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Retinal ganglion cell dendritic degeneration in a mouse model of Alzheimer's disease

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ABSTRACT

Retinal ganglion cells (RGCs) may be regarded as a target biomarker in Alzheimer's disease (AD). We therefore explored the possibility that RGC degeneration, rather than cell loss, is an early marker of neuronal degeneration in a murine model of AD. RGC dendritic morphology and dendritic spine densities of CA1 hippocampal pyramidal neurons were quantified in 14-month-old transgenic mice expressing the APP(SWE) (amyloid precusor protein-Swedish mutation) mutation (Tg2576). The dendritic integrity of RGCs was found to be significantly reduced in the absence of significant RGC loss in Tg2576 mice compared with age-matched wild-type controls. In hippocampal CA1 pyramidal neurons, we observed dendritic spines to be present at a lower frequency from the same animals, but this did not reach significance. Synaptic and mitochondrial protein expression markers (PSD95 [postsynaptic density protein 95], synaptophysin, and Mfn2 [mitofusin 2]) showed no significant changes in RGC synaptic densities but a highly significant change in mitochondrial morphology with a marked reduction in the integrity of the mitochondrial cristae. Our findings suggest that, in a well-characterized mouse model of AD, RGC dendritic atrophy precedes cell loss, and this change may be because of accumulations of amyloid-β. Because RGC dendrites are confined to the inner plexiform layer of the retina, imaging techniques that focus on this layer, rather than the loss of RGCs, may provide a sensitive biomarker for monitoring neural damage in AD.

1. Introduction

Alzheimer's disease (AD) remains a major cause of cognitive decline in the Western population. Considerable advances have been made in our understanding of the genetic basis of this disease, but the development of biomarkers for staging the disease remains a challenge. In the search for an optimal set of biomarkers, central nervous system imaging has shown the most promise (McEvoy and Brewer, 2010) and significant progress has been made in the use of magnetic resonance imaging in combination with ligands that can highlight areas of central nervous system pathology (Rowe et al., 2007, 2008) and positron emission tomography (Klunk et al., 2004) (reviewed in Furst and Kerchner, 2012). These methods rely either on the detection of amyloid plaques or on the neuronal and axonal loss. Although these are important stages in the disease process, there is compelling evidence that neurons undergo a prolonged period of degeneration before cell death, manifest as neurite loss and dendritic pruning (Anderton et al., 1998; Selkoe, 2002). The hippocampus and entorhinal cortex have consistently demonstrated early neuronal damage in AD, but the detection of this neurite loss in vivo in these areas is problematic.

The retina is the only tissue in which neurons can be imaged on a repeatable and long-term basis; the eye has, therefore, been considered as a "window on the brain" where optical imaging might enhance our ability to detect neurodegenerative disease (Koronyo et al., 2012; Koronyo-Hamaoui et al., 2011; Miller and Drachman, 2006). Recent developments in interference-based imaging methods, such as optical coherence tomography (OCT), indicate that it is possible to detect optical signals arising from changes in subcellular structures (Gossage et al., 2003; Kajic et al., 2010). Ultra–high-resolution OCT can provide retinal images at resolutions that are subcellular without the need for exogenous ligands (Drexler and Fujimoto, 2008; Povazay et al., 2009) raising the prospect that these imaging technologies could be used clinically to characterize retinal neuronal changes. Retinal ganglion cells (RGCs) in particular comprise an ideal candidate population

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(Cordeiro et al., 2010). They may be uniquely vulnerable to the effects of neurodegeneration because they have long axons for which the intraocular portion is unmyelinated thereby increasing energy requirements and cellular stress. Several investigators have tested the possibility that this cell population may be reduced in AD (Jindahra et al., 2010). In addition, amyloid-β (Aβ) deposits, a hallmark feature of AD, have been found in both AD patients and animal models of AD (Koronyo-Hamaoui et al., 2011). Postmortem studies in patients with AD have, however, been equivocal with regard to the loss of RGCs; some have reported significant reductions in RGC populations (Blanks et al., 1989, 1996b), but these have not been confirmed by others (Curcio and Drucker, 1993). Clinical imaging studies using OCT have reported significant loss of cells in the RGC layer with corresponding thinning of the retinal nerve fiber layer in patients with clinically manifest AD (Paquet et al., 2007). The development of novel ligands to identify in vivo RGC death driven by the experimental administration of Aβ peptide (1–40) raises the possibility that RGC loss could provide a valuable biomarker for AD (Guo et al., 2007).

Because a loss of synaptic connectivity is likely to be one of the earliest pathological changes in AD rather than neuronal loss (Selkoe, 2002), we reasoned that RGC dendritic integrity would provide a sensitive marker of neuronal degeneration. Reduced dendrite complexity has been reported in models of chronic RGC degeneration (Morgan et al., 2006; Weber et al., 1998; Williams et al., 2012). Because these changes are similar to those observed in AD (Scheff and Price, 2003; Scheff et al., 2006; Selkoe, 2002), we tested the hypothesis that RGC dendritic degeneration may provide a marker for central nervous system damage in a mouse (Tg2576) model of amyloid pathology. Animals were evaluated at 14 months of age by which time they have developed significant cognitive deficits associated with the deposition of Aβ plaques in cortical and limbic structures (Hsiao et al., 1996).

2. Materials and methods

2.1. Retinal preparation

All animals were in good health at the time of the study. Fourteen-month-old, female APP(SWE) Tg2576 mice (Tg, $n = 9$) (Chapman et al., 1999) and their age- and sex-matched control littermates (WT, $n = 8$) were killed by cervical dislocation, and the eyes were quickly enucleated and placed in chilled (4 °C) HBSS (Hank's balanced salt solution) (Invitrogen, UK). The eyes were punctured at the limbus and a slit cut in the sclera to remove the cornea and sclera anterior to the ora serrata, along with the lens and vitreous. Three cuts were made in the retina before it was flat-mounted, ganglion cell layer up, on a cell culture insert (Millipore, Billerica, MA, USA) and submerged in custom media (CM) containing Neurobasal media, 2% B-2 supplement, 1% N-27 supplement, and 0.5 mM glutamate (Invitrogen). Retinas were incubated at 37 °C and 4% CO_2 ready for DiOlistic labeling using a gene gun or labeling with Hoechst 33258 stain. The total time between death and DiOlistic labeling was less than 10 minutes. Because the APP(SWE) Tg2576 transgenic mouse line carries the retinal degeneration, "*rd*," mutation that adversely affect retinal anatomy, all mice were routinely genotyped to exclude any $rd^{-/-}$ mice from the study.

2.2. Brain slice preparation

The brains from all mice were sectioned sagittally in an ice-cold phosphate-buffered saline (PBS) at 250 μm using a Leica VT1000S vibratome (Leica Microsystems, UK). Approximately 3 consecutive sections from each brain hemisphere were immediately placed into CM in 6-well plates. The CM was then removed for DiOlistic labeling, replaced, and incubated at 37 °C and 4% CO_2. The total time between death and DiOlistic labeling of brain slices was less than 25 minutes.

2.3. DiOlistic labeling

DiOlistics, a modified gene-gun procedure, which has been previously used to quantify dendritic changes in AD models (Smith et al., 2009), relies on the affinity of carbocyanine dyes to the phospholipid membrane of cells. Using a relatively high pressure (typically greater than 100 psi), carbocyanine dyes adhered to tungsten particles are propelled through the barrel of the gene-gun into the tissue in culture (O'Brien and Lummis, 2004, 2006). This method gives 2 main advantages over common immunohistochemical (IHC) labeling or tracer studies: (1) labeling is random and typically with a low yield allowing for individual cells to be morphometrically analyzed; and (2) these dyes distribute along the membrane regardless of the cell's physiological status.

The setting for bead delivery and preparation for DiOlistic labeling has been described in detail elsewhere (Gan et al., 2000; Sun et al., 2002). Briefly, 100 mg of tungsten particles (1.7 μm; Bio-Rad, Hercules, CA, USA) was placed in a thin, even layer on a clean, glass slide. DiI (1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate) (80 μg; Invitrogen) was then mixed in 800 μL of methylene chloride and poured over the tungsten particles. The methylene chloride evaporated quickly to leave DiI-coated tungsten particles, which were then transferred onto clean wax-paper or tinfoil. This powder was then funneled into a length of 1.4 mm "Gold-Coat" tubing (Bio-Rad) and allowed to settle, resulting in a light application of the powder on the inside of the tubing. Excess powder was funneled off, and the tubing was cut into 1.2 cm lengths for storage in the dark at room temperature (RT) ready for use.

Retinas were shot once at 100 psi using a Helios gene gun (Bio-Rad) through a 3.0-μm pore size, high pore density, cell culture insert (Becton Dickinson, Franklin Lakes, NJ, USA) to block the passage of aggregated tungsten particles. The barrel of the gun was held 5 cm above the retinal explant. Retinas were then incubated for 30 minutes to facilitate dye diffusion before being placed in 4% PFA at RT for a further 30 minutes. Retinal preparations were then mounted RGC side up and coverslipped under ProLong Gold AntiFade Reagent (Invitrogen) containing 1 μg/mL Hoechst 33258 as a nuclear counterstain and sealed with nail polish. Hippocampal slices were shot at 150 psi from 5 cm with a 3.0-μm pore size cell culture insert before being incubated for 30 minutes to facilitate dye diffusion before being placed in 4% PFA at RT for a further 30 minutes. The slices were then mounted in ProLong Gold Anti-Fade Reagent and sealed with nail polish. Images were taken within 24 hours from both retinal and brain samples.

2.4. RGC dendrite morphological analysis

Image stacks of 142 RGCs (0.5 μm slice width) were obtained with a Zeiss LSM 510 confocal microscope (Carl Zeiss Ltd, UK) using a 20× objective to allow the capture of the entire dendritic tree in a single image. Dendritic morphologies were analyzed using ImageJ to measure dendritic field area (measured using the convex polygon tool to join the outermost points of the dendritic tree), an ImageJ plugin, NeuronJ to measure total dendritic length, and a custom Matlab macro to run a Sholl analysis (Gutierrez and Davies, 2007).

2.5. Ganglion cell layer cell counts

For RGC counts, the methods outlined by Jakobs et al. (2005) were used with minor modifications. All retinas were stained with Hoechst 33258 to allow for ganglion cell layer cell counts.