Indian Journal of Pharmacology 2003; 35: 47-50 **SHORT COMMUNICATION**

EVALUATION OF EFFECT OF SCOPOLAMINE ON STAGES OF ACTIVE AVOIDANCE LEARNING IN RATS

A. DAS, M. DIKSHIT, H.K. SINGH, C. NATH*

Division of Pharmacology, Central Drug Research Institute, Lucknow - 226 001.

Manuscript Received: 19.3.2002 Revised: 20.7.2002 Accepted: 25.8.2002

ABSTRACT

Objective: To investigate the effect of scopolamine administered at different stages of learning and memory process in active avoidance task in rats.

Methods: Adult male Sprague-Dawley rats were trained on active avoidance task. Each animal received a daily session of 15 trials for 5 days *i.e.,* a maximum of 75 trials. Scopolamine (3 mg/kg, *i.p*) was administered at different time points according to acquisition, consolidation and recall of active avoidance learning in the three groups (n=8). Increase in avoidance response on 5th session as compared to 1st session was taken as criteria of learning and failure of learning was considered as dementia.

Results: There was a significant increase in the avoidance response on 5th session as compared to 1st session in the control group. Among scopolamine treated groups, most marked dementia was observed in the group that received scopolamine after the last trial of 1st session.

Conclusion: Results of this study show that scopolamine preferentially affects acquisition and consolidation as compared to recall of memory in the learning process.

KEYWORDS Cognition dementia hyoscine memory acquisition memory consolidation memory recall

INTRODUCTION

On the basis of experimental as well as clinical evidences, central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions[1-2]. Cholinergic neuronal loss along with abnormal proteins, β-amyloid and tau, and associated impaired cognitive functions are the major features of senile dementia of Alzheimer type[3-4]. Blockade of central muscarinic acetylcholine receptor disrupts learning and memory functions in animals and human beings[5]. Anticholinergic drug (muscarinic blocker) such as scopolamine has been in use as potent dementic agent. It induces cognitive deficit in young volunteers which is qualitatively similar to that occurring naturally in aged subjects when tested on the same clinical battery. One such deficit was loss of memory in young subjects for recent (but not immediate) events[6]. Similarly, scopolamine injected into young monkeys caused memory deficits similar to those occurring naturally in aged monkeys[7]. In our earlier studies we have shown administration of scopolamine prior to first trial (acquisition) induced deficit in passive avoidance learning[8]. In the process of learning and memory, three important stages have been suggested *viz.,* acquisition, consolidation and recall of the learned task[9]. Inspite of extensive use of scopolamine as dementic agent in experimental studies, it is still not clearly defined as to which of the stage of learning and memory process is affected more by it in experimental models. Therefore, the present study was performed to investigate the effect of scopolamine administered at different stages of active avoidance learning in rats.

MATERIALS AND METHODS

Animals: Study was conducted on adult male Sprague-Dawley rats of 3-4 months (*wt.* 175-200 g). The animals were kept in polyacrylic cages

Correspondence: C. Nath
e-mail: cnathcdri@rediffmail.com

(38 x 23 x 10 cm) with 1-2 animals per cage and maintained under standard housing conditions (Room temperature 24-27ºC and humidity 60-65%) with 12 h light and dark cycle. The food in form of dry pellets and water were available *ad libitum*.

The animals were procured from the Laboratory Animal Services Division of Central Drug Research Institute. The animal experiments were performed according to internationally followed ethical standards and approved by the research ethics committee of Central Drug Research Institute.

Active avoidance training: The animals were trained on Active Avoidance Task in a computerized shuttle box (Columbus Instruments, Ohio, USA) provided with a software program PACS 30. Rat is placed in a compartment separated from the other one by a guillotine door in the shuttle box. After an exploration period of 2 min the guillotine door automatically opens. Thereafter, the trial starts. In each trial the animal is subjected after 30 s first to a light followed by sound stimulus at intensity of 8 (scale of 0 - off and 10 - maximum, provided in the PACS 30 software) for 10 s each in a total trial period of 1 min. Immediately after the sound stimulus, the rat receives a single low intensity foot shock (0.5 mA; 10 s) through the floor grid if it does not transfer to the other compartment (shock free). Infrared sensors monitor the transfer time from one compartment to another, which is recorded as avoid (after the stimulus either light alone or both light and sound) and escape (after the foot shock) response. Each animal received a daily session of 15 trials with an inter-trial duration of 15 s for 5 days *i.e.,* a maximum of 75 trials. The rats were evaluated for learning and memory functions on the basis of their performance in the last session *i.e.,* in the 5th session. The criterion for improved cognitive activity was taken as significant increase in the avoidance response on 5th session (retention) as compared to 1st session (training).

Scopolamine administration: The animals were divided into four different groups (n=8). Scopolamine (3 mg/kg, *i.p*) was administered at different time periods in the three groups as follows:

Group I - no scopolamine, (control),

Group II - scopolamine was administered 5 min prior to 1st Trial on 1st session (Training session),

Group III - scopolamine was administered 5 min after the 15th (*i.e.,* last) trial on 1st session (Training session), and

Group IV - scopolamine was administered 5 min prior to the 1st trial on the last session *i.e.,* 5th session (Retention test).

Dementic effect of scopolamine was evaluated on the basis of significant decrease in number of avoidance response in the treated groups as compared to that of control group in the last session *i.e.,* 5th session.

Statistical analysis: Mean values and standard error (SE) of mean were calculated for the numbers of avoid response. The significance of difference between the values of control and scopolamine treated groups for each session was determined by ANOVA (one-way) followed by Dunnett's test. The difference between the values of 1st session (training) and 5th session (retention test) of the same group was analysed by Student's paired ' *t* ' test. The data of percent change was analyzed by test of proportion, z test. P values < 0.05 were considered significant.

Chemicals: Scopolamine hydrobromide was purchased from Sigma Chemicals, USA.

RESULTS

There was a significant ($p<0.001$) increase in avoidance response on 5th session (9.0±1.29) as compared to 1st session (4.75±0.25) in the control group (group I) whereas the scopolamine treated groups, (group II-IV) did not show any significant increase in the avoidance responses on 5th session as compared to 1st session (Table 1).

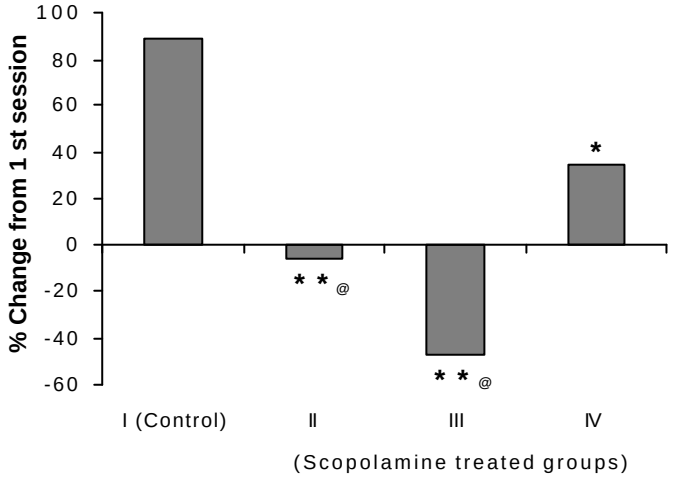
Group II (4.25±0.75) and III (3.50±1.32) *i.e.,* the one in which scopolamine was administered prior to first trial and after the last trial, respectively on 1st session showed a significant ($p<0.01$) decrease in avoidance response as compared to the control group (9.0±1.29) on 5th session (Table 1). In the scopolamine treated group II and III, there was 6 and 48 % decrease in avoidance response on 5th session from 1st session respectively, whereas scopolamine treated group IV and control (group I) group showed a 35 and 89 % increase in percent change in avoidance response on 5th session as compared to 1st session, respectively (Figure 1).

Table 1. Showing the number (mean±SEM) of avoidance responses in the control (group I) and scopolamine (scop) treated (group II - IV) groups (n=8) on each session of active avoidance training.

Groups	Sessions				
	1st	2nd	3rd	4th	5th
I (control)	4.75 ± 0.25	3.50 ± 1.04	4.75 ± 1.60	5.20 ± 1.08	9.0 ± 1.29*
II (scop)	4.50 ± 0.50	4.00 ± 0.71	4.25 ± 1.25	4.50 ± 0.50	4.25 ± 0.75@
III (scop)	6.75 ± 1.11	3.75 ± 1.43	3.00 ± 0.71	3.50 ± 1.19	3.50 ± 1.32@
IV (scop)	4.25 ± 1.11	4.00 ± 1.08	4.25 ± 1.11	5.75 ± 1.18	5.75 ± 1.50
One-way ANOVA					
P	>0.05	>0.05	>0.05	>0.05	<0.05
F	1.887	0.048	0.381	0.330	3.826
df	3, 28	3, 28	3, 28	3, 28	3, 28

@P<0.001, significant difference from the control in 5th session (retention) by Dunnett's test, and *P<0.01, significant difference between 1st session (training) and 5th session (retention) of the same group by Student's paired ' *t* ' test.

Figure 1. The percent change in avoidance response on 5th session from 1st session in control (group I) and scopolamine treated (group II-IV) groups (n=8). *P<0.01, **P<0.001, significant difference from the control group and @P<0.01, significantly different from scopolamine treated group IV (test of proportion [z test]).



DISCUSSION

Avoidance response has been suggested to reflect cognitive functions[10] and was investigated for its susceptibility to scopolamine treatment in rats. The different time points of scopolamine administration employed in the present study were based on the stages of learning and memory. Scopolamine treatment was aimed in group II for acquisition, group III for consolidation and group IV for recall of the learned task (avoidance response) in active avoidance test. Although there was a general decrease in performance in the active avoidance learning in the scopolamine treated groups as compared to control group the effect was more apparent when scopolamine was administered on the 1st session *i.e.,* group II (5 min prior to 1st Trial on 1st session) and III (5 min after the 15th *i.e.,* last trial on 1st session) as compared to its administration

on its last session *i.e.,* 5th session in group IV (5 min prior to the 1st trial on the last session *i.e.,* 5th session). Thus the acquisition and consolidation process of learned task rather than the recall is more susceptible to the effect of scopolamine.

There is also substantial clinical evidence that muscarinic receptor blockade results into disruptions of behavioral inhibition, working (short term) memory, retrieval from reference (long term) memory, attention, decisional processes, movement and strategy selection, and altered sensory processing[11]. Thus central cholinergic neurons are important in the acquisition and post-acquisition (consolidation) performance of a variety of learned behaviors[11]. The dementic effects of scopolamine observed in the present study are in concurrence with those observed in clinical situations. Therefore, the preferential effect of scopolamine on acquisition and consolidation of memory in the learning process, suggest that the time of administration of scopolamine is an important factor in the study of its effect on learning and memory.

ACKNOWLEDGEMENT

Dr. S. K. Mandal, Division of Biometry, deserves our special thanks for statistical analysis. One of the authors (AD) is grateful to CSIR (India) for providing Senior Research Fellowship.

REFERENCES

1. Vanderwolf CH. Cerebral activity and behavior: Control by central cholinergic and serotonergic system. *International Rev Neurobiol* 1988;**30**:225-340.
2. Blockland A. Acetylcholine: A neurotransmitter for learning and memory? *Brain Res Rev* 1996;**21**:285-300.
3. Enz A, Amstutz R, Boddeke H, Gmelin G, Malanowski J. Brain selective inhibition of acetylcholinesterase: A novel approach to therapy for Alzheimer's disease. *Prog Brain Res* 1993;**98**:431-8.
4. Siddiqui MF, Levey AI. Cholinergic therapies in Alzheimer's disease. *Drugs Fut* 1999;**24**:417-44.
5. Davis KL, Yamamura HI. Minireview: Cholinergic underactivity in human memory disorders. *Life Sci* 1978;**23**: 1729-34.
6. Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction: A critical review. *Science* 1982;**217**:408-17.
7. Fischer A. Potential animal models for senile dementia of Alzheimer's type, with emphasis on AF64A-induced cholinotoxicity. *Ann Rev Pharmacol Toxicol* 1986;**26**: 161-81.
8. Das A, Dikshit M, Nath C. Correlation between brain acetylcholinesterase activity and passive avoidance - learning in rats. *Annals Neurosci* 2001;**8**:31.
9. Guyton AC, Hall JE, editors. Text book of Medical Physiology. Singapore: Harcourt Asia Pte; 1999.
10. Reddy DS. Assessment of nootropic and amnestic activity of centrally acting agents. *Indian J Pharmacol* 1997; **29**:208-21.
11. Fibiger HC. Central cholinergic systems and memory. In: Squire LR, Lindenlaub E, editors. The biology of memory, Symposia Medica Hoechst 23. New York: Springer-Verlag; 1990.