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AN AGE-RELATED AXON TERMINAL PATHOLOGY AROUND THE FIRST OLFACTORY RELAY THAT INVOLVES AMYLOIDOGENIC PROTEIN OVEREXPRESSION WITHOUT PLAQUE FORMATION

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Abstract—The glomeruli are the first synaptic relay on the olfactory pathway and play a basic role in smell perception. Glomerular degeneration occurs in humans with age and in Alzheimer's disease (AD). The glomeruli heavily express β-amyloid precursor protein (APP), β-secretase (BACE1) and γ-secretase complex. However, extracellular β-amyloid peptide (Aβ) deposition occurs fairly rarely at this location in postmortem pathological studies. We sought to explore age-related glomerular changes that might link to alteration in amyloidogenic proteins and/or plaque pathogenesis in transgenic models of AD and humans. Focally increased BACE1 immunoreactivity (IR) in the glomerular layer was identified in several transgenic models, and characterized systematically in transgenic mice harboring five familiar AD-related mutations (5XFAD). These elements were co-labeled with antibodies against APP N-terminal (22C11) and Aβ N-terminal (3D6, 6E10) and mid-sequence (4G8). They were not co-labeled with two Aβ C-terminal antibodies (Ter40, Ter42), nor associated with extracellular amyloidosis. These profiles were further characterized to be most likely abnormal olfactory nerve terminals. Reduced glomerular area was detected in 6–12-month-old 5XFAD mice relative to non-transgenic controls, and in aged humans relative to young/adult controls, more robust in AD than aged subjects without cerebral amyloid and tau pathologies. The results suggest that olfactory nerve terminals may undergo age-related dystrophic and degenerative changes in AD model mice and humans, which are associated with increased labeling for amyloidogenic proteins but not local extracellular Aβ deposition. The identified axon terminal pathology might affect neuronal signal transmission and integration at the first olfactory synaptic relay.

Key words: aging, dementia, amyloidogenesis, axonal pathology, olfactory deficit.

INTRODUCTION

Since the seventies of the last century, the olfactory system has attracted much attention among pathologists and clinicians in research into brain aging and age-related neurodegenerative diseases (Peabody and Tinklenberg, 1985; Moberg et al., 1987; Altman, 1989; Haehner et al., 2009). For Alzheimer's disease (AD), olfactory deficits have been discussed in relevance to its etiology, pathogenesis, diagnosis and potential prevention/therapy (Kesslak et al., 1988; Altman, 1989; Kishikawa et al., 1994; Christen-Zaech et al., 2003; Wesson et al., 2010a). Olfactory functional impairments in AD include deficits in odor detection threshold, discrimination, identification, memory and associated behaviors (Kesslak et al., 1988; Nordin and Murphy, 1998; Devanand et al., 2000; Djordjevic et al., 2008; Li et al., 2010). Neuronal loss, neuritic dystrophy, amyloid deposition and tau pathology are reported in the olfactory epithelium, olfactory bulb, anterior olfactory nucleus, and olfactory high cortical centers (Esiri and Wilcock, 1984; Crino et al., 1985; Ohm and Braak, 1987; Struble and Clark, 1992; Davies et al., 1993; Kovacs et al., 1999; Devanand et al., 2000; Hoogland et al., 2003; Attems et al., 2005; Attems and Jellinger, 2006; Arnold et al., 2010; Bahar-Fuchs et al., 2010; Saiz-Sanchez et al., 2010; Mundiñano et al., 2011). Gross anatomical atrophy, reduced resting neuronal activity and blood supply as well as evoked responses are found in olfactory centers in AD individuals (Kareken et al., 2001; Johnson et al., 2006; Thomann et al., 2009; Bahar-Fuchs et al., 2010).

The relevance of olfactory deficits to aberrant amyloidogenic protein expression/processing has been also explored in Down's syndrome patients who carry an extra copy of β-amyloid precursor protein (APP) gene on chromosome 21 and suffer from early olfactory symptoms (Crino et al., 1985; Mann et al., 1986; Warner et al., 1988; Zucco and Negrin, 1994; Murphy and Jinich, 1996;

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Abbreviations: AD, Alzheimer's disease; APP, β-amyloid precursor protein; Aβ, β-amyloid peptide; EPL, external plexiform layer; FAD, familial AD; GAP43, growth-associated protein 43; GCL, granule cell layer; GL, glomerular layer; MAP2, microtubule-associated protein-2; NFL, nerve fiber layer; OMP, olfactory marker protein; PBS, phosphate-buffered saline; SEZ, subependymal zone; TH, tyrosine hydroxylase; VGLUT2, vesicular glutamate transporter-2.

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Nijjar and Murphy, 2002; Chen et al., 2006). More recently, studies have shown early-onset olfactory dysfunction and olfaction-related behavioral deficits in transgenic models of AD that carry familial AD (FAD)-related APP/presenilin mutations. Thus, odor discrimination and olfactory memory deficits have been reported in several mouse strains, such as the Tg2576 mice (Young et al., 2009; Wesson et al., 2010a), 2XFAD mice (Phillips et al., 2011; Rey et al., 2012) and 3xTg-AD mice (Cassano et al., 2011).

The primary olfactory system expresses a full set of the amyloidogenic machinery, with APP and its two cleavage enzymes, β-secretase-1 (BACE1) and γ-secretase, concentrated at the glomeruli (Clarris et al., 1995; Iwai et al., 1995; Cai et al., 2001; Yan et al., 2004, 2007; Xiong et al., 2007a; Zhang et al., 2010; Rajapaksha et al., 2011; Cao et al., 2012). Such a pattern raises a compelling question whether the expression of the amyloidogenic proteins may underlie or associate with a certain pathogenic process around the olfactory glomeruli. Postmortem human data indicate that amyloid plaque pathology occurs predominantly in the anterior olfactory nucleus and high cortical centers in AD and Down's syndrome patients, while tau pathology appears to be common in the bulb (Esiri and Wilcock, 1984; Ohm and Braak, 1987; Struble and Clark, 1992; ter Laak et al., 1994; Bahar-Fuchs et al., 2010). Although some studies show a small amount of β-amyloid peptide (Aβ) deposition in the bulb, the lesion is not common around the glomeruli (Kovacs et al., 1999; Attems et al., 2005; Mundiñano et al., 2011). In transgenic AD models, extracellular Aβ deposition can develop in the bulb with age, but is largely restricted to the granule cell layer (GCL) (Zhang et al., 2010; Cassano et al., 2011; Phillips et al., 2011; Rey et al., 2012).

Human olfactory glomeruli undergo apparent age-related degeneration that might be more prominent in AD cases. This change involves loss and disruption of the glomerular structure as well as aberrant sprouting of the olfactory nerve terminals (Meisami et al., 1998; Hoogland et al., 2003). Because the glomeruli serve as the first synaptic relay and integrative center on the olfactory pathway, which is crucial for odor-evoked neurotransmission and all olfactory functions (Johnson and Leon, 2007), we wondered whether glomerular degeneration exists in transgenic AD models, and if so, whether this change relates to the expression of amyloidogenic proteins or amyloid pathogenesis. We explored these issues by examining immunoreactivities with APP, BACE1 and Aβ antibodies relative to olfactory nerve terminal markers and glomerular morphometry. These analyses were carried out systematically in the 5XFAD mice and non-Tg cohorts from 1–12 months of age. We also explored the existence of the pathology in the olfactory bulbs in three other transgenic mouse strains (Tg2576, 2XFAD, and 3xTg-AD) and humans.

EXPERIMENTAL PROCEDURES

Transgenic and control mice

5XFAD [B6SJL-Tg (APPSwFlLon, PSEN1∗M146L∗L286V) 6799Vas/J] transgenic mice were purchased from the Jackson Laboratory (Bar Harbor, ME) (Oakley et al., 2006), and examined at 1, 2, 4, 6, 8 and 10–12 months of age (n = 6 per age point or period). C57BL/6 J (n = 6/point) mice at matched age points were used as controls (Xiong et al., 2007b; Zhang et al., 2009). Bulbs from other transgenic AD model mice (Tg2576, 2XFAD, and 3xTg-AD) were examined during our recent studies (Zhang et al., 2009, 2010; Cai et al., 2012). Animal use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Animal Care and Use Committee of Southern Illinois University at Carbondale.

Postmortem human olfactory bulbs

Human olfactory bulbs were collected from teaching cadavers and human brain bank collections in accordance with the willed body/brain donation program approved by the Education/Research Human Ethics Committee of Xiangya School of Medicine. Detailed clinical records were not released by the hospitals, but patient age, sex, occupation and final diagnosis of death cause (including information about senile dementia) were documented. Cadavers were perfused 13–36 h postmortem with 10% formalin in saline via heart, and stored in body chambers (4 °C) for 3–24 months before brain removal. One olfactory bulb and a slice (approximately 0.5 cm thick) of the middle temporal lobe were collected from each brain for the present study. Bulbs and cortical blocks were soaked with four changes of 0.01 M phosphate-buffered saline (PBS, pH = 7.4) for 2 days, and transferred subsequently into 30% sucrose until sunk at 4 °C before histological preparation. Samples from a total of 64 individuals died at 20–83 years of age were initially prepared. Given that the amyloid and tau pathologies are currently used as pathological diagnostic standards for AD, we examined these lesions in the temporal cortex and hippocampal formation using 6E10, BACE1, and two p-tau antibodies. Cases were selected based on the presence of BACE1 labeling in the hippocampal mossy fiber terminals in order to exclude samples with poor preservation of antigenicity. We thus identified 40 usable cases for the present study (Table 1). The young/adult group consisted of 17 cases died at 20–50 years (mean = 33.5 years, 11 males and six females). The aged "non-AD control" group consisted of 13 cases from 65–78 years old (mean = 69.2 years, seven males and six females), without amyloid and tau pathologies detectable in temporal lobe structures. The "AD" group included 10 cases from 65–80 years of age (mean = 75.7 years, five males and five females); all had amyloid and tau pathologies in the temporal cortex and hippocampal formation.

Tissue preparation

Mice were perfused transcardially with 4% paraformaldehyde in PBS under overdose anesthesia (sodium pentobarbital 100 mg/kg, i.p.). Brains were dissected out, bisected, postfixed in the perfusion fixative overnight, and cryoprotected in 30% sucrose at 4 °C. Hemi-brains were cut coronally and sagittally using a cryostat. For sagittal sectioning, the bulb and frontal cortex were cut with the initial cutting plane set parallel to the median cerebral surface. Fiducial markers (needle hole or small corner cutting) were made on/in brain blocks or during cutting, to help later identification of sections from different cases after a same batch of immunolabeling. Eight sets of 30-μm thick sections were collected in order in PBS in cell culture plate, for immunolabeling with avidin–biotin complex (ABC) method and Nissl stain. Additional 10 sets of 6-μm thick sections were collected by thaw-mounting on positively charged microslides, specifically for double immunofluorescence. Thus, each set contained equally-spaced sections ~300 μm apart. Human bulbs and temporal cortex were cut perpendicular to the long axis of the bulb and approximately at the frontal cerebral plane, respectively. Ten sets of adjacent sections at 40 μm thickness were collected free-floating and 10 sets of adjacent 6 μm thaw-mounted on slides. One-millimeter thick tissue was then trimmed off, followed