DIFFERENT EFFECTS OF SCOPOLAMINE ON MEMORY OF YOUNG MALE AND FEMALE RATS

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Summary

The interaction of both male and female sex hormones with neurotransmitters has been widely investigated. In the present study different effect of scopolamine on learning and memory of male and female rats was investigate. Forty rats were divided into four groups: (1) Male, (2) Male-Scopolamine (Male-Sco), (3) Female and (4) Female-Scopolamine (Female-Sco). On a training trial, the rats were placed in the light compartment and when entered completely into the dark compartment received an electric shock . The latency time to enter the dark compartment was recorded and defined as retention trial. The animals of Male-Sco and Female-Sco groups received scopolamine(2 mg/kg, ip) 30 min before each retention trial. The time latency to enter the dark compartment in both Male-Sco and Female-Sco groups was lower than male and female groups respectively (P<0.001). The time latency in Female-Sco group was higher than Male-Sco group (P<0.001). These results suggest that deleterious effects of scopolamine on memory are gender dependent and therefore male or female hormones have probably an interaction with cholinergic system.

Keywords: Male, Female, Memory, Scopolamine, Gender, Difference

Introduction

The sex dependent difference in anatomy and the functions of several regions of the brain have been widely reported(1). It has been well known that the brain of males is larger then of female with the same body size however, it seems that the volume of gray matter in women is greater than men (2, 3). The sex dependent difference in the hippocampus, and important part of the brain involved in cognition and memory, and therefore the difference in cognitive performance has also been reported (4-6). The presence of high density of both male and female sex hormone receptors in brain regions such as the hippocampus may confirm the relationship between sex hormones and cognitive functions of the brain(7-11). Several neurotransmitter systems such as glutamatergic, srotonergic, adrenergic, dopaminergic and cholinergic are involved in cognition, memory and learning and also, the interaction of sex hormones with these neurotransmitters has been reported (12-19).The central cholinergic system is the most important neurotransmitter system which is involved in various aspects of cognition and memory (19-22). The cholinergic basis of memory dysfunction and cognitive decline in diseases such as Alzheimer's has also been well documented (23-27).

The interaction of estrogen with the central cholinergic system, the most important neurotransmitter system involved in cognitive functions has also been suggested. Estrogen has been reported to stimulate choline acetyltransferase expression and activity, the activity of acetylcholine esterase and potassium-stimulated acetylcholine release in rat hippocampus (28-30). The interaction of male gonadal hormones with cholinergic system has also been reported(31).

Scopolamine, a muscarinic acetylcholine receptor antagonist, has been frequently used to produce learning and memory impairments which mimic several aspects of cognitive impairment due to aging and dementia (32-35). Regarding to the possible sex dependent difference in cholinergic system with both male and female hormones(36), the aim of present study was to elucidate different effects of scopolamine on learning and memory of male and female rats using passive avoidance test.

Material and methods

Animals and groups

Forty, 8- weeks male female Wistar rats (200±10 g), were obtained from Razi vaccine and serum research institute of Mashhad. All rats were housed 4 per standard cage at room temperature (23± 1 °C) on a 12 h light/dark cycle with free access to water and food ad libitum. Rats were given one week to adapt with new environment before any procedure was initiated. Animal handling and all related procedures were approved by the Mashhad Medical University Committee on Animal Research. The animals were randomly divided to following groups: (1) Male, (2) Male-Scopolamine (Male-Sco), (3) Female and (4) Female-Scopolamine (Female-Sco).

Behavioral procedures

The animals were handled for 1 week before starting the experiments. Passive avoidance learning test based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a light and a dark compartment with a grid floor adjoining each other through a small gate. The rats were accustomed to the behavioral apparatus for 5 min during 2 consecutive days before the training session. On the third day, the animals were placed in light compartment and the time latency to enter the dark compartment was recorded .On a training trial, the rats were placed in the light compartment facing away from the dark compartment. When the rats were entered completely into the dark compartment, they received an electric shock (1 mA, 2s duration). Then, the rats were returned to their home cage. 1, 3, 24 and 48 hours later, the rats were placed in the light compartment and the latency time to enter the dark compartment as well as, the times spent by the animals in dark and light compartments was recorded and defined as retention trial. The animals of Male-Sco and Female-Sco groups received scopolamine(2 mg/kg, ip) 30 min before each retention trial. In Male and Female groups 1m/kg saline was injected instead of scopolamine.

Statistical analysis

Data were expressed as mean ± SEM. The statistical analysis was done by one-way and repeated measures ANOVA, followed by post hoc comparisons test. The criterion for statistical significance was $p < 0.05$.

Results

As shown in Figure 1, before receiving shock, there was no significant difference in time latency to enter the dark compartment between groups. Figure 2 shows that the time latency to enter the dark compartment in both Male - Sco and Female - Sco groups was lower than of Male and Female groups($P<0.001$). 48 hr after receiving shock the time lentency to enter the dark compartment in Female group was higher than of Male group($P<0.01$). In this retention trial, the time latency in Female-Sco group was also greater than Male –Sco group($P<0.001$). The total time spent in dark compartment by the animals of both Male –Sco and Female Sco groups was significantly higher than Male and Female groups respectively (Figure 3, $P<0.001$). 48 hours after receiving shock the animals of Male -Sco group spent more times in dark compartment in comparison with Female-Sco group ($P<0.001$); however, there was no significant difference between Male group compared to Female group (Figure 3). The total time spent in light compartment by the animals of Male-Sco and Female-Sco groups was lower than their controls ($P<0.001$) (Figure 4). There was no significant difference between Male group in comparison with Female group. 48 hours after shock the animals of Female-Sco group spent more times in light compartment in comparison Male-Sco group ($P<0.001$) (Figure 4).

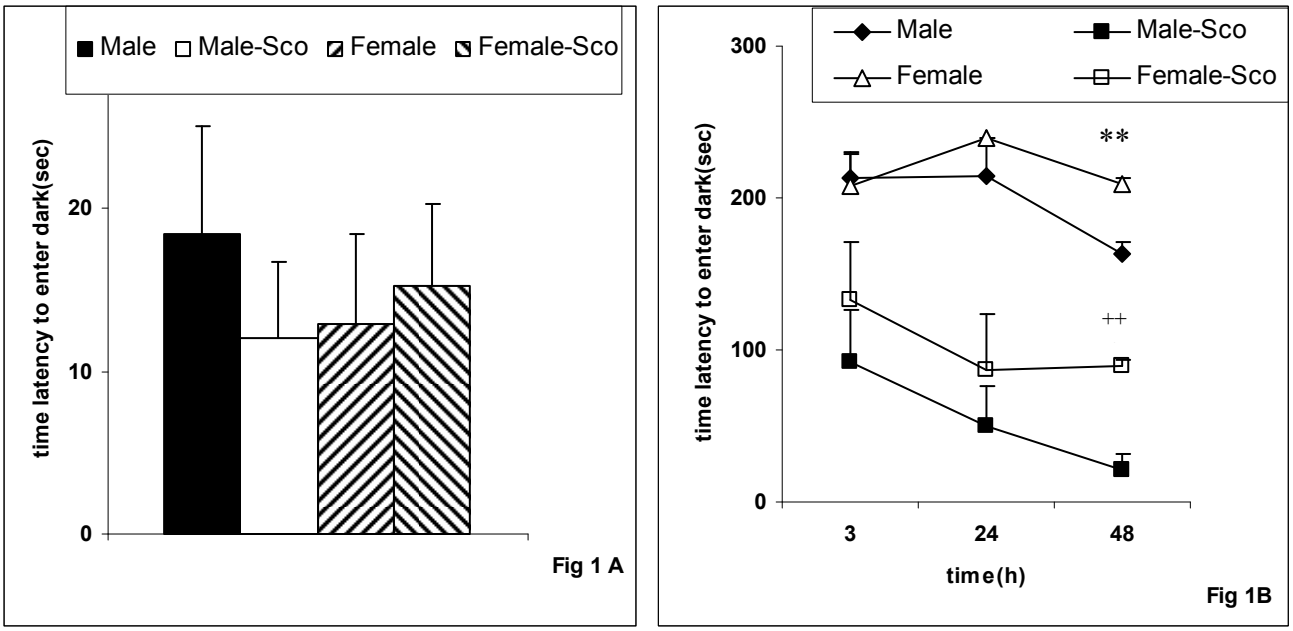

Figure 1. Comparison of time latency to enter the dark compartment before (1A) and 3, 24 and 48 hours after (1B) receiving shock1A) and af in groups: Male Male-Scopolamine (Male-Sco), Female and Female- Scopolaimne (Female-Sco).

Data were expressed as mean ± SEM. $^{**}P<0.01$ when Female group was compared with Male group, $^{+++}P<0.001$ when Female-Sco group was compared with Male-Sco group.

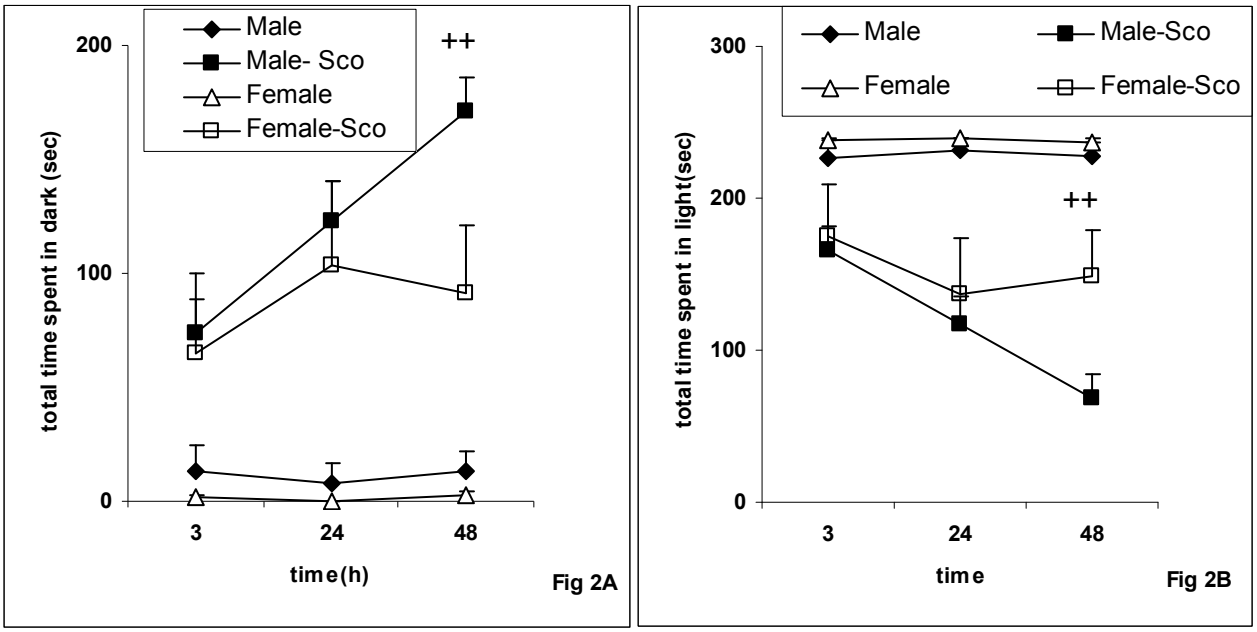

Figure 2. Comparison of the total time spent in dark (2A) and light(2B) compartments, 3, 24 and 48 hours after receiving shock, in experimental groups. $^{++}P<0.001$ when Female-Sco group was compared with Male-Sco group.

Discussion

Formation of memory is a complex process which needs the contribution of several neuronal pathways and neurotransmitters. It is well known that the cholinergic system plays an important role in learning and memory in humans and animals (19, 20, 37). Several of these neurotransmitters systems have been shown to have interactions with both male and female hormones (12, 17, 18). Sex dependent differences in learning and memory have also been reported. Numerous studies obtained from animals and humans have demonstrated that males have better spatial abilities in comparison with females(38). In other tasks, such as visual memory and object recognition and the non-spatial version of the water radial-arm maze females have better performances than males(39, 40). The differences between male and female rats in passive avoidance learning and memory has also been reported(41). In the present study 48 hours after receiving shock the time latency to enter dark compartment in female group was longer than male group. These results are agree with the results of Kemble (42) who showed the superiority of females over males. However the results obtained from other studies showed no significant difference in passive avoidance learning and memory between male and female(43, 44).

The negative effects (45, 46) or no effect (47-49) of estradiole on learning and memory have been widely reported. Regarding the present study it seems that the physiological levels of estradiole has positive effects on memory retrieval in passive avoidance test. The mechanism(s) by which estrogen regulates memory functions has been widely investigated. It has been reported that elevated levels of circulating estrogen in female rats result in increased spine and synaptic density and parallel increases in NMDA receptor binding in area CA1 of the hippocampus (50, 51). The increase in spine density is associated with increased sensitivity of CA1 pyramidal cells to NMDA-receptor mediated synaptic input (52), suggesting that the new spines and synapses induced by estrogen are enriched in NMDA-

receptors (53). There are also evidences showing that ovariectomy decreases NMDA binding density in the hippocampal CA1 region and dentate gyrus and estradiol restored and increased NMDA binding density in the CA1 region(54). The NMDA receptor has been implicated in the induction of hippocampal long-term potentiation (LTP) and its highest density is in hippocampus which is associated with certain forms of learning (55). It has been reported that the passive avoidance learning and memory depends on normal function of the central cholinergic transmission. It seems that the muscarinic cholinergic synaptic elements located in the posteroventral region of hippocampus and the basal lateral part of the posterior amygdale has an important role in this kind of memory and learning (56, 57). In the present study sex dependent difference in memory impairment by scopolamine was investigated. The results showed that the latencies to enter the dark compartment in scopolamine - treated female rats were greater than male. The time spent in dark by the animals of female-scopolamine group was also lower than male-scopolamine. It seems that the deleterious effects of scopolamine in female groups are moderate in comparison with male. There are strong evidences approving the effects of ovarian steroid hormones on learning and memory (58). The results of present study indicated that estrogen probably attenuates deleterious effects of scopolamine on memory retention; time latency to enter the dark compartment in scopolamine treated female animals was higher than male treated. Gibbs et al also showed that estrogen replacement prevents scopolamine-induced impairment in passive avoidance acquisition (59). The presence of estrogen receptors on neurons of the basal forebrain region and hippocampus confirms the interaction of cholinergic system with estrogen in cognition and memory (60-62). The results obtained from young female rats showed that estrogen reversed the memory impairment due to disruption of the cholinergic transmission however, it was not effective in older female rats (63). Estrogen has been shown to influence cholinergic neurochemistry in the basal forebrain and hippocampus and it has been previously suggested that the ability of estrogen to alter NMDA receptor binding to CA1 is related to its ability to alter cholinergic system (64)**.**

Based on the results of present study it is suggested that the deleterious effects of scopolamine on learning and memory is different in male and female rats

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