The Effect of Scopolamine on Avoidance Memory and Hippocampal Neurons in Male Wistar Rats

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A B S T R A C T

Introduction: Cholinergic systems are involved in learning and memory. Scopolamine, a muscarinic acetylcholine receptor antagonist, is used as a standard/reference drug for inducing cognitive deficits in healthy humans and animals. The purpose of this study was to evaluate the effects of scopolamine on avoidance memory and number of neurons in rat's hippocampus. .

Methods: Thirty five male albino Wistar rats (200 ± 20 g) were used in this study. The rats were divided randomly into five groups: control group (healthy samples), sham (saline) and 3 experimental groups 0.2, 0.5 and 1 mg/kg (intraperitoneally - single dose of Scopolamine). Animals were tested by passive avoidance method (shuttle box). After one week, a memory test was taken from rats. Finally, with dissection of the rats' brains and tissue operations, neurons were stained with cresyl violet. Photographs of the samples in hippocampal areas were prepared, and neurons were counted.

Results: Our results showed that the number of neurons in all experimental groups was lower than that in the control group. The highest decrease in number of neurons was shown in response to 1 mg/kg scopolamine compared to the control group in all regions of hippocampus. Also, we found that in comparison to the saline-treated animals, the injection of scopolamine to rats after training, caused memory destruction.

Discussion: We concluded that memory impairment-induced by scopolamine is probably associated with neuronal loss and this decrease was dose dependent.

1. Introduction

Today, it is accepted that memory is one of the most essential roles of the brain. Memory is vital for survival because it is the process by which organisms are able to record their experiences and use this information to adapt their responses to the environment (Dunning & During, 2003). Gradual loss of memory and impaired cognitive functions are the major features of Alzheimer's disease (AD) (Siddiqui & Levey, 1999).

The neurotransmitter of the central cholinergic system is involved in cognitive functions (Blokland, 1995), and plays an important role in learning and memory for humans and animals (Morris, Anderson, Lynch, & Baudry, 1986; Sutherland, Whishaw, & Regehr, 1982; Winkler, Suhr, Gage, Thal, & Fisher, 1995).

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Loss of memory is often the most disabling aspect of many disorders, impairing the normal daily activities of the patients. Animal models of memory impairment can help us understand the molecular basis of memory and its therapeutic targets. The model of scopolamine- induced amnesia in rodents is one of the well-established animal models of memory dysfunction (Blokland, 2005).

Scopolamine, a non-selective muscarinic receptor antagonist, confers impairment of learning acquisition and short-term memory (Izquierdo, 1989) and reduces acetylcholine level in the hippocampus.

Similarities have been reported in the memory deficit between Alzheimer's disease patients and scopolamine treated animals. Thus, it has been anticipated that scopolamine, can provide a useful pharmacological tool to generate a partial model of the disorder (Azami, Jahanshahi, & Babapour, 2010).

The passive avoidance model has been used to study learning and memory for a stressful stimulus. The practice is based on the innate preference of rodents for the dark section of the apparatus and the suppression of this innate preference following exposure to an inescapable shock; that is, passive avoidance performance is an adaptive response to a stressful experience that serves as a measure of learning and memory (Tsuji, Takeda, & Matsumiya, 2003).

Recent neuroscience research demonstrated that in the mammalian brain two regions have neurogenesis: the subventricular zone of the anterior lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus (Jahanshahi, Khoshnazar, Azami, & Heidari, 2011).

Experimental lesion of cholinergic nuclei leads to the suppression of neurogenesis in rat's hippocampus (Cooper-Kuhn, Winkler, & Kuhn, 2004; Van der Borght et al., 2005). Thus, it is considered that activation of the central cholinergic system enhances hippocampal neurogenesis, and this may contribute to the improvement of cognitive function (Kotani, Yamauchi, Teramoto, & Ogura, 2006).

Therefore, the aim of this study was to evaluate the effects of scopolamine (as muscarinic receptor antagonist) on avoidance memory and determination of scopolamine side effects on the number of neurons in male Wistar rats' hippocampal formation.

2. Materials and Methods

2.1. Animals

Thirty five male Wistar rats (Pasteur institute, Amol, Iran), weighting 200 ± 20 g at the time of training, were used in passive avoidance task. They had free access to food and water, were housed seven in a cage, and kept at controlled temperature ($22 \pm 2°C$) under a 12/12 h light-dark cycle (light beginning at 7:00 a.m.) at least 1 week before the beginning of the experiments. The rats were randomly distributed into five groups ($n = 7$) as follows:

1. Control: Without receiving scopolamine and behavioral test.

2. Saline-control: received saline + behavioral test.

3. Exp. 0.2 mg/kg: received scopolamine (0.2 mg/kg IP) + behavioral test.

4. Exp. 0.5 mg/kg: received scopolamine (0.5 mg/kg IP) + behavioral test.

5. Exp. 1 mg/kg: received scopolamine (1 mg/kg IP) + behavioral test.

All experiments were carried out during the light phase between 9:00 and 15:00. Experimental groups consisted of 7 animals and each animal was tasted once only. All procedures were performed in accordance with institutional guidelines for animal care and use.

2.2. Drugs

Scopolamine hydrobromide (sigma, USA) was used in the present study. Scopolamine was dissolved in saline (NaCl 0.9%) at final concentrations of 0.2, 0.5 and 1 mg/kg, and was injected intraperitoneally one day after the training.

Inhibitory Avoidance Apparatus

The step-through inhibitory avoidance apparatus consisted of two compartments of the same size (20 × 20 × 30 cm3). In the middle of a dividing wall, a guillotine door (7.9 cm2) could be lifted manually. The walls and floor of one compartment consisted of white opaque resin and the walls of the other compartment were dark. Stainless steel bars (3 mm in diameter and 1 cm intervals) constituted the floor of the dark compartment. Intermittent electric shocks (50 Hz, 3 s, 1.5 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator.

Behavioral Procedures

Our previous study (Azami, et al., 2010) described passive avoidance as follows: All animals were allowed to habituate in the experimental room (with light and sound attenuated) for at least 30 min prior to the experiments. Then, each animal was gently placed in the brightly lit compartment of the apparatus; after 5 s the guillotine door was opened and the animal was allowed to enter the dark module.

The latency with which the animal entered the dark chamber was recorded. Animals that waited more than 120 s to enter the dark chamber were excluded from the experiments.

Once the animal entered with all four-paws to the next chamber, the guillotine door was closed and the rat was immediately withdrawn from the compartment. This trial was repeated after 30 min. As in the acquisition trial, after 5s the guillotine door was opened, and as soon as the animal entered the dark (shock) compartment the door was closed; and a foot shock (50 Hz, 1 mA and 3 s) was immediately delivered to the grid floor of the dark room. After 20 s, the rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials; if the rat did not enter the dark compartment during 120 s, a successful acquisition of inhibitory avoidance response was recorded. Otherwise, when the rat entered the dark compartment (before 120 s) a second time, the door was closed and the animal received the shock again. After retesting, if the rat learned inhibitory avoidance response successfully, it was moved to the cage and 24h after the training, received scopolamine or saline (i.p.). On the test day (one week after training) each animal gently placed in the light compartment and after 5 s the door was opened, and step through latency (sec) was recorded in the absence of electric foot shocks, as indicator of inhibitory avoidance behavior.

Histology

24 h after the testing sessions, rats were anaesthetized with chloroform, decapitated and their brains were removed from the skull and stored in paraformaldehyde (4%) for two weeks, then, the brains were placed in tissue processor apparatus for tissue procedures. Following this session, samples of the brain were embedded in paraffin and kept in refrigerator. Then the brains were sliced coronally into 8 μm sections (from Bregma –2.5 mm to –4.5 mm of the hippocampal formation)

with a rotary microtome (MK 1110) and the sections were stained with cresyl violet in accordance with routine laboratory procedures (Bancroft & Gamble, 2008). A photograph of each section was produced using an Olympus BX 51 microscope and a DP 12 digital camera under a magnification of 1000 for Dentate Gyrus (DG) area and 400 for the other areas. An area of 4800 μm2 for DG and 30000 μm2 were selected in the lower horn of the dentate gyrus in all sections. To measure the area density of the granule cells, the images were transferred to the computer. Using OLYSIA Autobioreport software, Olympus Co, the appropriate grids were superimposed on the pictures and the cells were counted manually. To perform an unbiased measurement, the individual was double-blinded and only the cells with significant granule cell characteristics were counted (Jahanshahi, Golalipour, & Afshar, 2009; Jahanshahi, et al., 2011; Jahanshahi, Sadeghi, Hosseini, Naghdi, & Marjani, 2008).

Statistical Analysis

All data were entered into and analyzed by SPSS software. The data were expressed as mean ± SEM. The statistical analysis was performed using one and two way analysis of variance (ANOVA). Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate.

The level of statistical significance was set at $P < 0.05$.

3. Results

In the experimental groups the rats were trained and were tested 7 days later. Table 1 shows the results of the memory test. As the table shows, latency of response in the sham group was 128.57 ± 97.48 s while for the experimental groups it was a much shorter time (0.2 = 52.86 ± 9.51 s, 0.5 = 50.86 ± 57.32 s and 1mg/kg = $26.57 \pm 34.07.48$ s). Data showed a significant difference between sham and experimental groups ($p < 0.05$). We found no significant difference between the experimental groups with the other.

Tables 2, 3 and 4 show the results of cell counts in different groups. The number of neurons in all experimental groups was lower than in the control group and these differences were significant ($P < 0.05$).

The highest decrease in the number of neurons was shown in response to scopolamine with dose of 1 mg/kg compared to the control, in all regions of the hippocampus.

Table 1. Latency of entrance (in seconds) to dark chamber in different groups of rats

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7
Sham	300	40	91	79	62	231	97
0.2 mg/kg	59	54	58	57	39	63	40
0.5 mg/kg	73	29	32	4	35	12	171
1 mg/kg	40	4	10	23	6	5	98

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Table 2. Number of neurons in CA1 area of Hippocampus (Mean and SD)

Groups – CA1	mean	Std. Deviation	P value
Control	31.87	7.158	
Saline-control	33.88	6.354	0.059
Exp. 0.2 mg/kg	32.4	5.936	0.616
Exp. 0.5 mg/kg	28.58	4.749	0.002
Exp. 1 mg/kg	27.97	5.227	0.000

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Table 3. Number of neurons in CA3 area of Hippocampus (Mean and SD)

Groups – CA3	Mean	Std. Deviation	P value
Control	23.15	5.374	
Saline-control	25.63	6.244	0.003
Exp. 0.2 mg/kg	20.73	4.704	0.004
Exp. 0.5 mg/kg	20.33	3.112	0.001
Exp. 1 mg/kg	27.97	3.504	0.000

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**Table 4.** Number of neurons in DG area of Hippocampus (Mean and SD)

Groups – DG	Mean	Std. Deviation	P value
Control	20.98	3.789	
Saline-control	19.33	3.564	0.004
Exp. 0.2 mg/kg	18.47	2.819	0.000
Exp. 0.5 mg/kg	19.5	2.168	0.001
Exp. 1 mg/kg	19.82	3.045	0.000

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4. Discussion

We found some increase in the number of neurons in CA1 and CA3 areas of hippocampus in sham-saline group compared to the control group, however, this increase was only significant in CA3.

This study was performed to assess whether scopolamine can decrease the number of neurons proliferating in the hippocampal formation, and if this can affect its function as inhibitory memory latency. We observed
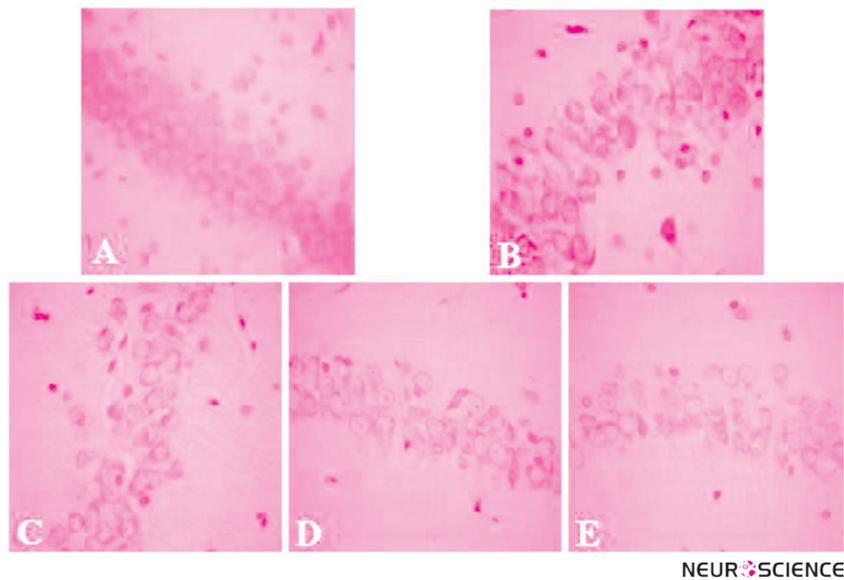
Figure 1. CA1 area of hippocampus in all groups. A= Control, B= Sham, C= 0.2 mg/kg, D= 0.5 mg/kg and E= 1 mg/kg

good consistency between neurogenesis reduction and shortness of inhibitory memory latency.

A review of the literature on scopolamine, clearly shows that this drug is capable of inducing various behavioral changes in several animal species (Robinson, Harbaran, & Riedel, 2004; Santi & Weise, 1995; Savage, Faust, Lambert, & Moerschbaecher, 1996; Spinelli, Ballard, Feldon, Higgins, & Pryce, 2006), which shows scopolamine can cause very potent performance impairment on tests of learning and memory (Hodges Jr, Lindner, Hogan, Jones, & Markus, 2009; Sambeth, Riedel, Smits, & Blokland, 2007; Spinelli, et al., 2006). This would corroborate the view that scopolamine is an amnesic drug that disrupts central cholinergic neurotransmission.

Limbic system has an undeniable role in memory. For example, to clarify the role of cholinergic neurons in the amygdala on learning and memory, topical scopolamine injection into the bilateral amygdala of mice suggested that the site of action for scopolamine to cause anterograde amnesia is the amygdala, and that cholinergic neurons projecting to the amygdala play an important role in memory acquisition in the two passive avoidance tasks (Nomura, Nishiyama, Saito, & Matsuki, 1994).

Riekkinen in 1995, by examining the role of anterior and posterior cingulate cortical muscarinic receptors, suggested that muscarinic acetylcholine receptor antagonist may modulate passive avoidance performance via cholinergic receptors located in anterior cingulate cortex and the ability to develop a spatial navigation strategy via muscarinic receptors is located in the posterior cingulate (Riekkinen, Kuitunen, & Riekkinen, 1995).

Similar to our study, Gacar showed that scopolamine significantly shortened the second day latency compared to the control group (Gacar et al., 2011). Also bilateral injection of scopolamine (2 and 4 µg/rat) into the dorsal hippocampus (intra-CA1) of rats decreased memory consolidation and induced amnesia (Jamali-Raeufy, Nasehi, Ebrahimi-ghiri, & Zarrindast, 2011).

Many researchers have addressed the potential effectiveness of neurogenesis as a cause of hippocampal functions in learning and memory (Gage, Kempermann, Palmer, Peterson, & Ray, 1998; Shors et al., 2001; Van Praag, Kempermann, & Gage, 1999).

Similar to our observations in animal model system, Kotani in 2006 showed that scopolamine (0.75, 3 mg/day for four weeks) decreases the number of BrdU-positive cells in the DG, compared to the control group, and scopolamine suppresses the survival of newborn cells in the DG via CREB signaling without affecting neural progenitor cell proliferation and the neuronal differentiation. This is the first evidence that pharmacological manipulation of the cholinergic system can modulate adult hippocampal neurogenesis (Kotani, et al., 2006).

As a drug model, Konar in 2011 found that scopolamine hydrobromide (1, 3, 6, 10 mg/kg), administered intraperitoneally to mice, caused dose and time dependent down regulation of the BDNF and GFAP expression (Konar et al., 2011).

Conclusion

We concluded that scopolamine, as a non-selective muscarinic receptor antagonist, can significantly shorten the latency compared to the saline-control group in test day. Also it can reduce the number of neurons in sub-regions of hippocampal formation.

Acknowledgments

References

Azami, N. S., Jahanshahi, M., & Babapour, V. (2010). The role of CA1 α-adrenoceptor on scopolamine induced memory impairment in male rats. Physiology and Pharmacology, 14(1), 66-77.

Bancroft, J., & Gamble, M. (2008). Theory and practice of histological techniques: Elsevier Health Sciences.

Blokland, A. (1995). Acetylcholine: a neurotransmitter for learning and memory? Brain Research Reviews, 21(3), 285-300.

Blokland, A. (2005). Scopolamine-induced deficits in cognitive performance: A review of animal studies. Scopolamine Rev, 1-76.

Cooper-Kuhn, C. M., Winkler, J., & Kuhn, H. G. (2004). Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. Journal of neuroscience research, 77(2), 155-165.

Dunning, J., & During, M. J. (2003). Molecular mechanisms of learning and memory. Expert Reviews in Molecular Medicine, 5(25), 1-11.

Gacar, N., Mutlu, O., Utkan, T., Komsuoglu, I., Gocmez, S. S., & Ulak, G. (2011). Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and Morris water maze tests in rats. Pharmacology, biochemistry, and behavior, 99(3), 316-323.

Gage, F. H., Kempermann, G., Palmer, T. D., Peterson, D. A., & Ray, J. (1998). Multipotent progenitor cells in the adult dentate gyrus. Journal of neurobiology, 36(2), 249-266.

Hodges Jr, D. B., Lindner, M. D., Hogan, J. B., Jones, K. M., & Markus, E. J. (2009). Scopolamine induced deficits in a battery of rat cognitive tests: comparisons of sensitivity and specificity. Behavioural pharmacology, 20(3), 237.

Izquierdo, I. (1989). Mechanism of action of scopolamine as an amnestic. Trends in pharmacological sciences, 10(5), 175-177.

Jahanshahi, M., Golalipour, M., & Afshar, M. (2009). The effect of Urtica dioica extract on the number of astrocytes in the dentate gyrus of diabetic rats. Folia Morphologica, 68(2), 93-92.

Jahanshahi, M., Khoshnazar, A. K., Azami, N. S., & Heidari, M. (2011). Radiation-induced lowered neurogenesis associated with shortened latency of inhibitory avoidance memory response. Folia Neuropathol, 49(2), 103-108.

Jahanshahi, M., Sadeghi, Y., Hosseini, A., Naghdi, N., & Marjani, A. (2008). The effect of spatial learning on the number of astrocytes in the CA3 subfield of the rat hippocampus. Singapore Med J, 49(5), 388-391.

Jamali-Raeufy, N., Nasehi, M., Ebrahimi-ghiri, M., & Zarrindast, M. R. (2011). Cross state-dependency of learning between WIN55, 212-2 and scopolamine in rat dorsal hippocampus. Neuroscience letters, 491(3), 227-231.

Konar, A., Shah, N., Singh, R., Saxena, N., Kaul, S. C., Wadhwa, R., & Thakur, M. K. (2011). Protective Role of Ashwagandha Leaf Extract and Its Component Withanone on Scopolamine-Induced Changes in the Brain and Brain-Derived Cells. PloS one, 6(11), e27265.

Kotani, S., Yamauchi, T., Teramoto, T., & Ogura, H. (2006). Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. Neuroscience, 142(2), 505-514.

Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature, 319(6056), 774-776.

Nomura, Y., Nishiyama, N., Saito, H., & Matsuki, N. (1994). Role of cholinergic neurotransmission in the amygdala on performances of passive avoidance learning in mice. Biological & pharmaceutical bulletin, 17(4), 490-494.

Riekkinen, P., Kuitunen, J., & Riekkinen, M. (1995). Effects of scopolamine infusions into the anterior and posterior cingulate on passive avoidance and water maze navigation. Brain research, 685(1-2), 46-54.

Robinson, L., Harbaran, D., & Riedel, G. (2004). Visual acuity in the water maze: sensitivity to muscarinic receptor blockade in rats and mice. Behavioural brain research, 151(1-2), 277-286.

Sambeth, A., Riedel, W. J., Smits, L. T., & Blokland, A. (2007). Cholinergic drugs affect novel object recognition in rats: Relation with hippocampal EEG? European journal of pharmacology, 572(2-3), 151-159.

Santi, A., & Weise, L. (1995). The effects of scopolamine on memory for time in rats and pigeons. Pharmacology Biochemistry and Behavior, 51(2), 271-277.

Savage, U., Faust, W., Lambert, P., & Moerschbaecher, J. (1996). Effects of scopolamine on learning and memory in monkeys. Psychopharmacology, 123(1), 9-14.

Shors, T. J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., & Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. Nature, 410(6826), 372-375.

Siddiqui, M. F., & Levey, A. I. (1999). Cholinergic therapies in Alzheimer's disease. Drugs Future, 24(4), 417-424.

Spinelli, S., Ballard, T., Feldon, J., Higgins, G. A., & Pryce, C. R. (2006). Enhancing effects of nicotine and impairing effects of scopolamine on distinct aspects of performance in computerized attention and working memory tasks in marmoset monkeys. Neuropharmacology, 51(2), 238-250.

Sutherland, R. J., Whishaw, I. Q., & Regehr, J. C. (1982). Cholinergic receptor blockade impairs spatial localization by use of distal cues in the rat. Journal of Comparative and Physiological Psychology, 96(4), 563-573.

Tsuji, M., Takeda, H., & Matsumiya, T. (2003). Modulation of Passive Avoidance in Mice by the 5-HT1A Receptor Agonist Flesinoxan: Comparison with the Benzodiazepine Receptor Agonist Diazepam. Neuropsychopharmacology, 28(4), 664-674.

Van der Borght, K., Mulder, J., Keijser, J. N., Eggen, B. J. L., Luiten, P. G. M., & Van der Zee, E. A. (2005). Input from the medial septum regulates adult hippocampal neurogenesis. Brain research bulletin, 67(1-2), 117-125.

Van Praag, H., Kempermann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature neuroscience, 2(3), 266-270.

Winkler, J., Suhr, S. T., Gage, F. H., Thal, L. J., & Fisher, L. J. (1995). Essential role of neocortical acetylcholine in spatial memory. Nature, 375(6531), 484–487.