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Original research article

A comparison of mecamylamine and bupropion effects on memory-related responses induced by nicotine and scopolamine in the novel object recognition test in mice

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ABSTRACT

Background: The aim of the present study was to evaluate the involvement of the cholinergic receptors ligands in the memory-related responses in mice, using the novel object recognition (NOR) test.
Methods: The NOR test is based on natural, exploratory abilities of animals exposed to a new environment. In the first session, two copies of the same object were presented. In the next sessions (30 min and 24 h after), one of the familiar object and a new object were presented.
Results: The mice injected with nicotine (0.035 and 0.175 mg/kg, free base, sc) before the first session spent more time exploring the new object than the familiar one at the second and third session, indicating that nicotine improved cognition. In turn, the mice injected with scopolamine (0.3 and 1 mg/kg, ip) before the first session spent less time exploring the new object than the familiar one at the second and third trial, indicating that scopolamine impaired the memory performance. Additionally, the acute injection of drugs used in smoking cessation in humans: mecamylamine (0.5 and 1 mg/kg) and bupropion (5 and 10 mg/kg), prior to injections of nicotine (0.035 mg/kg) or scopolamine (1 mg/kg), significantly prevented nicotine-induced memory improvement or scopolamine-induced memory impairment, at the second and third session.
Conclusions: The results of our studies unveiling neuronal mechanisms for cholinergic system of memory processes, via both nicotinic and muscarinic cholinergic receptors, will be useful for development of more effective pharmacotherapies for memory impairment-like treatment of human disorders in which cholinergic pathways have been implicated.

Introduction

The central cholinergic system via neurotransmitters and receptors is highly involved in memory and learning processes. One of the neurotransmitters essential for cognition is acetylcholine (ACh). Two main classes of cholinergic receptors, that mediate the action of ACh and play an important role in memory processing, exist in the human brain, i.e. muscarinic (mAChRs) and nicotinic (nAChRs) receptors [18,25,63].

Previous studies concluded that there is a strong correlation between the level of synaptic ACh and improvement in cognitive functions. The data reports that the inhibition of the activity of acetylcholinesterase, an enzyme which is breaking down ACh, leads to increased level of ACh in brain, especially in the two major areas which are involved in cognitive processes (i.e. the hippocampus and cerebral cortex) [11,25,37]. Moreover, it has been demonstrated that abnormally regulated cholinergic system, a decline in the number of cholinergic neurons in the basal forebrain and decrease in the activity of choline acetyltransferase contribute to the cognitive symptoms of neuropsychiatric disorders, e.g. Alzheimer's disease (AD) [15]. Thus, many of acetylcholinesterase inhibitors improve performance in several cognitive models in humans and rodents, whereas anticholinergic drugs have been demonstrated to impair learning and memory in a variety of experimental paradigms [4,32]. Indeed, the first clinical trials in patients suffering from AD revealed that there is a significant decrease in nAChRs density, especially in the areas of the cerebral cortex and hippocampus [57].

Based on the data mentioned above, much effort has been directed toward investigating the memory-related effects of

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substances affecting the cholinergic system (i.e. nicotine and scopolamine) in mice. Additionally, based on previous findings demonstrating that there is a commonality in the molecular mechanisms and the brain regions involved in drug addiction and memory-related processes, and that nAChRs are involved in cognition, we aimed to investigate and compare the influence of mecamylamine and bupropion, drugs acting by nAChRs and commonly used in smoking cessation in humans [3,23,26,30], on the nicotine- and scopolamine-induced memory and learning effects in mice.

In our experiments memory-related responses were measured using the novel object recognition (NOR) test. This NOR test allows a rapid evaluation of memory performance in rodents and bases on natural, exploratory abilities of animals exposed to a new environment [2,5,61,62]. Although many studies on the potential cognitive effects of cholinergic agents have been conducted, this subject seems not to be investigated sufficiently as long as the NOR test is considered.

Results from our experiments may contribute to a better understanding of cholinergic neuronal mechanisms important for modulation of memory and learning processes. This knowledge allows developing more effective pharmacotherapy for a variety of neuropsychiatric disorders, including human memory disturbances associated with cholinergic system.

Materials and methods

Animals

The experiments were carried out on naive male Swiss mice (Farm of Laboratory Animals, Warsaw, Poland) weighing 20–30 g. The animals were maintained under standard laboratory conditions (12-h light/dark cycle, room temperature 21 ± 1 °C) with free access to tap water and laboratory chow (Agropol, Motycz, Poland) in their home cages, and adapted to the laboratory conditions for at least one week. Each experimental group consisted of 8–12 animals. All behavioral experiments were performed between 8:00 and 15:00, and were conducted according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Council Directive for the Care and Use of laboratory animals of 24 November 1986 (86/609/EEC), and approved by the local ethics committee.

Drugs

The compounds tested were (-) nicotine hydrogen tartrate (0.035, 0.175 and 0.35 mg/kg, reported in freebase nicotine weight) (Sigma–Aldrich, St. Louis, MO, USA); scopolamine hydrochloride (0.1, 0.3 and 1 mg/kg) (Sigma–Aldrich, St. Louis, MO, USA); mecamylamine hydrochloride (0.5, 1 and 2 mg/kg) (Sigma, St. Louis, MO, USA) and bupropion hydrochloride (5, 10 and 20 mg/kg) (Sigma–Aldrich, St. Louis, MO, USA). All compounds were dissolved in saline solution (0.9% NaCl). Except for nicotine, drug doses refer to the salt form. The pH of the nicotine solution was adjusted to 7.0. All agents were administered subcutaneously (s.c.) or intraperitoneally (i.p.) at a volume of 10 ml/kg. Control groups received saline injections at the same volume and by the same route.

Experimental procedure

Memory-related responses were measured using the NOR test, which evaluated the rodent's ability to recognize a novel object in the environment. The apparatus consisted of a box (36 cm × 22 cm × 18 cm) made of white Plexiglas. This box was located in an isolated testing room and illuminated by a dim light. The day before testing, animals were allowed to explore the test box for 15 min. No object was placed in the box during the habituation session. All experiments were conducted blind to drug administration conditions.

Novel object recognition testing

In the first session, i.e. *introductory session (session I)* the mice were placed in the middle of the arena and presented with two identical objects (A1 and A2) for 10 min. Objects A1 and A2 had identical textures, colors and sizes. Both objects were placed on the opposite back corners of the arena. After 30 min and 24 h delay in the home cage, the mice were again placed in the same arena used before for 10 min and presented with two objects, the old familiar A1 and a novel object B for 5 min (*recognition session*) (*session II and III*). Objects A1 and B had different textures, colors and sizes. Both, the objects and the apparatus, were cleaned with water after each trial to remove the olfactory cues.

The NOR task is based on natural, exploratory abilities of rodents exposed to a new object. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Time spent exploring the objects was measured manually using stopwatch. The difference in exploration between a previously seen object and a novel object was taken as an index of memory performance (recognition index, RI) [2,5,61,62].

RI was calculated for each animal in the recognition session, and reported as the ratio:

$$\text{RI}\ (\%) = \frac{B \times 100\%}{\text{A1} + B}$$

A1 – time spent exploring the familiar object A1 during the recognition session (session II or session III); *B* – time spent exploring the novel object B during the recognition session (session II or session III).

Treatment

The first step of experiment was designed to estimate the influence of nicotine, scopolamine, mecamylamine and bupropion on memory-related responses using the NOR test in mice. Nicotine (0.035, 0.175 and 0.35 mg/kg, sc), scopolamine (0.1, 0.3 and 1 mg/kg, ip), mecamylamine (0.5, 1 and 2 mg/kg, ip), bupropion (5, 10 and 20 mg/kg, ip) and saline was administered 30 min before the introductory session. Recognition session was tested both 30 min and 24 h after the introductory session. The second step of experiment was designed to examine and compare the influence of mecamylamine and bupropion on memory-related responses induced by nicotine or scopolamine administration. For this purpose, mecamylamine (0.5 and 1 mg/kg, ip), bupropion (5 and 10 mg/kg; ip) and saline was administered 15 min prior to nicotine (0.35 mg/kg, sc) or scopolamine (1 mg/kg, ip), and the mice were then tested 30 min and 24 h after the introductory session.

Experimental doses and procedures were chosen accordingly to those frequently used in literature, including our previous study, in which we examined the cognitive effects of nicotine and scopolamine in mice [6–8,33,44,45,58].

Statistics

The data were expressed as the means ± SEM. For the NOR test, we measured RI [%], i.e. the difference in exploration between a previously seen object and a novel object. The statistical analysis were performed using two-way analysis of variance (ANOVA) for the factors of pretreatment, treatment and treatment interaction or one-way analysis of variance (ANOVA). *Post hoc* comparison of means was carried out with the Tukey's test for multiple comparisons, when appropriate. The data were considered statistically significant at confidence limit of $p < 0.05$.