common form of senile dementia. However, limited information is available on the effect of age, the major risk factor of AD, on Reelin expression. Here, we report that normal aging in rodents and primates is accompanied by accumulation of Reelin-enriched proteinous aggregates in the hippocampal formation that are related to the loss of Reelin-expressing neurons. Both phenomena are associated with age-related memory impairments in wild-type mice. We provide evidence that normal aging involves loss of Reelin neurons, reduced production and elimination of the extracellular deposits, whereas a prenatal immune challenge or the expression of AD-causing gene products, result in earlier, higher, and more persistent levels of Reelin-positive deposits. These aggregates co-localize with non-fibrillary amyloid-plaques, potentially representing oligomeric Aβ species. Our findings suggest that elevated Reelin plaque load creates a precursor condition for senile plaque deposition and may represent a critical risk factor for sporadic AD.

Keywords: Mus musculus; Rattus norvegicus; Callithrix jacchus; Hippocampus; Stratum lacunosum-moleculare; Entorhinal cortex; Piriform cortex; Episodic-like memory; Radial arm maze; GABAergic interneurons; PolyIC; Neuroinflammation; 3xTg-AD mice; SynGAP; GFAP; Dab1; F4/80; Immunohistochemistry; Alzheimer's disease

1. Introduction

Reelin is a large extracellular glycoprotein secreted by Cajal-Retzius cells and mediating proper positioning of neurons during development through the activation of the apolipoprotein E receptor 2 (ApoER2) and very-low-density lipoprotein receptor (VLDLR, D'Arcangelo et al., 1995, 1999; Hiesberger et al., 1999; Howell et al., 1997; Trommsdorff et al., 1999). Transduction of the signal involves the interaction of the adapter protein Dab1 with the intracellular NPxY motiv of these receptors, resulting in tyrosine phosphorylation of Dab1, activation of Src family of non-receptor tyrosine kinases, and triggering a downstream cytosolic kinase cascade beginning with the activation of phosphatidylinositol-3-kinase (PI3K) and ending with the inhibition of glycogen synthase kinase 3β (GSK3β; for recent review, see Herz and Chen, 2006). The signal is terminated with Reelin targeted to the lysosome and Dab1 degraded by the proteasome (Arnaud et al., 2003; Bock et al., 2004; Morimura et al., 2005). In the adult brain, Reelin expression is maintained by GABAergic interneurons and glutamatergic pyramidal neurons in layer II of the entorhinal cortex (Alcantara et al., 1998; Miettinen et al., 2005; Pesold et al., 1998; Ramos-Moreno et al., 2006) and the same signaling cascade is used in adult synapses to modulate neuronal function and synaptic plasticity by regulating glutamate receptor activity through the phosphorylation of intracellular tyrosine

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residues (Beffert et al., 2005, 2006; Chen et al., 2005; Weeber et al., 2002).

In line with its critical role in synaptic transmission and learning and memory, several recent findings suggest that alterations in Reelin signaling may contribute to neuronal dysfunction associated with Alzheimer's disease (AD). This most common type of dementia is characterized by progressive cognitive decline and two histopathological hallmarks including amyloid-beta (Aβ) plaques, which are caused by an imbalance in Aβ metabolism, and neurofibrillary tangles that result from abnormal phosphorylation and aggregation of Tau (Glenner and Wong, 1984; Grundke-Iqbal et al., 1986). Reelin binding to its receptors potently downregulates the activity of GSK3β, a major Tau kinase, and as a result mutant mice with defects in the Reelin signaling show increased levels of hyperphosphorylated Tau (Beffert et al., 2004; Hiesberger et al., 1999; Ohkubo et al., 2003). In addition, the Reelin receptors are also targets of the common ApoE isoform ε4, a major genetic risk factor associated with sporadic AD (Corder et al., 1993; Schmechel et al., 1993). The finding that recombinant ApoE ε3 and ε4 inhibit Reelin binding by 50–60% (D'Arcangelo et al., 1999) indicates that lipid-associated ApoE could compete with Reelin for lipoprotein receptor activation. As a consequence, impaired ApoE receptor signaling may result in a dysbalance in cholesterol homeostasis, which has been shown to profoundly affect the production and trafficking of Aβ (Simons et al., 1998) and hypothesized to underlie accelerating synaptic loss and onset of dementia (Herz and Chen, 2006; Raber et al., 1998; Weeber et al., 2002). Further support for a direct link between Reelin and amyloid precursor protein (APP) processing has recently been proved by Hoe and colleagues by demonstrating that Reelin increases the intracellular interaction of Dab1 with ApoER2 and APP and promotes their cleavage, resulting in reduced toxic Aβ production (Hoe et al., 2006a). Moreover, recent data from AD patients reporting altered Reelin expression levels in both CSF and cortical tissue (Botella-Lopez et al., 2006; Saez-Valero et al., 2003) point to profound alterations in Reelin processing and signaling potentially underlying AD-related neuronal dysfunction. Although Reelin, ApoE receptors, and APP functionally converge at synaptic sites likely joining forces to control glutamate receptor activity and regulate neurotransmission, cognition and memory, it is currently unknown whether Reelin expression is affected by age; the major risk factor of AD. We therefore set out a comprehensive investigation on Reelin expression in different species during normal and pathological forms of aging.

2. Methods and materials

2.1. Animals

All procedures were approved by the local authorities and were performed in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). Six different groups of animals were employed (Table 1). *Group 1*: Adult (4–5 months), middle-aged (11–14 months), and old

Table 1
Animals employed

Group	Species	Strain	Genotype	Treatment	Breeding Colony	Age	Number	Experiment
1	Mice	C57B6 Jico	Wild type	None	Janvier, Le Genest-Saint Isle, France	4–5 months	7 ♂	Behavioural testing (RAM)
						11–14 months	10 ♂	
						20–23 months	16 ♂	Reelin-IHC: C/P
2		C57B6J	$SynGAP^{+/+}$ $SynGAP^{+/-}$	None	ETH Zurich	3 months 6 months 12 months 24 months	4 ♂, 3 ♀ 6 ♀ 4 ♂, 4 ♀ 8 ♂ per genotype	Reelin-IHC: C/P
3		C57B6J	Wild type	Prenatal PolyI:C or vehicle exposure	ETH Zurich	6 months	10 ♂, 10 ♀	Reelin-IHC: C/P
							5 ♂, 5 ♀	
4		129/C57B6J	3xTg-AD Non-Tg controls	None	ETH Zurich	15 months	8 ♂ 10 ♂	Reelin-IHC: P
5	Rats	Wistar	WIST:Hanlbm	None	ETH Zurich	6 months 20 months	3 ♂ 3 ♂	Reelin-IHC
6	Monkeys	Callithrix jacchus		None	ETH Zurich	1 years	1 ♀	Reelin-IHC
					Anthropology University Zurich	10 years	1 ♀	

RAM: radial arm maze task; IHC: immunohistochemistry; C: quantification of Reelin-pos neurons; P: quantification of Reelin-pos plaques.