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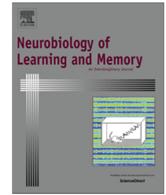

Cholinergic neurodegeneration in an Alzheimer mouse model overexpressing amyloid-precursor protein with the Swedish-Dutch-Iowa mutations

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder that is mainly characterized by beta-amyloid (Aβ) plaque deposition, Tau pathology and dysfunction of the cholinergic system causing memory impairment. The aim of the present study was to examine (1) anxiety and cognition, (2) Aβ plaque deposition and (3) degeneration of cholinergic neurons in the nucleus basalis of Meynert (nbM) and cortical cholinergic innervation in an Alzheimer mouse model (APP_SweDI; overexpressing amyloid precursor protein (APP) with the Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutations). Our results show that 12-month-old APP_SweDI mice were more anxious and had more memory impairment. A large number of Aβ plaques were already visible at the age of 6 months and increased with age. A significant decrease in cholinergic neurons was seen in the transgenic mouse model in comparison to the wild-type mice, identified by immunohistochemistry against choline acetyltransferase (ChAT) and p75 neurotrophin receptor as well as by in situ hybridization. Moreover, a significant decrease in cortical cholinergic fiber density was found in the transgenic mice as compared to the wild-type. In the cerebral cortex of APP_SweDI mice, swollen cholinergic varicosities were seen in the vicinity of Aβ plaques. In conclusion, the present study shows that in an AD mouse model (APP_SweDI mice) a high Aβ plaque load in the cortex causes damage to cholinergic axons in the cortex, followed by subsequent retrograde-induced cell death of cholinergic neurons and some forms of compensatory processes. This degeneration was accompanied by enhanced anxiety and impaired cognition.

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1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder that accounts for most cases of dementia in the elderly population. The neuropathological hallmarks of AD are beta-amyloid (Aβ) plaque deposits, Tau neurofibrillary tangles, neuronal and synaptic loss, inflammation and cerebrovascular damage. Senile Aβ plaques consist mainly of insoluble deposits of 40- or 42-amino acid long Aβ peptides that are produced by sequential cleavage of the transmembrane glycoprotein amyloid precursor protein (APP) (Selkoe, 2001). It has been shown that mutations in human APP are associated with early onset familial AD (Chartier-Harlin, Crawford, Houlden, et al., 1991; Goate et al., 1991; Murrell, Farlow, Ghetti, & Benson, 1991). The exact processes of genesis of Aβ plaques in AD is not entirely understood, but the Aβ cascade is the most prominent hypothesis for the development of AD (Hardy & Higgins, 1992; Marchesi, 2005; Reitz, 2012), suggesting that the excessive accumulation of insoluble Aβ peptides induces a series of events that leads to neuronal dysfunction and death.

Degeneration of the basal forebrain cholinergic system is an important pathophysiology of AD (Perry et al., 1978; Schliebs & Arendt, 2006). Cholinergic neurons provide the main source of acetylcholine (ACh) in the cortex and a decline in ACh directly correlates with cognitive impairment (Perry et al., 1978). The number of cholinergic neurons has consistently been found to be reduced in the basal forebrain of advanced AD (Davies & Maloney, 1976; Whitehouse, Price, Struble, & Clarke, 1982), accompanied by decreases in choline acetyltransferase (ChAT) activity (Lehéricy et al., 1993; Wilcock, Esiri, Bowen, & Smith, 1982). A lack of ACh in the cerebral cortex directly correlates with cognitive decline. These findings led to the cholinergic hypothesis of AD (Francis, Palmer, Snape, & Wilcock, 1999), proposing that the loss of basal

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forebrain cholinergic neurons and the associated decline in cholinergic innervation of cortical areas is causal for cognitive dysfunction in AD patients.

Murine transgenic mouse models are the state of the art for exploring mechanisms of plaque genesis in AD pathology. A variety of transgenic murine models for AD have been generated by overexpression of human mutated APP (Games et al., 1995). In the present study we used an AD mouse strain expressing human APP with the Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutations (APP_SweDI). These mice develop plaques within four to six months in all areas of the brain and also in vessels, called amyloid angiopathy (Davis et al., 2004). However, these mice lack any Tau pathology. Previous analysis of cholinergic neuropathology in another APP transgenic mouse strain (the PDAPP mice) showed an age-related significant reduction in the density of cholinergic nerve terminals in the cerebral cortex, but no decrease in basal forebrain cholinergic somata (German et al., 2003). However, so far, cholinergic neurodegeneration has not been investigated in the APP_SweDI AD mouse model.

The aim of the present study was to explore in this AD mouse model whether the deposition of plaques in the brain directly correlates with a decline in cholinergic neurons and innervation. As the neurotransmitter ACh is responsible for memory and learning, we want to study whether these mice are also impaired in learning and memory in the classical labyrinth. To support these findings the mice will also be tested for anxiety in the black/white box and the plus maze. These data should provide more insights into the complex interaction between plaques, cholinergic innervation and memory and anxiety.

2. Methods

2.1. Transgenic Alzheimer mice

Wildtype (WT, C57BL/6N) and transgenic APPSweDI (TG, expressing amyloid precursor protein (APP) harboring the Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutations; C57BL/6-Tg(Thy1-APPSwDutIowa) BWevn/Mmjax) mice were purchased from The Jackson Laboratory (MMRRC, Bar Harbor, ME, USA) and housed at the Medical University of Innsbruck animal facility providing open access to food and water under 12 h/12 h light/dark cycles. The mice were generated and extensively characterized by Davis et al. (2004). All animals were genotyped according to standardized methods. All animal experiments were approved by the Austrian Ministry of Science and Research (BMWF-66.011/0044-II/3b/2011 and BMWF-66.011/0059-II/3b/2011) and conformed to the Austrian guidelines on animal welfare and experimentation. All possible steps were taken to reduce suffering and the number of animals used during the experiment.

2.2. Black/white test box

Anxiety was assessed using the black/white (B/W) box, also known as the light/dark exploration test (Fig. 1B). This model consists of two inter-connected compartments that vary in color (white/black) and illumination (light/dark) (Fig. 1B). In the first week (day 1) mice were adapted to the environment and the researcher; anxiety was recorded on day 5 (Fig. 1A). Memory of anxiety was tested in week 3 on day 1. (Fig. 1A). Mice were placed in the black, shaded area where they naturally feel safer than in the illuminated white area. To quantify anxiety the first move from the black to the white area and the time spent in the black area were recorded.

2.3. Classical mouse labyrinth

Spatial learning and memory were assessed in a well-established simple classical mouse labyrinth (Fig. 1C). On the first day of week 2 mice were adapted to the maze and the environment. On day 2 mice were trained to find a target. Mice were conditioned to 28 ± 2 mg chocolate (Ferrero© Nutella). The memory to find this target was recorded on day 5 (week 2) as well as in week 4 on day 1 (Fig. 1A). Quantification of cognitive performance (spatial learning and memory) was done by measuring the time taken by the mice to find the target and by calculating the number of working memory errors made by entering a wrong maze arm.

2.4. Plus maze discriminative avoidance task

To support the data obtained with the anxiety model and the classical maze we additionally performed the plus maze discriminative avoidance task as reported by Silva and Frussa-Filho (2000). This model permits learning/memory and anxiety to be measured simultaneously. On the first day, the training session was performed. Each mouse was placed in the centre of the apparatus (Fig. 1D), where they had 10 min time to explore the maze. The apparatus consists of two enclosed arms (one of them with aversive stimuli from a 100-W light bulb and an 80-dB noise every time a mouse entered the arm) and two open arms. The noise was induced by beating a metal stick against the wooden wall of the enclosed aversive arm and at the same time the light was turned on for as long as the animal stayed in this arm. Additional visual cues (stickers showing arrows, plus signs, hands, stop sign) that the mice could use to differentiate between the arms were placed on each side of the maze. Twenty-four hours after the training session the test session was performed. The mice were again placed in the middle of the maze, but had only 3 min time to explore the maze without being given any aversive stimuli. By measuring the time spent in each arm, memory related to the learned aversive stimuli was quantified. At the same time information about the anxiety level can also be given by measuring the time the mice spent in the open arms of the apparatus. To exclude olfactory cues, all of the apparatuses were cleaned with 70% EtOH after every trial.

2.5. Tissue collection

At the end of the experiment (one week after the last behavioral testing), animals were anesthetized by subcutaneous sodium thiopental (12.5 mg/ml, 1 ml) injection. A transcardial perfusion was performed with 20 ml of phosphate-buffered saline (PBS) containing EDTA and heparin and then subsequently with 50 ml of 4% paraformaldehyde (PFA) in PBS. The brain was removed, post-fixed in 4% PFA for 3 h at 4 °C, and then immersed in a 20% sucrose/PBS solution overnight. Brains were frozen in a CO_2 stream and cryosectioned (40 μm).

2.6. Immunohistochemistry

Immunohistochemistry was performed as previously described under free-floating conditions (Ullrich, Pirchl, & Humpel, 2010). The sections were washed with PBS and incubated in PBS/0.1% Triton (T-PBS) for 30 min at 20 °C while shaking. To quench endogenous peroxidase, sections were treated with PBS/1%H_2O_2/5% methanol. After incubation, the sections were blocked in T-PBS/20% horse serum (GIBCO Invitrogen)/0.2% BSA (SERVA) for 30 min at 20 °C while shaking. Following blocking, brain sections were incubated with primary antibody (beta-amyloid, 1-16 (6E10) Covance SIG-39300; p75 neurotrophin receptor Promega G3231; choline acetyltransferase ChAT Millipore AB144P) in T-PBS/0.2% BSA for two days at 4 °C. The sections were then washed

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