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Hyposensitivity to the amnesic effects of scopolamine or amyloid β_{25-35} peptide in heterozygous acetylcholinesterase knockout ($AChE^{+/-}$) mice☆

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ABSTRACT

We examined the sensitivity of $AChE^{+/-}$ mice to the amnesic effects of scopolamine and amyloid β peptide. $AChE^{+/-}$ and $AChE^{+/+}$ littermates, 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 $A\beta_{25-35}$ 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.

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 concomittant 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 α_7 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 (129 sv strain), tested at 5–9 weeks of age, failed to show any difference

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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 Aβ_{25-35} 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).

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. Aβ_{25-35} and scrambled Aβ_{25-35} (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 ($Fr = 2.12$, $p > 0.05$; Fig. 1B). Interestingly, V-treated 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 Aβ_{25-35} peptide. Aβ_{25-35} 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 Aβ_{25-35} 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 Aβ_{25-35} 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 Aβ_{25-35} 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.