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Selective degeneration of septal and hippocampal GABAergic neurons in a mouse model of amyloidosis and tauopathy

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by brain accumulation of amyloid-β peptide and neurofibrillary tangles, which are believed to initiate a pathological cascade that results in progressive impairment of cognitive functions and eventual neuronal death. To obtain a mouse model displaying the typical AD histopathology of amyloidosis and tauopathy, we generated a triple-transgenic mouse line (TauPS2APP) by overexpressing human mutations of the amyloid precursor protein, presenilin2 and tau genes. Stereological analysis of TauPS2APP mice revealed significant neurodegeneration of GABAergic septo-hippocampal projection neurons as well as their target cells, the GABAergic hippocampal interneurons. In contrast, the cholinergic medial septum neurons remained unaffected. Moreover, the degeneration of hippocampal GABAergic interneurons was dependent on the hippocampal subfield and interneuronal subtype investigated, whereby the dentate gyrus and the NPY-positive interneurons, respectively, were most strongly affected. Neurodegeneration was also accompanied by a change in the mRNA expression of markers for inhibitory interneurons. In line with the loss of inhibitory neurons, we observed functional changes in TauPS2APP mice relative to WT mice, with strongly enhanced long-term potentiation in the medial-perforant pathway input to the dentate gyrus, and stereotypic hyperactivity. Our data indicate that inhibitory neurons are the targets of neurodegeneration in a mouse model of amyloidosis and tauopathy, thus pointing to a possible role of the inhibitory network in the pathophysiological and functional cascade of Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative condition in adult humans, characterized by a slow-progressing decline in cognitive function that results in dementia and eventually death. AD is clinically divided into an early onset or familial (FAD) form, and a sporadic or late onset form (SAD). FAD is associated with genetic mutations in amyloid precursor protein (APP) and presenilin (PS) 1 or 2 (Bertram and Tanzi, 2005; Goate, 2006; Sherrington et al., 1995), making models based on these human mutations highly relevant for investigations on the pathophysiology of FAD. The late-onset cases have been ascribed to apolipoprotein E (APOE) gene mutations that are now considered to be the most important risk factor for the development of SAD (Bertram et al., 2010; Corder et al., 1993; Strittmatter et al., 1993). The histopathological changes occurring in the brains of AD patients include the formation of β-amyloid plaques and tau neurofibrillary tangles. Accordingly, transgenic mouse lines expressing mutated forms of APP, PS or tau provide suitable models for the investigation of AD pathophysiology. However, when mice transgenically express mutated APP either alone or in combination with PS, there is an increase both in Aβ levels and plaque formation, but no development of tau neurofibrillary tangles (Games et al., 1995; Holcomb et al., 1998; Hsiao et al., 1996; Sturchler-Pierrat et al., 1997). While mutations in human tau are yet to be reported in AD patients, tau dysfunction has been linked to neurodegeneration in frontotemporal dementia (Hutton et al., 1998). Moreover, mice overexpressing mutated forms of tau show neurofibrillary pathology and neurodegeneration (Götz and Ittner, 2008). Thus, in order to establish a mouse model displaying the relevant histopathological features of AD, we recently generated a triple-transgenic mouse line (TauPS2APP) expressing mutated forms of tau, PS2 and APP (Grueninger et al., 2010). These mice display an age-dependent accumulation of Aβ, with formation of Aβ-plaques and neurofibrillary tangles in the hippocampus. Notably, accumulation of Aβ in TauPS2APP mice leads to accelerated tau pathology and increased phosphorylation of tau at serine 422 when compared to tau single-transgenic mice. Moreover, TauPS2APP mice are hyperactive and show impaired spatial learning in the Morris water maze. However,

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stereological analysis of pyramidal neurons within the hippocampus did not reveal any signs of neuronal loss (Grueninger et al., 2010). In the present study, we provide a comprehensive analysis of neurodegeneration in TauPS2APP mice in the basal forebrain, an important source of GABAergic and cholinergic input for the hippocampus, and in the hippocampus itself. In addition, we performed electrophysiological characterization of TauPS2APP mice, as well as an age-dependent evaluation of their behavioral activity. Our results show that GABAergic neurons undergo neurodegeneration in a triple transgenic mouse model for amyloidosis and tauopathy, thus suggesting a role for the inhibitory network in the pathophysiology of AD.

Material and methods

Transgenic mice

The transgenic APP (line 147.72H) is homozygous for the human APP Swedish FAD transgene, the transgenic PS2 (line PS2.30H) is homozygous for human PS2 N141I and the transgenic PS2APP (line B6.152H) is homozygous for both human PS2 N141I and APP Swedish FAD transgenes. The double transgenic mouse line was created by co-injection of both transgenes into C57BL/6 zygotes, as previously described (Ozmen et al., 2009). The transgenic TauP301L mouse (line pR5) was generated on a mixed C57Bl/6, DBA/2 background (Gotz et al., 2001a, 2001b) and backcrossed for seven generations into C57Bl/6 before generation of the triple transgenic (TauPS2APP) mice. TauPS2APP mice were obtained as recently described (Grueninger et al., 2010). Mice were screened by PCR using oligonucleotide pairs specific for the transgenes that overlap promoter and coding regions, resulting in the amplification of fragments that are only present in the transgenic mice. Age-matched C57Bl/6 mice were used as wild-type (WT) controls. Mice were housed under a 12-h light/12-h dark cycle (lights on at 6:00 a.m., unless otherwise stated) at 20–22 °C, with ad libitum access to food and water. All procedures were conducted in strict adherence to the Swiss and German federal regulations for animal protection and to the rules of the Association for Assessment and Accreditation of Laboratory Animal Care, with the explicit approval of the local veterinary authority.

Histology and immunohistochemistry

All animals assigned to morphological analyses were anesthetized with a mixture of ketamine, xylazine and acepromazine (0.3 ml/20 g body weight) and transcardially perfused with 4% paraformaldehyde in PBS. Coronal brain sections (50 μm) were cut on a vibratome and the sections stored in 0.1 M PB. Histological examination of fornix morphology was performed on routine Nissl-stained vibratome sections at different rostro-caudal levels. For immunohistochemistry, free-floating sections were treated with 3% H_2O_2 in 0.1 M PB for 15 min, followed by pre-incubation in a solution containing 5–10% normal serum of the species in which the secondary antibody was raised (see secondary antibodies below), 2% BSA, and 0.1% Triton X in 0.1 M PB for 1 h at room temperature (RT). The following primary antibodies were used: polyclonal anti-choline acetyltransferase (ChAT, 1:100 dilution; Millipore); monoclonal anti-GAD67 (1:20,000 dilution; Millipore); polyclonal anti-parvalbumin (PV, 1:5000 dilution; Swant); polyclonal anti-Somatostatin (SOM, 1:5000 dilution; Bachem); polyclonal anti-calretinin (CALRET, 1:5000 dilution; Swant) and polyclonal anti-neuropeptide Y (NPY, 1:1500 dilution; ImmunoStar Inc., Hudson, WI, USA). After incubation with primary antibodies the sections were transferred to solutions containing biotinylated goat anti-rabbit IgG (for parvalbumin, somatostatin, calretinin and NPY), biotinylated rabbit anti-mouse IgG (for GAD-67), or biotinylated rat anti-goat IgG (for ChAT; all secondary antibodies: Vector Laboratories, Burlingame, CA), diluted 1:250 in PB for 2 h at RT. Subsequent visualization of the labeling by an avidin–biotin complex (1:250; Elite Vectastain ABC kit, Vector Laboratories, Burlingame, CA) was performed using DAB as a substrate. Immunofluorescence stainings were used for double labeling of sections using ptau (PS422, 1:500) or Aβ (BAP-2, 1:1000) antibodies and GAD67 antibody, respectively. These sections were labeled with primary antibodies and visualized with cy3- (dilution 1:800; Jackson ImmunoResearch Laboratories INC., West Grove, PA, USA) or Alexa 488-labeled (dilution 1:250; Invitrogen, Karlsruhe, Germany) secondary antibodies.

The cholinergic fiber density in the hippocampus was examined using acetylcholine esterase (AChE)-histochemistry according to previous protocols (Eckenstein and Sofroniew, 1983), with acetylcholine as the substrate and ethopropazine as the inhibitor of non-acetylcholinesterase.

Stereological cell counts

Stereological cell counts (Stereo Investigator software: MicroBrightField, Inc., Colchester, VT; version 4.31) were performed to determine the number of ChAT- and PV-immunoreactive (ir) neurons in the medial septum-vertical limb of the diagonal band complex, and the respective number of GAD-67-, PV-, SOM-, CALRET- and NPY-ir interneurons in the hippocampus. Every second serial section (50 μm) of the medial septum/vertical limb of the diagonal band and every third serial section of the hippocampus was used for stereological analysis. The optical disector/fractionator method (OF) was applied to each region as previously described (Guijarro et al., 2006; Naumann et al., 2002; West et al., 1991). Briefly, sections were visualized on a computer screen attached to an Olympus BX60 microscope F5 (Olympus Optical Co. Ltd, Düsseldorf, Germany) with a computer-controlled stepper motor stage and focus assembly that allow movement in the x-, y- and z-axes. Cell counts were performed using Stereo Investigator software (version 3.0; MicroBrightField, Inc., Colchester, USA). The region of interest was first outlined in each section at low magnification (4× objective, nA: 0.10) and the following parameters were implemented: a counting frame of 50×30 μm; a guard zone of 2 μm; and a counting depth of 8 μm. The view was then switched to high magnification (100× oil objective, NA: 1.35,), where immuno-positive cells that fulfilled the criteria of the unbiased counting rules (e.g. presence of a recognizable soma within the counting frame, somata showing a distinct nucleus; cf. Coggeshall and Lekan, 1996) were marked and added to the probe run list. Total cell numbers estimated by the OF were statistically analyzed by a two way-analysis of variance (ANOVA; for details see Naumann T et al., 2002). Statistical significance was analyzed for the corresponding classifiers and classes (WT, TauPS2APP).

Quantification of AChE staining

The density of cholinergic fibres in subregions of the dorsal and ventral hippocampus (i.e. CA1, CA3, dentate gyrus) was determined by optical densitometry measurements (n = 6 per genotype). After delineation of the relevant region of interest on digital images the mean optical density (OD) of AChE-ir was measured using image analysis software (Olympus SIS, Stuttgart, Germany). The mean OD in the region of the corpus callosum in each section was considered as 'background' and subtracted from all mean ODs measured in the regions of interest.

Ultrastructural analysis

For electron microscopy (EM), 12-month-old WT and TauPS2APP mice ($n = 3$ for each genotype) were deeply anesthetized as described above and transcardially perfused with 0.9% saline followed by a fixative containing 4% PFA and 1% glutaraldehyde in 0.1 M PB, pH 7.4. Brains were removed and postfixed in the same fixative for 12 h. Tissue blocks were washed with PB and horizontal sections (50 μm) were cut on a vibratome. Sections were treated with OsO4,