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Involvement of cholinergic receptors in the different stages of memory measured in the modified elevated plus maze test in mice

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Abstract:
Background and Methods: Several lines of evidence support a strong relationship between cholinergic pathways and memory. The aim of our experiments was to examine the mechanisms involved in the formation of different memory stages, to evaluate the impact of substances, which affect the cholinergic system in mice, with an employment of the modified elevated plus maze (mEPM) test. This test allows examining different processes of memory (acquisition, consolidation and retrieval), depending on the time of drug treatment. The time period, necessary for mice to move from the opened arm to the enclosed arm (i.e., transfer latency, TL) was used as an index of memory.
Results: Our findings revealed that in both memory acquisition and consolidation, nicotine, an agonist of cholinergic receptors (0.035 and 0.175 mg/kg, free base, *sc*), reduced TL on the second day of the experiment (TL2), thus improving memory. In turn, scopolamine, an antagonist of cholinergic receptors (0.3 and 1.0 mg/kg, *ip*), significantly increased TL2 values, impairing cognition. Subsequently, we evaluated the influence of mecamylamine, a non-selective antagonist of nicotinic cholinergic receptors (nAChRs) and of varenicline, an $\alpha 4\beta 2$ partial nAChRs agonist, on memory-related behaviors induced by nicotine and scopolamine. Acute injections of mecamylamine (0.5 and 1.0 mg/kg, *ip*) and varenicline (0.5 and 1.0 mg/kg, *ip*), prior to the injections of nicotine (0.035 mg/kg) or scopolamine (1.0 mg/kg), significantly suppressed nicotine-induced memory improvement or scopolamine-induced memory impairment.
Conclusion: Our studies indicate that the cholinergic system plays a crucial role in memory processes. Pharmacological manipulation of cholinergic transmission can be the base to develop more effective pharmacotherapies for these memory disturbances in which cholinergic receptors are involved.

Key words:
nicotine, scopolamine, mecamylamine, varenicline, memory and learning, modified elevated plus maze

Abbreviations: ACh – acetylcholine, AD – Alzheimer's disease, DA – dopamine, GABA – γ-aminobutyric acid, mAChRs – muscarinic cholinergic receptors, mEPM – modified elevated plus maze, nAChRs – nicotinic cholinergic receptors, NMDA – N-methyl-D-aspartate, TL – transfer latency, VTA – ventral tegmental area

Introduction

A fair number of studies imply some role of the cholinergic system in cognitive functions, specifically in attention and memory encoding. Spatial memory is one

of the most essential forms of higher cognitive processes, demanding a certain capacity to record environmental and spatial orientation data. Thus, spatial memory formation represents a simple form of episodic-like memory in rodents that engages both the basal cholinergic system and its target structures [17, 18, 24].

It is known that acetylcholine (ACh) is a neurotransmitter essential for these cognitive functions. Previous studies concluded a strong correlation between synaptic ACh levels and cognitive function improvement, providing evidence that acetylcholinesterase (an ACh breaking down enzyme), lead to increased ACh levels in the brain, especially in cortical and hippocampal brain regions, i.e., the two major areas involved in cognitive processes. Accordingly, many acetylcholinesterase inhibitors improve performance levels in several cognitive models, involving humans or rodents [34].

Muscarinic (mAChRs) and nicotinic (nAChRs) receptors, i.e., the two main classes of cholinergic receptors that mediate ACh actions and play a crucial role in memory processing, are localized in the human brain [32, 64]. Out of them, it is the nAChRs which are mainly involved in memory processes. The characteristic structure of nAChRs includes a ring of five subunits, arranged around a ligand-gated excitatory ion channel. To date, 12 individual subunits of nAChRs (α2–α10) and (β2–β4) have been identified. The two main neuronal categories, defined by their function and pharmacology, include heterologous pentamers, formed from the combinations of α- and β-subunits, and homologous pentamers, formed from one subunit type, α7, α8 or α9 [13, 58]. Out of all the central nAChRs subtypes, both the α4β2 combination and α7 subunits seem to play the most important role in memory-related responses [35]. A growing body of evidence reveals a certain involvement of the α4β2 subtype of nAChRs in cognitive processing [49]. Furthermore, metanicotine, a selective α4β2 nAChRs agonist, enhances reference and working memory in the radial arm maze performance in rats with ibotenic acid forebrain lesions and also improves passive avoidance retention in rats with scopolamine-induced amnesia [44, 45]. Consistent with the role of α7 nAChRs in cognition, some deficits in olfactory working memory were also revealed in α7 knockout mice *vs.* wild-type controls [75]. Additionally, another research with the use of methyllycaconitine, a specific antagonist of α7 nAChRs, infused into the ventral hippocampus, demonstrates that this type of nAChRs is essential for working memory-related responses, measured in the radial arm maze test in rats [2, 3, 37].

An abnormally regulated cholinergic system, with a decline of cholinergic neurons in the basal forebrain, have been hypothesized as being responsible for the cognitive symptoms of neuropsychiatric disorders, e.g., Alzheimer's disease (AD) [18, 32]. The first clinical trials in patients, suffering from AD, revealed no changes in the number, the structure or the function of mAChRs, while significantly decreased levels of α4β2 nAChRs subtypes were observed, especially in the cortex and the hippocampus [18, 29, 32, 41]. That reported decline may be appropriate for the cognitive deficits that characterize the condition. Therefore, an administration of drugs, which improve cholinergic signaling, is able to enhance cognitive functions [29, 43]. What is more, literature data suggest that agonists and partial agonists of various subtypes of nAChRs are probably an interesting therapeutic target for the treatment of various central nervous system disorders, such as AD [2, 29, 43].

Based on the above-mentioned data, much effort has been undertaken toward investigating memory-related effects of the two cholinergic system affecting substances (nicotine and scopolamine) in mice. Additionally, assuming the reported data that α4β2 and α7 ligands may represent a new generation of compounds to treat memory disorders, such as AD, we aimed to investigate and compare the influences, exerted on the memory and learning processes in mice, by mecamylamine, a non-selective nicotinic receptor antagonist, and by varenicline, a partial α4β2 nAChR agonist, which has recently been approved as a pharmacological aid to cease the smoking habit [14, 33]. In our experiments, we used the recently developed modified elevated plus maze (mEPM) memory animal model. The mEPM test is a simple method that evaluates spatial memory and, moreover, this memory model allows investigating different stages of memory processes [30, 31, 54, 70]. We wanted to ascertain whether memory acquisition or consolidation processes were by those drugs in any way affected.

The experiments may contribute to a better understanding of the cholinergic neuronal mechanisms, important for the modulation of memory and learning processes. The knowledge about the mechanisms of action of cholinergic ligands (not only mecamylamine and varenicline) enables a development of more effective pharmacotherapies for human memory disorders associated with cholinergic pathways.