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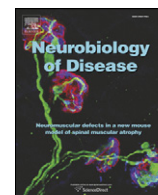

Functional cholinergic damage develops with amyloid accumulation in young adult APPswe/PS1dE9 transgenic mice

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ABSTRACT

We investigated the functional characteristics of pre- and postsynaptic cholinergic transmission in APPswe/PS1dE9 double transgenic mice at a young age (7-10 weeks) before the onset of amyloid plaque formation and at adult age (5-6 months) at its onset. We compared brain slices from cerebral cortex and hippocampus with amyloid deposits to slices from striatum with no amyloid plaques by 6 months of age. In young transgenic mice we found no impairments of preformed and newly synthesized [^{3}H]-ACh release, indicating intact releasing machinery and release turnover, respectively. Adult transgenic mice displayed a significant increase in preformed [^{3}H]-ACh release in cortex but a decrease in hippocampus and striatum. The extent of presynaptic muscarinic autoregulation was unchanged. Evoked release of newly synthesized [^{3}H]-ACh was significantly reduced in the cortex and hippocampus but unchanged in the striatum. Carbachol-induced G-protein activation in cortical membranes displayed decreased potency but normal efficacy in adult animals and no changes in young animals. These results indicate that functional pre- and postsynaptic cholinergic deficits are not present in APPswe/PS1dE9 transgenic mice before 10 weeks of age, but develop along with β-amyloid accumulation in the brain.

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Introduction

The characteristic post-mortem morphological finding in Alzheimer's disease (AD) is the presence of senile plaques comprising β-amyloid peptides as their main constituent and neurofibrillary tangles in the cerebral cortex and hippocampus. However, these pathological changes are also found in post-mortem brains of elderly persons with no history of clinical symptoms of the disease (Snowdon, 2003). The primary event in the pathogenesis of AD is considered to be increased production of noxious β-amyloid fragments composed of 39–43 amino acids. The biologically active form of β-amyloid consists of soluble oligomers (Haass and Steiner, 2001; Klein et al., 2001) that appear in the brain earlier than amyloid plaques and neurofibrillary tangles.

In rare familial cases of AD overproduction of β-amyloid fragments is due to known defects in amyloid precursor protein (APP), presenilin 1, or presenilin 2 genes (Selkoe, 2001). However, the reason for increased production of β-amyloid is unknown in the sporadic form of the disease that represents the overwhelming majority of cases. Original neurochemical findings in Alzheimer's disease brains pointed out disturbances of acetylcholine metabolism (Bowen et al., 1976; Davies and Maloney, 1976; Perry et al., 1977a,b; Sims et al., 1980, 1981; Francis et al., 1985 and 1999) and led to formulation of the “cholinergic hypothesis” of Alzheimer's disease (Bartus et al., 1982). Since then, a large body of evidence has accumulated either in support of or questioning this hypothesis (Bartus, 2000). The crucial question is whether disturbances of cholinergic mechanisms are present and play a role at the beginning of the pathogenesis of AD or simply reflect a general neurodegeneration that afflicts many neurotransmitter systems in the late or terminal stage of the disease. This is a very important issue because in addition to the involvement of muscarinic neurotransmission in cognitive functions, stimulation of M_1 and M_3 subtypes of muscarinic receptors leads to non-amyloidogenic cleavage of the amyloid precursor protein (Buxbaum et al., 1992; Nitsch et al., 1992). We have recently demonstrated in the cerebral cortex of a transgenic APPswe/PS1dE9 mouse model of AD (Jankowsky et al., 2004) a reduction of vesicular acetylcholine transporter protein levels and a functional decline of muscarinic neurotransmission (Machová et al., 2008). These deficits were already apparent in 7-month-old transgenic animals and deteriorated further with aging. APPswe/PS1dE9 mice demonstrate appearance of plaques at about 4 months of age (Shemer et al., 2006) and manifest cognitive deficits between 10–15 months of age (Savonenko et al., 2005; Minkeviciene et al., 2008).

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Cholinergic neurotransmission depends on the ability of cholinergic nerve terminals to synthesize, store and release acetylcholine (ACh), and on the ability of postsynaptic cells to adequately respond to released ACh. Our objective was to determine the functional integrity of cholinergic terminals in young (7–10 weeks) and adult (5–6 months) APPswe/PS1dE9 mice, i.e. before and at an early stage of amyloid pathology. We performed two types of *ex vivo* experiments on cortical, hippocampal and striatal slices. In the first set of experiments we investigated the release of previously stored (preformed) ACh and its presynaptic autoregulation. These experiments tested the releasing machinery and function of muscarinic inhibitory autoreceptors. In the second set of experiments we estimated the capacity to maximally release newly synthesized ACh that is additionally limited by the supply of substrates for ACh synthesis and loading of ACh to synaptic vesicles. In addition, we probed the capacity of the ACh analog carbachol in activating muscarinic receptors/G-protein coupling in cortical membranes that show age and transgene-dependent deterioration in mice between 7 and 17 months of age (Machová et al., 2008).

Methods

Animals

The APPswe/PS1dE9 founder mice were obtained from the Johns Hopkins University, Baltimore, MD, USA (D. Borchelt and J. Jankowsky, Dept. of Pathology) and a colony was established at the University of Kuopio as described previously (Machová et al., 2008). The housing conditions (National Animal Center, Kuopio, Finland and Animal Facility of the Institute of Physiology, v.v.i. in Prague, Czech republic) were controlled (temperature 22 °C, light from 07:00 to 19:00; humidity 50–60%), and fresh food and water were freely available. Female transgenic mice and littermate controls were transported to Prague by air and left to accommodate for at least 2 weeks before experiments. Two age groups of mice were used, young (7–10 weeks) and adult (5–6 months). The experiments were conducted according to the Council of Europe (Directive 86/609) in accordance with the Declaration of Helsinki.

Release of preformed ACh

Cortical, hippocampal, and striatal slices were prepared from 5- to 6-month-old female mice using McIlwain tissue chopper set at a width of 0.35 mm. Tissues (brain cortex from left hemisphere, left and right striatum, and left and right hippocampus) were dissected and chopped in two perpendicular directions. Superfusion experiments were done essentially as described previously (Lazareno et al., 2004; Machová et al., 2007). Brain slices were loaded with $[^3H]$ choline (Amersham, UK; SRA 82 Ci/mmol) in Krebs buffer (in mM: NaCl 138; KCl 3; $CaCl_2$ 1.2; $MgCl_2$ 1; NaH_2PO_4 1.2; $NaHCO_3$ 25; glucose 10; saturated with mixture of 5% CO_2/95% O_2; final pH adjusted to 7.4) for 30 min, washed in superfusion medium, and loaded to a superfusion apparatus (Brandel, USA). Superfusion medium contained 10 μM hemicholinium-3 to prevent re-uptake of labeled choline. In experiments on striatal slices 500 nM domperidone (RBI, USA) was included in superfusion medium to prevent dopamine D_2 receptor-mediated inhibition of acetylcholine release (Doležal et al., 1992). Slices were superfused at a rate of 0.5 ml/min and 4-min fractions were collected after 1 h washout of free radioactive substances. The release of $[^3H]$-ACh was evoked by mild field electrical stimulation (sixty 2-ms rectangular monopolar pulses, 1 Hz, 25 mA) at the beginning of the third, ninth and fifteenth fractions denoted S_1, S_2, and S_3, respectively. These conditions do not induce autoinhibition of the release by endogenous ACh. The first stimulation was always control. The second stimulation was in the presence of 10 μM carbachol to maximally stimulate autoinhibition and the third stimulation in the presence of 1 μM atropine to prevent any stimulation of presynaptic muscarinic autoreceptors.

Release of newly synthesized ACh

The release of newly synthesized $[^3H]$-ACh was done as described previously (Doležal and Tuček, 1991). Briefly, brain slices were preincubated in Krebs buffer containing 2 μM choline and 50 μM paraoxon to irreversibly inhibit cholinesterases for 1 h and then washed three times to remove paraoxon. Slices were then separated into duplicate incubation vials and incubated for 60 min in 0.3 ml of normal Krebs buffer or depolarizing Krebs buffer with 50 mM KCl (at the expense of NaCl to maintain isoosmolarity) containing 2 μM choline, 1 μM atropine, and $[^3H]$choline (1 μCi/ml). In case of striatal slices incubation medium also contained 500 nM of the dopamine D2 receptor antagonist domperidone, both during preincubation and incubation. Preincubations and incubations were at 37 °C. At the end of incubation tissues and media were separated by centrifugation (2 min, 400 g). Tissues were extracted into 0.3 ml of 10% trichloroacetic acid. Concentrations of $[^3H]$-ACh in incubation media and tissue trichloroacetic acid extracts were determined as described (Doležal and Tuček, 1991). Trichloroacetic acid was removed from tissue extracts with ether. Two 0.1 ml aliquots were taken from each extract and medium. One of them was incubated with choline oxidase (0.2 U; Sigma) to remove choline and the other with choline oxidase plus acetylcholinesterase (type V, 2 IU; Sigma) to remove both choline and ACh. Final volume of 0.2 ml was made up with 100 mM Tris buffer (final concentration 50 mM and pH 8). After 20 min at 37 °C, 0.2 ml of sodium tetraphenylboron dissolved in butyronitrile (10 mg/ml) was added, the mixture was vigorously shaken for 1 min, the organic phase was separated by centrifugation, and the radioactivity in 0.1 ml aliquots of organic layer was measured. The radioactivity corresponding to $[^3H]$-ACh was calculated as the difference between the radioactivities recovered in the organic phases from the two samples.

GTP-γ^{35}S binding

Determinations of carbachol-stimulated GTP-γ^{35}S binding as a postsynaptic marker of cholinergic transmission were done in membranes prepared from right hemisphere cortices. They were homogenized on ice in 1.5 ml of buffer containing 100 mM NaCl, 10 mM Mg Cl_2, 20 mM Hepes, 10 mM EDTA, and pH 7.4 using a glass homogenizer. Homogenates were centrifuged at 30 000 g and 4 °C for 30 min. Supernatants were then collected, membrane pellets were resuspended in 1.5 ml of the same buffer without EDTA, and again centrifuged under the same conditions. Supernatants were removed and pooled with previous ones. Crude membrane pellets and supernatants were stored in −80 °C until assayed. Pooled supernatants were used to confirm increase in concentration of soluble amyloid β_{1-40} and amyloid β_{1-42} in transgenic animals.

Muscarinic receptor-induced activation of G-proteins was determined as an increase of GTP-γ^{35}S binding to membranes caused by the muscarinic receptor agonist carbachol, essentially as described earlier (Jakubík et al., 2006; Machová et al., 2008). Briefly, 50 μl aliquots of membranes containing 10 μg protein were incubated for 15 min at 30 °C in 150 μl of reaction buffer containing 100 mM NaCl, 10 mM Mg Cl_2, 20 mM Hepes, 1 mM DTT, 50 μM GDP, and the muscarinic agonist carbachol at a concentration range 300 nM–100 μM. After this preincubation, 50 μl aliquots of GTP-γ^{35}S (Perkin Elmer, USA; SRA 1250 Ci/mmol) were added to give a final concentration of 500 pM and incubation continued for another 60 min. Total content of G-proteins in membranes was determined as GTP-γ^{35}S binding in the absence of GDP. Aliquots of membrane suspension containing 5 μg of protein were incubated for 60 min

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