Hyposensitivity to the amnesic effects of scopolamine or amyloid β25–35 peptide in heterozygous acetylcholinesterase knockout (AChE+/−) mice

Julie Espallergues a,b,c, Laurie Galvan a,b,c, Laurence Lepourry c,d,e, Béatrice Bonafos c,d,e, Tangui Maurice a,b,c, Arnaud Chatonnet c,d,e, *

a INSERM U. 710, 34095 Montpellier, France
b EPHE, 75017 Paris, France
c University of Montpellier II, 34095 Montpellier, France
d INRA UMR 866, 34060 Montpellier, France
e University of Montpellier I, 34967 Montpellier, France

1. Introduction

Acetylcholinesterase (AChE) is the main catabolic enzyme of acetylcholine (ACh), responsible for the synaptic clearance of the neurotransmitter. Decrease in AChE expression or activity results in increased cholinergic tonus in the brain or periphery, with concomitant regulation of nicotinic and muscarinic receptors expression [1]. Increasing the cholinergic tonus through inhibition of AChE activity is the main symptomatic therapy currently used in Alzheimer's disease (AD). AD is a progressive neurodegenerative process, due to deposition of senile plaques containing amyloid proteins and neurofibrillary tangles formed of hyperphosphorylated Tau protein [2,3]. Amyloid toxicity severely affects cholinergic pathways, particularly originating from the magnocellularis basalis nucleus. Sustaining cholinergic transmission by specific inhibitors, such as donepezil, rivastigmine or galantamine, helps to maintain the cognitive scores in AD patients [4]. Moreover, cholinergic receptors may also mediate protection against amyloid toxicity. Activation of nicotinic α7 receptors, particularly, has been shown to protect neurons against Aβ peptide toxicity, through activation of the PI3 kinase/Akt pathway [5,6].

We used AChE knockout mice [7,8] and characterized the behavioral phenotype of heterozygous animals, particularly focusing on memory functions. Male and female, AChE+/− and AChE+/+ littermate controls (129sv strain), tested at 5–9 weeks of age, failed to show any difference in locomotion, exploration and anxiety in the open-field test, or in-place learning in the water-maze. However, when treated with the muscarinic receptor antagonist scopolamine (0.5, 5 mg/kg s.c.) 20 min before each water-maze training session, learning impairments were observed at both doses in AChE+/+ mice, but only at the highest dose in AChE+/− mice. The central injection of Aβ25–35 peptide (9 nmol) induced learning deficits only in AChE+/+ but not in AChE+/− mice. Therefore, the hyper-activity of cholinergic systems in AChE+/− mice did not result in increased memory abilities, but prevented the deleterious effects of muscarinic blockade or amyloid toxicity.

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in locomotion, exploration and anxiety, in the open-field test. Animals were trained to locate an invisible platform in the water-maze, a procedure assessing spatial reference memory, using either a ‘sustained acquisition’ protocol (3 swims/day, 5 days) or a ‘mild acquisition’ protocol (2 swims/day, 9 days). Learning profiles and probe test performances were similar in AChE+/− and AChE+/+ control mice (article in preparation). In the present study, we examined whether male AChE+/− mice are sensitive to amnesic treatments induced by muscarinic receptor blockade or by central injection of Aβ peptide. Mice were treated with the muscarinic receptor antagonist scopolamine (0.5, 5 mg/kg s.c., 20 min before behavioral testing, or received a central injection of Aβ25–35 peptide (9 nmol), 1 week before testing. We observed that the increase in cholinergic tonus did not result in increased memory abilities in AChE+/− mice, but provided significant protection against the deleterious effects of muscarinic blockade or amyloid toxicity.

2. Materials and methods

2.1. Animals

AChE+/− and wild-type littermates were bred in the laboratory and used at the CompAn behavioral phenotyping facility (University of Montpellier). Mice were maintained in a temperature and humidity controlled room, under a 12-h light:12-h dark cycle (lights on at 07:00 a.m.). Animal procedures were conducted in adherence to the European Council Directive of 24 Nov. 1986 (86–609). For Scop (0.5); Fr=2.78, p>0.05 for Scop (5); Fig. 1A). In AChE+/− mice, the treatments with saline and lowest dose of scopolamine did not interfere with maze learning, as indicated by similar decreases in acquisition latency over trials (Fr=11.76, p<0.01 for V; Fr=9.84, p<0.01 for Scop (0.5); Fig. 1B), with significant differences between trials 3–4 and 1. On the contrary, the scopolamine (5 mg/kg) treatment blocked the decrease in acquisition latencies (Fr=4.15, p>0.05 for each trial). AChE+/− mice showed lower swimming latency than V-treated wild-type animals, although no significant difference was measured (p>0.05 for each trial). AChE+/− mice therefore showed a non-significant tendency to learn faster than wild-type controls animals.

2.2. Drugs

Scopolamine hydrobromide was from Sigma–Aldrich (France). The drug was solubilized in physiological saline and injected subcutaneously (s.c.) in 100 μl/20 g b.w., 20 min before testing. Aβ25–35 and scrambled Aβ25–35 (Sc.Aβ) peptides were from NeoMPS (France). They were dissolved in distilled water at 3 mg/ml and kept at −20°C. Peptides were incubated at 37°C for 4 days and injected intracerebroventricularly (i.c.v.) in a volume of 3 μl per mouse, as described [9].

2.3. Water-maze procedures

The water-maze was a circular pool (diameter 150 cm, height 30 cm). Water temperature (21 ± 1°C), light intensity, external cues in the room and water opacity (obtained by suspension of lime carbonate) were unchanged throughout testing. Four departure positions were set at opposite positions and a transparent Plexiglas platform (diameter 10 cm) could be immersed at the centre of each pool quadrant defined by the departure positions. The quadrant where the platform was located was termed the training (T) quadrant and others, opposite (O), adjacent right (AR), and adjacent left (AL). Swimming was recorded using the Videotrack® II software (Viewpoint, France), trajectories being analyzed as latencies and distances. Training consisted of 3 swims per day for 4 or 5 days, with 15 min intertrial time. Start positions were randomly selected. Mice were allowed a 90 s swim to find the platform and left on it for 20 s. Two hours after the last swim, the probe test was performed. The platform was removed and each animal was allowed a free 60 s swim. The percentage of time spent in each quadrant was determined. Median latency, expressed as mean ± S.E.M., was calculated for each training day and analyzed using the Friedman repeated measure non-parametric ANOVA, post hoc comparisons being made using Dunn’s test. Presence in the T quadrant during the probe test was analyzed vs. chance level (25%) using the Wilcoxon test.

3. Results

Heterozygous AChE KO mice were first tested for their sensitivity to the amnesic effect of scopolamine. The muscarinic receptor antagonist was injected at 0.5 and 5 mg/kg s.c. Wild-type AChE+/− mice treated with physiological saline vehicle solution (V) showed a decrease in swimming latency during acquisition (Fr=4.19, p<0.05; Fig. 1A) with a significant difference between trial 4 and 1, indicating that animals correctly learned the platform location in the maze. Animals treated with either 0.5 or 5 mg/kg scopolamine showed maze learning deficits, indicated by a lack of diminution of latency over training trials (Fr=4.15, p>0.05 for Scop (0.5); Fr=2.78, p>0.05 for Scop (5); Fig. 1A). In AChE+/− mice, the treatments with saline and lowest dose of scopolamine did not interfere with maze learning, as indicated by similar decreases in acquisition latency over trials (Fr=11.76, p<0.01 for V; Fr=9.84, p<0.01 for Scop (0.5); Fig. 1B), with significant differences between trials 3–4 and 1. On the contrary, the scopolamine (5 mg/kg) treatment blocked the decrease in acquisition latencies (Fr=4.15, p>0.05 for each trial). AChE+/− mice showed lower swimming latency than V-treated wild-type animals, although no significant difference was measured (p>0.05 for each trial). AChE+/− mice therefore showed a non-significant tendency to learn faster than wild-type controls animals.

During the probe test, the scopolamine treatment at 0.5 or 5 mg/kg resulted in a decrease of the time spent in the T quadrant, as compared with V-treated AChE+/− mice (Fig. 1C). In AChE+/− mice, only the treatment with the highest dose of scopolamine resulted in a blockade of the preferential exploration in the T quadrant (Fig. 1C). AChE+/− mice were then tested for their sensitivity to the amnesic effect of Aβ25–35 peptide. Aβ25–35 or Sc.Aβ peptide (9 nmol) was injected i.c.v. 1 week before the initiation of place learning in the water-maze. Sc.Aβ-treated AChE+/− mice showed a decrease in swimming latency (Fr=16.29, p<0.01; Fig. 2A) with a significant difference between trials 4–5 and 1. Animals treated with Aβ25–35 failed to show a significant decrease in latency over training trials (Fr=8.78, p>0.05; Fig. 2A). In AChE+/− mice, both Sc.Aβ and Aβ25–35 treated groups showed significant decreases in acquisition latencies over trials (Fr=10.07, p<0.05 and Fr=24.63, p<0.0001, respectively; Fig. 2B) with significant differences between trials 3–5 and 1. During the probe test, the Aβ25–35 treatment induced a decrease of the time spent in the T quadrant in AChE+/− mice but not AChE+/− mice (Fig. 2C), confirming that the latter are insensitive to the amnesic effect of the amyloid peptide.
Fig. 1. AChE+/− mice were less sensitive to the memory impairing effect of scopolamine in the water-maze test. Acquisition profiles for (A) AChE+/+ and (B) AChE+/− mice and (C) probe test performances. Scopolamine was administered at 0.5 or 5 mg/kg subcutaneously 20 min before the first swim each training day. (A and B) Acquisition profiles, *p < 0.05, **p < 0.01 vs. trial 1 latency. (C) Probe test, the presence in the T quadrant is shown. The number of mice per group is shown within the T column. *p < 0.05, **p < 0.01 vs. chance level (dotted line).

Fig. 2. AChE+/− mice were less sensitive to the memory impairing effect of amyloid Aβ25–35 peptide, in the water-maze test. Acquisition profiles for (A) AChE+/+ and (B) AChE+/− mice and (C) probe test performances. Mice received Sc.Aβ or Aβ25–35 peptides (9 nmol) intracerebroventricularly 7 days before the first training day. (A and B) Acquisition profiles, *p < 0.05, **p < 0.01 vs. trial 1. (C) Probe test, the number of mice per group is shown within the T column. *p < 0.05, **p < 0.01 vs. chance level (dotted line).
4. Discussion

We observed that scopolamine was less potent in inducing learning impairments in AChE−/− mice. Indeed, a 0.5 mg/kg dose, effective in wild-type mice, did not prevent maze acquisition in AChE−/− mice, which required a 10× higher dose before showing clear signs of impaired learning. This effect was observed in male (this data) as well as female mice (not shown). Therefore, although the hyper-cholinergy resulting from the inactivation of AChE did not directly result in learning enhancement, it appeared sufficient to provoke a shift in the effective dose of scopolamine. AChE−/− mice appeared less sensitive to muscarinic receptor blockade. Indeed, Volpicelli-Daley et al. [1] reported that M1, M2 and M4 muscarinic receptors showed a 50–80% decrease in expression in the hippocampus and cortex of AChE−/− mice, brain regions associated with memory. In addition, muscarinic receptors showed decreased presynaptic, cell surface, and dendritic distributions and increased localization to intracellular puncta. Furthermore, muscarinic agonist-induced activation of extracellular signal-regulated kinase (ERK), a signaling pathway associated with synaptic plasticity and amyloidogenesis, was diminished in the hippocampus and cortex of AChE−/− mice [1]. Therefore, chronic diminution of ACh metabolism and resulting hyper-cholinergic tonus results in adaptations of ACh receptor expression and function and thus hyposensitivity to muscarinic antagonists.

We also report that central injection of Aβ25–35 peptide failed to induce learning impairments in AChE−/− mice, 1 week after injection. This observation deserves a more extensive study, but suggests that adaptations affecting nicotinic or muscarinic ACh receptors are not sufficient to conteract the sustained activation of endogenous protection pathways by ACh. Indeed, protection could be induced by activation of either α7 nicotinic receptors, through the phosphatidylinositol 3-kinase (PI3K)-Akt pathway [5,6] or M1 receptors, through the phospholipase C (PLC)/protein kinase C (PKC) pathway [10].

In conclusion, AChE−/− mice appear to be a promising model to analyze the regulations induced by increased ACh tonus on neurotoxicity and to understand how the downregulation of nicotinic and muscarinic receptors may contribute to the limited efficacy of AChE inhibitors in Alzheimer’s disease.

References