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Effect of Scopolamine-based Amnesia on the Number of Astrocytes in the Rat's Hippocampus

Efecto de la Amnesia Inducida por Escopolamina sobre el Número de Astrocitos en el Hipocampo de Ratas

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SUMMARY: As neuron–astrocyte interactions play a crucial role in the adult brain, it is thought that astrocytes support learning and memory through specific mechanisms. In this study, the effect of scopolamine based amnesia on the number of astrocytes in rats' hippocampus was studied. Adult male albino Wistar rats were bilaterally cannulated into the CA1 region and animals received saline or different doses of scopolamine (0.5, 1 and 2 mg/ rat, intra - CA1), immediately after training. Then all the rats were sacrificed and coronal sections were taken from the dorsal hippocampal formation of the right cerebral hemispheres and stained with PTAH. The area densities of the astrocytes in dentate gyrus were measured and compared in the all groups ($p < 0.05$). Data showed that post-training scopolamine (0.5, 1 and 2 μg/rat, intra-CA1) dose-dependently reduced the step-through latency in the inhibitory avoidance task, showing scopolamine-induced amnesia. Also we found different response of astrocytes in different subfields of hippocampal formation. In dentate gyrus the number of astrocytes was increased, but in other areas scopolamine can decreased the density of astrocytes. We concluded that scopolamine can cause amnesia and this phenomenon can have an effect on astrocyte numbers in the rats hippocampal formation.

KEY WORDS: Scopolamine; Amnesia; Astrocyte; Hippocampus; Rat.

INTRODUCTION

Inhibitory avoidance tasks, which are widely used in pharmacological studies of long-term memory in rodents, have been anticipated to rely mainly on the dorsal hippocampus (Mahmoodi *et al.*, 2010). As the cholinergic system plays an important role in learning and memory, a loss of cholinergic neurons and reduced choline acetyltransferase activity in the cerebral cortex and hippocampus are consistent with findings in Alzheimer's disease (Kwon *et al.*, 2009). Alzheimer's disease (AD) is a progressive neurodegenerative disorder. AD patients have cognitive deficits, impaired long-term potentiation (LTP) and learning and memory (Cachard-Chastel *et al.*, 2008).

The importance of cholinergic systems in learning and memory has been shown previously. Evidence suggested that acetylcholinesterase inhibitors, which enhance the availability of acetylcholine in the synaptic cleft improve performance in several cognitive models in both rodents and humans, whereas anticholinergic drugs impair learning and memory in a variety of tasks (Azami *et al.*, 2010). Scopolamine is a muscarinic cholinergic receptor antagonist that impairs memory performance that has been proposed as an animal model of dementia (Collerton, 1986; Jensen *et al.*, 1987). Some similarities between Alzheimer patients and scopolamine treated animals in the memory deficits have been reported (Azami *et al*.). In addition, along with cholinergic atrophy, monoamines are reduced in Alzheimer's disease and the possibility may exist that enhancement of monoaminergic functions may elicit beneficial effects on behavior and cortical activity (Dringenberg, 2000).

In the past astrocytes were considered to be only of structural importance, but now the scientists believe that astrocytes play a essential role in brain functions, such as neuronal path finding, potassium buffering, PH regulation and production of energy substrates for neurons and also neurotransmitter synthesis and uptake (Emamian *et al.*, 2009).

Neuron–astrocyte interactions play a crucial role during development and in the adult brain. Astrocytes

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modulate synaptic transmission, as well as the regulation of neurosteroidogenesis and neurogenesis (Jahanshahi *et al.*, 2008; Emamian *et al.*).

Based on previous findings the aim of this study was to investigate the effects of bilateral microinjections of CA1 region of the dorsal hippocampus on scopolamine-induced amnesia and scopolamine state-dependent memory, by using an inhibitory avoidance task and also the number of astrocytes in different subfield of hippocampus after amnesia was investigated.

MATERIAL AND METHOD

Adult male (8 weeks) Wistar rats (Pasteur Institute, Tehran, Iran) weighing 220– 270 g were used. They were housed four in a cage and had free access to food and water, and kept at (22 ± 2) ?C under a 12/12 h light-dark cycle (light beginning at 7:00 a.m.). All experiments were carried out during the light phase between 8:00 and 14:00. Experimental groups consisted of eight animals and each animal was tested once.

Animals were anaesthetized intraperitoneally with a ketamine/xylazine mixture (100 and 10 mg/kg, respectively) and placed in a stereotaxic frame (David Kopf Instruments, USA) with flat-skull position. A midline incision was made and the skin and underlying periosteum retracted. Stereotaxic coordinates for the CA1 regions of dorsal hippocampi were AP: -3 mm from bregma, L: ±2 mm from midline and V: -2.8 mm from the skull surface (Paxinos & Watson, 2007). The cannulae were anchored to the skull with dental cement, and then stainless steel stylets (27 gauge) were inserted into the guide cannulae to maintain patency prior to microinfusions.

All procedures were performed in accordance with institutional guidelines for animal care and use.

Drugs and microinfusions. Scopolamine hydrobromide (Tocris, UK) was dissolved in sterile saline and injected into CA1 of dorsal hippocampus. For bilateral drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 27-gauge injection needles (1mm below the tip of the guide cannula). The injection solutions were administered in a total volume of 1 ml/rat (0.5 ml in each side) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs (Azami *et al.*).

For behavioral test we used a step-through inhibitory avoidance apparatus consisted of two compartments of the same size (20 × 20 x 30 cm^3). In the middle of a dividing wall, a guillotine door (7.9 cm^2) could be lifted manually. The walls and floor of one compartment consisted of white opaque resin; 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, 3s, 1 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator (Azami *et al.*).

The method of our test was based on previous studies (Zarrindast *et al.*, 2002, 2005). Effect of scopolamine on memory retrieval in this experiment, the effect of post-training and pre-test administration of scopolamine on inhibitory avoidance response was examined. Four groups of animals received saline or different doses of scopolamine (0.5, 1 and 2 mg/ rat, intra - CA1), immediately after training. On the test day, the animals received saline (1 ml/rat, intra - CA1) 5 min before the test. The other groups of animals received saline (1 ml/rat, intra - CA1) or scopolamine (2 mg/ rat, intra-CA1), immediately after training and pre-test (5 min before the test) injections of different doses of scopolamine (0.5, 1 and 2 mg/ rat, intra - CA1).

Following behavioral testing, animals were decapitated under diethyl ether (Merck, Germany) anesthesia. Brains were removed and postfixed for 2 weeks in 10% formaldehyde for histological assessment. Next steps were dehydration using different degrees of alcohol and clarification with xylol. After histological processing, tissue was impregnated and then embedded in paraffin wax.

Histological and staining process was done on the brain sections of all six experimental groups. To quantify the number of astrocytes in the dentate gyrus, 15 slides from the proximity of cannula implantation region was obtained for each rat.

Finally, 7 μm thick coronal slices were cut with a Leitz rotary microtome (HM 325, Microm International GmbH, Walldorf, Germany). Mounted on slides, the last section of each 10 serial section was selected for staining; therefore, about 15 slides for morphometric measurement were obtained for each rat. Continuously, astrocytes were stained using phosphotanguestic acid hematoxylin (PTAH) (Jahanshahi, Sadeghi *et al.* 2008; Jahanshahi, Golalipour *et al.* 2009).

We preferred PTAH because it is the special staining method for astrocyte cell bodies and processes. In this method the astrocytes appear blue and the neurons become pink. Morphometric measurements were carried out using

an Olympus DP 12 digital camera and BX 51 microscope (Olympus Optical, Tokyo, Japan). We selected a field (20,000 μm^2) within the inferior horn of dentate gyrus.

Randomly selected, non-overlapping photographs were taken from the designated areas using a 40¥ objective lens. Images were saved by the Bioreporter program and further processed using the Adobe Photoshop 6.0 program (Adobe System Inc., San Jose, CA, USA). For cell counts, photographs at a magnification of 40× (objective lens) were taken throughout the longitudinal axis of dentate gyrus and further processed as described above. All of the astrocytes shown on this field (20,000 μm^2) were counted. The mean average of number of astrocytes from 15 slides of each rat was calculated (Jahanshahi *et al.*, 2008, 2009).

Data analysis. The data are expressed as mean ± S.D. 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 $P<0.05$. Calculations were performed using the SPSS statistical package.

RESULTS

Effects of Scopolamine on memory retrieval. Our data shows the effects of post-training or pre-test intra-CA1 administration of scopolamine on step-through latency. One-way ANOVA revealed that post-training scopolamine (0.5, 1 and 2 μg/rat, intra-CA1) dose-dependently reduced the step-through latency in the inhibitory avoidance task, showing scopolamine-induced amnesia. Furthermore, there was no significant difference between the number of trials to acquisition in animals that received scopolamine after training or before testing by itself and thus confirmed their uniformity (Fig. 1).

Effects of Scopolamine on hippocampal astrocytes. The mean and standard deviation of astrocytes in all groups showed in Tables (I, II and III). We found that in CA1 and CA3 areas of hippocampus the number of astrocytes decreased after exposure of scopolamine in all groups, and these differences were significant ($P<0.05$).

Our data showed that the differences of astrocytes

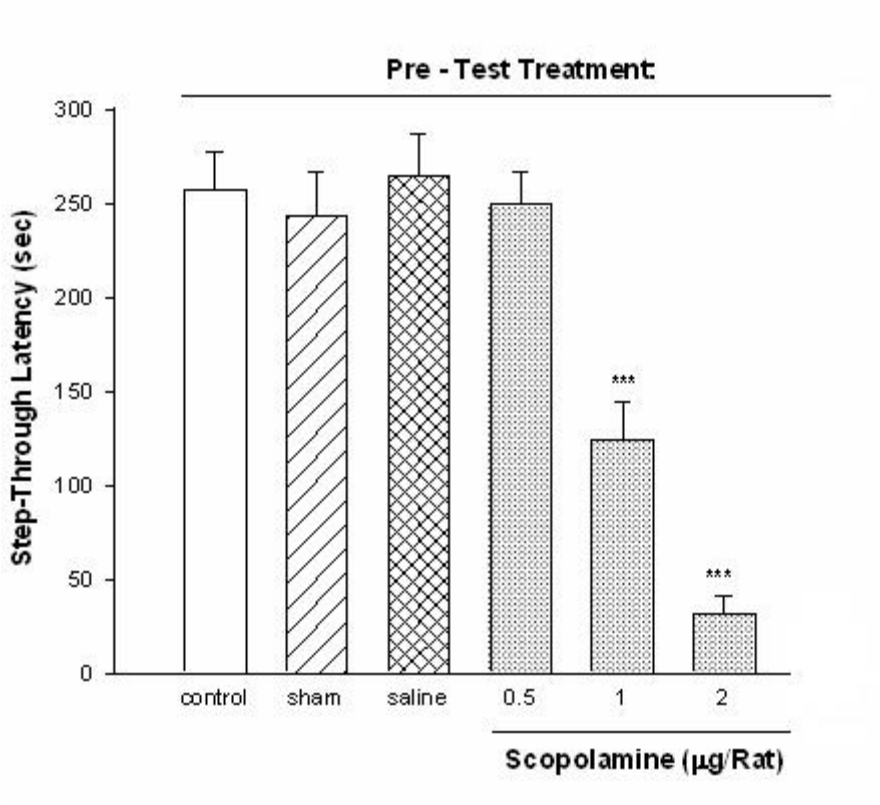

Fig. 1 indicates that animals in which retrieval was impaired due to post-training administration of scopolamine (scopolamine-induced amnesia), pre-test scopolamine (0.5, 1 and 2 μg /kg) restored the retrieval to the control level.

Table I. The number of astrocytes of all groups in CA1 area Groups were compared with control.

CA1	Mean of astrocytes	Std. Deviation	P-Value
Control	19.03	5.44	
Sham (surgery stress)	11.15	3.65	.000
Sham saline-saline	10.42	3.11	.000
Sco 2 - saline	11.97	4.11	.000
Sco 1 - saline	8.90	2.45	.000
Sco 0.5 - saline	9.20	3.23	.000
Sco 2 - 2	8.82	2.91	.000
Sco 2 - 1	12.88	5.36	.000
Sco 2 – 0.5	9.70	3.36	.000

Table II. The number of astrocytes of all groups in CA3 area Groups were compared with control.

CA3	Mean of astrocytes	Std. Deviation	P-Value
Control	25.30	7.74	
Sham (surgery stress)	9.80	3.75	.000
Sham saline-saline	10.68	2.85	.000
Sco 2 - saline	12.63	5.66	.000
Sco 1 - saline	9.23	1.91	.000
Sco 0.5 - saline	9.38	3.24	.000
Sco 2 - 2	9.60	2.62	.000
Sco 2 - 1	12.25	5.49	.000
Sco 2 – 0.5	9.00	1.67	.000

Table III. The number of astrocytes of all groups in DG area Groups were compared with control.

Dentate gyrus	Mean of astrocytes	Std. Deviation	P-Value
Control	17.02	6.50	
Sham (surgery stress)	35.88	14.03	.000
Sham saline-saline	19.62	9.62	.989
Sco 2 - saline	23.45	12.57	.171
Sco 1 - saline	19.97	11.88	.973
Sco 0.5 - saline	24.95	13.13	.028
Sco 2 - 2	20.15	8.43	.959
Sco 2 - 1	23.35	10.90	.189
Sco 2 – 0.5	13.28	6.39	.873

number in Dentate gyrus between control group with (17.02±6.5) and surgery stress sham group with (35.88±14.03) and also between control group and Sco2-0.5 group with (13.28±6.39) were significant, but the differences of astrocytes number between control group and the other groups were not significant, however the density of astrocytes in these groups were more than the control group.

DISCUSSION

Step-through is a model of inhibitory avoidance task which is widely used in pharmacological studies of long-term memory in rodents (Izquierdo & McGaugh, 2000; Izquierdo *et al.*, 2006). The present data indicated that post-training or pre-test intra-CA1 administration of scopolamine by itself impaired memory retrieval on the test day. In agreement with our present studies, several lines of evidence have been reported that acetylcholine is a crucial mediator of learning and memory (Blokland, 1995).

As we show in dentate gyrus the density of astrocytes in all groups was increased but this increase in animals that affected in surgery stress was more than the other groups. This phenomenon probably is due to relationship between stress and astrocytes reaction. Also we found that the density of astrocytes in Sco 2-0.5 group decreased ratio to control group.

Also we found different response of astrocytes in different subfields of hippocampal formation. It seams in dentate gyrus following the neurogenesis phenomenon astrocytes increased, but in other areas scopolamine can decreased the density of astrocytes.

Several pharmacological studies showed that the scopolamine as muscarinic cholinergic receptor antagonist, causing destruction of memory and learning in different learning models, and this degradation is directly related to reduced cholinergic system performance (Bartus, 2000).

In this regard, some studies have shown that not only the reduced levels of acetylcholine, but also the increase in immune reactivity of IL-1b-induced beta-amyloid peptide Ab1-42, caused defects in learning and memory. IL-1 released from astrocytes in culture medium inhibits long-term memory in the hippocampus. Therefore, the correlation between the

number of immunoreactive astrocytes to GFAP and spatial memory deficit has also been reported (Yan *et al*., 2001).

Other studies showed that injection of a-GPC (cholinergic precursors) before the behavioral test of learning prevent deficit caused by scopolamine, because it is possible that scopolamine associated with increased synthesis and release of acetylcholine in hippocampus. Treatment with a-GPC reduce glial reaction levels in the hippocampus that it is possible to cause neuronal protection and astroglial dynamic effects and some important neurodegenerative disorders such as brain aging, stroke, ischemic brain disease Parkinson's and Alzheimer's to be effective (Bramanti *et al.*, 2008).

As was stated earlier the scopolamine induced amnesia. Many studies have shown that Alzheimer's disease is associated with neuronal apoptosis. Recently, Bcl-2 gene as a major inhibitor of apoptosis considered that associated with learning and memory ability is the central nervous system (Niu *et al.*, 2007).

About that the mechanisms associated with the effect of scopolamine in 2005, Zhong *et al*. (2005) showed that potassium channels are important to regulate neuronal excitability and synaptic transmission. Several studies in various experimental models have proven important role of potassium channels in learning and memory mechanisms.

In conclusion, considering the results, it can be concluded that a dose-dependent scopolamine (0.5, 1, 2 μg / rat, intra-CA1) injections, after training or before testing, reduces the delay of arrival in black chamber of passive avoidance, this is represents scopolamine induced amnesia. Also we concluded that the scopolamine have different effect on the number of astrocytes in different subfields of hippocampus.

JAHANSHAHI, M.; AZAMI, N. S. & NICKMAHZAR, E. Efecto de la amnesia inducida por escopolamina sobre el número de astrocitos en el hipocampo de ratas. ***Int. J. Morphol., 30(2)***:388-393, 2012.

RESUMEN: Las interacciones neuronas-astrocitos desempeñan un papel crucial en el cerebro adulto, y se cree que los astrocitos apoyan el aprendizaje y la memoria a través de mecanismos específicos. Fue estudiado el efecto de amnesia inducida por escopolamina en el número de astrocitos del hipocampo de ratas. Ratas Wistar albinas macho adultas fueron canuladas bilateralmente en la región CA1 recibiendo solución salina o diferentes dosis de escopolamina (0,5, 1 y 2mg/rata, intra - CA1), inmediatamente después del entrenamiento. Luego, todas las ratas se sacrificaron y se tomaron secciones coronales de la formación del hipocampo dorsal del hemisferio cerebral derecho y se tiñeron con PTAH. Las densidades de área de los astrocitos en el giro dentado fueron medidas y comparadas en todos los grupos ($p < 0,05$). Los datos mostraron que la escopolamina (0,5, 1 y 2 mg / rata, intra-CA1) dosis-dependiente post-entrenamiento redujo el paso de latencia de la tarea de evitación inhibitoria, mostrando amnesia inducida por escopolamina. También encontramos diferentes respuestas de los astrocitos en los distintos subcampos de la formación hipocampal. En el giro dentado, el número de astrocitos se incrementó, pero en otras áreas la escopolamina pudo disminuir la densidad de los astrocitos. Se concluye que la escopolamina puede causar amnesia y este fenómeno puede afectar el número astrocitos en la formación hipocampal de ratas.

PALABRAS CLAVE: Escopolamina; Amnesia; Astrocitos; Hipocampo; Rata.

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