Cholinergic axon length reduced by 300 meters in the brain of an Alzheimer mouse model

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Received 9 May 2011; accepted 10 May 2011

Abstract

Modern stereological techniques have been used to show that the total length of the cholinergic fibers in the cerebral cortex of the APPswe/PS1deltaE9 mouse is reduced by almost 300 meters at 18 months of age and has a nonlinear relationship to the amount of transgenetically-induced amyloidosis. These data provide rigorous quantitative morphological evidence that Alzheimer’s-like amyloidosis affects the axons of the cholinergic enervation of the cerebral cortex.

Keywords: VAChT; Stereology; Space balls; Axons; Fractionator

1. Introduction

Disturbances in the cholinergic enervation of the brain are consistent features of Alzheimer’s disease (AD). These include the loss of cholinergic neurons in the basal forebrain and reductions in the cholinergic markers in the cerebral cortex and hippocampal region (Davies and Maloney, 1976; Mufson et al., 1987; Whitehouse et al., 1981). Cholinergic axons from basal forebrain neurons (Mesulam et al., 1983) synapse directly on principal and interneurons in cortex (Freund and Buzsáki, 1996) and presynaptically regulate a number of transmitter systems (Vizi and Kiss, 1998). These disturbances can be expected to contribute to the cognitive decline that accompanies AD. Although there is accumulating evidence that disturbances in axonal transport-related AD amyloidosis, are involved (Salehi et al., 2007), the quantitative relationship between the amyloidosis associated with AD and disturbances in the cholinergic system is not clear.

Transgenic mouse models of AD-like amyloidosis that harbor genes related to mutations found in familial forms of AD, similarly show evidence of disturbances found in the cholinergic systems. These include reductions in acetylcholine (ACh) markers (Savonenko et al., 2005), including choline acetyltransferase (ChAT) (Perez et al., 2007) and vesicular acetylcholine transporter (VAChT)-positive boutons (Wong et al., 1999); aberrant cholinergic fibers in the vicinity of amyloid plaques (Boncristiano et al., 2002; Perez et al., 2007); decreases in the density of ACh fibers (Perez et al., 2007) and synaptic vericosities (Boncristiano et al., 2002; Bronfman et al., 2000); and increases in the size of cell bodies (Boncristiano et al., 2002; Perez et al., 2007; Salehi et al., 2007). In many of these models, including the APP/PS1deltaE9 mouse model studied here, these disturbances occur in the absence of neuron and synapse loss in glutamatergic systems (Volianskis et al., 2010; West et al., 2009). The relative specificity of these alterations in the APP/PS1deltaE9 mouse provide an opportunity to study the cellular mechanisms by which aberrant amyloid metabolism results in neurodegenerative changes in the cholinergic system, to dissect their inductive and responsive roles in the pathogenesis of AD, and to better understand the basis of the selective vulnerability of the cholinergic fibers.

To provide a more rigorous structural framework in which to study the effect of AD like amyloidosis on the
cholinergic enervation of the forebrain, we have estimated the total length of the cholinergic fibers in the cerebral cortex of a transgenic mouse model of AD-like amyloidosis at 3 different ages.

2. Methods

2.1. General

The total length of the cholinergic fibers was estimated in the cerebral cortex of a transgenic mouse model of AD-like amyloidosis using space ball probes and fractionator sampling. Mean lengths of VChT immunoreactive fibers were compared for groups of transgenic (Tg) mice and wild-type (Wt) littermates of different ages using analysis of variance (ANOVA). All aspects of the animal studies were carried out in accordance with the guidelines for animal experiments in Denmark.

2.2. Animals

Groups of 4-month-old (Wt, n = 10; Tg, n = 6), 10-month-old (Wt, n = 8; Tg, n = 6), and 18-month-old (Wt, n = 7; Tg, n = 7), heterozygotic, female APPswe/PS1dE9 transgenic mice (Borchelt et al., 1996, 1997), line 85 that express chimeric mouse/human AbetaPP-695 with mutations KM 593/594 NL and PS1 (dE9: deletion of exon 9) (Jankowski et al., 2001), and age- and gender-matched Wt mice were used in the study. This transgenic mouse starts to develop amyloid plaques in the cerebral cortex at 4 months of age and an age-related increase in Abeta_{42} (A \beta_{42}) levels and in the ratio A \beta_{40} to A \beta_{42}.

2.3. Tissue preparation

The mice were anesthetized and transcardially perfused with 10 mL phosphate buffer followed by 10 mL Bouin’s fixative. The brains were removed and postfixed for 4 hours in Bouin’s fixative and transferred to a 70% alcohol solution for 1 to 3 days. The brains were then divided at the midline and the right hemisphere was exhaustively sectioned in the frontal plane into 50-μm thick slabs with a vibratome. A systematic random series of every sixth section was selected for the analysis of each mouse, resulting in an average of 22 sections per mouse hemisphere.

2.4. Immunostaining

Each series of sections was immunohistochemically stained with the anti-vesicular acetylcholine transporter protein vesicular acetylcholine transporter (VAcHT) (139103; Synaptic Systems, Goettingen, Germany) to visualize the cholinergic axons, using an antibody concentration of 1:1000 (Fig. 1). Salient aspects of the staining procedure included incubating the free-floating sections with avidin and then biotin. After preincubation with 0.2% milk in tris-buffered saline (TBS) pH 7.4 with 1% Triton X-100, the sections were incubated with primary antibody, rabbit anti-VAcHT, in TBS with 0.2% milk, and 1% Triton X.

2.5. Stereology

2.5.1. Delineation of the cerebral cortex

The estimates of fiber length were based on samples taken from the entire extent of the cerebral cortex dorsal to the rhinal fissure, including cingulate cortex, infralimbic cortex, dorsal peduncular cortex (except at levels rostral to interaural [ia], distance +5.7), dorsal tenia tecta, medial orbital cortex, ventral orbital cortex (except at ia Level +5.78), and excluding claustrum using the mouse brain atlas of Franklin and Paxinos (2008) in the section series from the right side of the brain.

2.5.2. Sampling scheme

Hemispheric space ball probes (Fig. 1) with a diameter of 4 mm were used for all of the estimates of total ACh fiber length (Mouton et al., 2002). Accordingly, the surface area of the probe was 100.5 μm². A systematic random sampling scheme was used to sample each of the sections with the space ball probes. The distance between the probed positions was 1000 μm, so that the area associated with each probe, A_{step}, was 1 million μm². The coefficients of error (CEs) were calculated taking into account the variability, between sections and within sections, in the number of intersections between VAcHT stains and the surface of the space ball probes (Slomianka and West, 2005). This re-
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