6-HYDROXY-L-NICOTINE FROM *ARTHROBACTER NICOTINOVORANS* FACILITATE SPATIAL MEMORY FORMATION IN RATS

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Abstract: Effects of 6-hydroxy-L-nicotine derived from nicotine catabolism in *Arthrobacter nicotinovorans* on learning and memory processes were examined in adult male Wistar rats. 6-hydroxy-L-nicotine (0.3 mg/kg, i.p., 7 consecutive days chronic administration) significantly increased spontaneous alternation in Y-maze task and working memory in radial arm-maze task, suggesting effects on short-term memory, without affecting long-term memory, explored by reference memory in radial arm-maze task. Taken together, our results suggest that 6-hydroxy-L-nicotine sustain memory formation and may provide the opportunity to manage neurological abnormalities in neurodegenerative diseases.

INTRODUCTION

Nicotine is a naturally occurring alkaloid found in many plants. The principal sources of nicotine exposure are through the use of tobacco, nicotine containing gum and nicotine replacement therapies. Nicotine is an amine composed of pyridine and pyrrolidine rings. It has been shown that nicotine crosses biological membranes and the blood brain barrier easily. The absorbed nicotine is extensively metabolized in the liver to form a wide variety of metabolites including nicotine N-oxide and cotinine N-oxide. These are the products of mixed function oxidase system. Nicotine is also converted to some biologically important compounds during harvesting. Among these are the nitrosamines specific to tobacco. Nicotine has been shown to affect a wide variety of biological functions ranging from gene expression, memory, regulation of hormone secretion and enzyme activities (Hefco et al., 2003; Hritcu et al., 2009; Yildiz, 2004). Studies on nicotine metabolism were advanced by the use of several methods involving enzyme purification, purification of specific antibodies, immunochemical and biochemical methods, and high pressure liquid chromatography (HPLC) (Nakayama, 1988). Studies on cytochrome P450 and flavin adenine dinucleotide (FAD) containing monooxygenases produced remarkable progress on analysis of nicotine metabolism (Nakayama, 1988). The biochemical techniques suggested the participation of cytochrome P-450 and FAD-containing monooxygenases in microsomal nicotine metabolism (Peyton et al., 1988). Metabolism of nicotine in living organisms is complicated. Pathways of nicotine metabolism could be discussed as phases I and II metabolism of nicotine. The phase I metabolism involves the microsomal oxidation of nicotine and falls into four groups. The phase II metabolism involves N- and O-glucuronidation of nicotine and its metabolites. However, almost all of the latter studies have focused primarily on the peripheral metabolism of nicotine, and relatively few investigations have addressed the important issue of whether nicotine biotransformation products are present in brain after peripheral nicotine administration. Nicotine metabolites in brain are of particular importance, because of their potential contribution to the neuropharmacological effects resulting from nicotine exposure (Crooks et al., 1997). Nicotine and some of its metabolites may effectively reduce the amyloidal β-peptide aggregation in the brain and stimulate dopamine release, so nicotinic drug treatment may be a novel protective therapy in Alzheimer's and Parkinson’s diseases.

Arthrobacter nicotinovorans is a Gram-positive bacterium that uses nicotine as carbon and nitrogen source (Brandsch, 2006). Within *A. nicotinovorans*, nicotine degradation is based on the catabolic machinery encoded by *pAO1* megaplasmid (Brandsch, 2006). In *A. nicotinovorans pAO1*, nicotine metabolism is controlled by the nicotine metabolite 6-hydroxy-nicotine (6HNic), which interacts with the 6-hydroxy-nicotine oxidase repressor (HdnoR), thereby disrupting its binding to the specific operator site and inducing the production of downstream metabolic enzymes (Sandu et al., 2003). In the present study, we are primarily interested if 6-hydroxy-L-nicotine, a nicotine metabolite derived from nicotine catabolism in *Arthrobacter nicotinovorans* has possible effects on central nervous system (CNS) activity, with relevance for management of cognitive-related abnormalities in neurodegenerative disorders.

MATERIALS AND METHODS

Animals

20 male Wistar rats weighing 200-250 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethic Committee and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

Y-maze task

Spatial memory performance was assessed by recording spontaneous alternation behavior in a single session in Y-maze. The procedure was the same as previously described (Hritcu and Nabeshima, 2009), as follows: each rat was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The spontaneous alternation percentage was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two) X 100.

Radial arm-maze task

Spatial working and reference memory were tested by using a radial arm-maze apparatus as previously described (Hritcu et al., 2007). Briefly, three or four rats were simultaneously placed in the radial maze and allowed to explore for 5 min and take food freely. The food was initially available throughout the maze, but was gradually restricted to the food cup. The animals were trained for 4 days to run to the end of the arms and consume the baits. To evaluate basal activity of rats in radial arm-maze, the rats were given 5 consecutive training trials per day to run to the end of the arms and consume the baits. The training trial continued until all the 5 baits had been consumed or until 5 min has elapsed. Criterion performance was defined as consumption of all 5 baits or until 5 min had elapsed. All rats were trained 1 trial per day. The following data were recorded: 1) number of working memory errors (entering an arm containing food, but previously entered); 2) number of reference memory errors (entering an arm that was not baited); 3) time taken to consume all five baits and 4) entries to repeat (number of arms entered until a repeat entry was made).

Drug administration

6-hydroxy-L-nicotine (6HLNic) was generously provided by Dr. Roderich Brandsch (University of Freiburg, Institute of Biochemistry and Molecular Biology, Germany). 6HLNic was dissolved in sterile saline and injected i.p. in a volume of 1ml/kg b.w. 6HLNic was chronically administrated, daily, 7 consecutive days, at a dose of 0.3 mg/kg b.w. Controls animals received i.p. an equal volume of sterile saline (1 ml/kg b.w.).

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student's "t" test (T- test: Paired Two Sample for Means). $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISSCUSIONS

1. Effect of chronic 6-hydroxy-L-nicotine on learning and memory

6-hydroxy-L-nicotine improve short-term memory, in 6HLNic alone treated rats, as evidenced by a significant increase of the spontaneous alternation percentage ($p<0.00001$) in Y-maze test (Fig. 1). This effect could not be attributed to decreased motor activity, because 6HLNic induced an increase in locomotor activity ($p<0.00001$) as it can be deduced from number of arm entries in Y-maze test (Fig. 1).

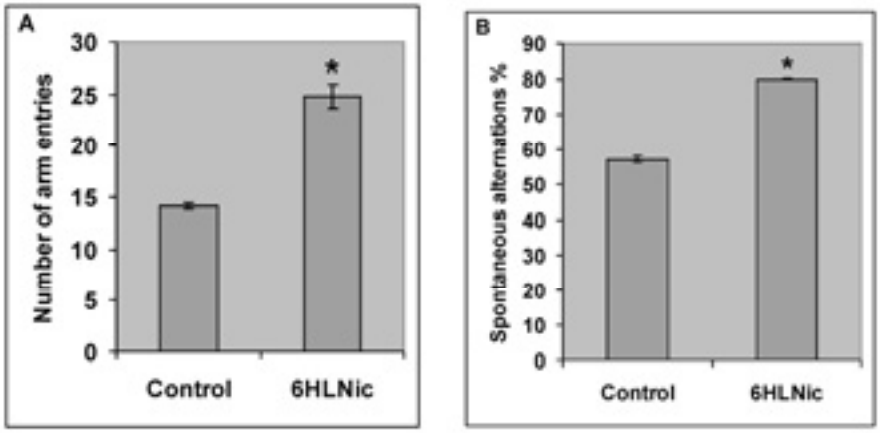

Fig. 1. Effects of 6HLNic treatment on number of arm entries (A) and on spontaneous alternation % (B) in Y-maze task. Data are means ± S.E.M. (n=10 per group). *P<0.00001 vs. control group.

In radial arm maze-task, rats chronically treated with 6HLNic showed significant decrease ($p<0.0003$) of the number of working memory errors (Fig. 2A) compared to control group, during 7 days training, suggesting effects on short-term memory. Furthermore, in 6HLNic treated-groups, short-term memory improvement resulted in non-significant decrease of entries to repeat (Fig. 2C) and in significant decrease ($p<0.0008$) of time taken to consume all five baits (Figure 2D) compared to control group.

Regarding long-time memory, explored by the number of reference memory errors (Fig. 2B), was unimpaired during 7 days training of rats exposed to 6HLNic ($p>0.05$), suggesting no effects of long-term memory.

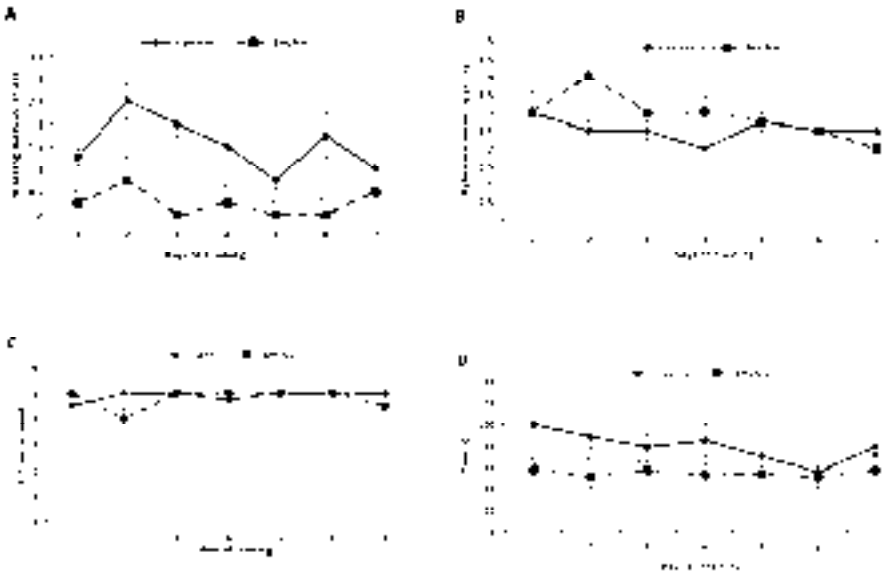

Fig. 2. Effects of 6HLNic treatment on the number of working memory errors (A), the number of reference memory errors (B), entries to repeat (C) and time taken to consume all five baits (D) during 7 days training in radial arm-maze task. Values are means ± S.E.M. (n=10 animals per group).

Previous studies suggested that some of nicotine close derivates have major application in the therapy of some neurodegenerative disorders and diseases (e.g. Alzheimer's disease, Parkinson's disease, Tourette's syndrome, schizophrenia etc.) (Pogocki et al., 2007). The present study highlights several important findings regarding the effect of chronic 6HLNic administration on memory processes in male Wistar rats.

Our results showed that 6HLNic administration induce a board spectrum of behavioral effects. 6HLNic induced a significant increase in short-term memory (significant increase of spontaneous alternation percentage in Y-maze test) and working memory (explored through the radial arm-maze test) without affecting significantly reference memory (explored through radial arm-maze test) compared to control groups. 6HLNic increase performance accuracy in treated rats. All training was done after 6HLNic administration, so the positive effect of 6HLNic supports the hypothesis that this nicotine metabolite contributes to spatial memory formation.

Short response time of 6HLNic-treated rats could be a cause of memory improvement, evidenced by a significant decrease of time taken to consume all 5 baits in radial arm-maze task compared to control rats.

A specific effect of 6HLNic treatment was choice accuracy. Therefore, in the radial arm-maze task, we concluded that increased motivation to respond for food might be associated with improved choice accuracy.

CONCLUSIONS

On the basis of our results obtained by 6HLNic administration, we can conclude that in the rats, peripheral administration of chronic 6HLNic improved short-term memory in normal rats without significantly affecting long-term memory. This study also suggests the antianmesic effect of 6HLNic can also result from its action at nicotinic receptors, and could be used as nicotine in therapy of some neurodegenerative diseases and neuropsychiatric disorders.

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