BASIC RESEARCH

The effect of chronic administration of L-arginine on the learning and memory of estradiol-treated ovariectomized rats tested in the morris water maze

Mahmoud Hosseini,[1] Raheleh Headari,[2] Sharbanoo Oryan,[2] Mosa Alreza Hadjzadeh,[1] Fatima Saffarzadeh,[1] Majid Khazaei[3]

1 Department of Physiology and Neuroscience Research Center, School of Medicine, Mashhad University of Medical Sciences – Mashhad/Iran. **2** Department of Biology, Faculty of Science, Tarbiat Moallem University of Tehran – Tehran/Iran. **3** Department of Physiology, School of Medicine, Isfahan University of Medical Sciences – Isfahan/Iran.

OBJECTIVE: The present study was carried out to evaluate the effect of L-arginine on the learning and memory of estradiol-treated ovariectomized (OVX) rats.

METHODS: Forty-eight rats were divided into six groups: (1) sham, (2) OVX, (3) sham-Est, (4) OVX-Est, (5) sham-Est-LA, and (6) OVX-Est-LA. The animals of the sham-Est and OVX-Est groups were treated by weekly injection of estradiol valerate (2mg/kg). The sham-Est-LA and OVX-Est-LA groups were treated in the same manner but with an additional daily injection of L-arginine (200mg/kg). After eight weeks, animals of all groups were tested in the Morris water maze. The escape latency and path traveled to reach the platform were compared between groups.

RESULTS: Time latency and path length in the OVX group were significantly higher than in the sham group ($P<0.05$). The OVX-Est group had a significantly shorter traveled path length and time latency compared to the OVX group ($P<0.001$). Time latency and path length in the sham-Est group was significantly higher than in the sham group ($P<0.001$). Time latency and path length in the OVX-Est-LA group were significantly higher than in the OVX-Est group.

CONCLUSIONS: These results allow us to propose that chronic treatment with estradiol enhances the spatial learning and memory of OVX rats, and that long term L-arginine treatment attenuates the effects of improvement produced by estradiol in OVX rats.

KEYWORDS: Estradiol; L-arginine; Morris water maze; Ovariectomy.

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E-mail: hosseinim@MUMS.ac.ir

Tel.: 98 511 8828564-5

INTRODUCTION

Nitric oxide (NO) is a free radical gas that plays important physiological roles in biological systems; specifically, it acts as a diffusible intercellular signaling molecule in the central nervous system. NO is synthesized from L-arginine by NO synthase (NOS) and acts as a critical mediator in synaptic plasticity, long-term potentiation (LTP) and the consolidation of long-term memory.[1,2] The relationship between NMDA receptors and the NO system in learning and memory has been well documented.[2,3] Several studies have indicated that NOS inhibitors impair consolidation of memory[4,5] and block the induction of LTP.[6] L-arginine, a NO precursor, improves memory formation and reverses the effect of NOS inhibitors.[7]

There is strong evidence documenting the positive effects of ovarian steroid hormones on learning and memory.[8] Several experiments show that estradiol replacement after ovariectomy enhances memory in ovariectomized (OVX) rats. This enhancement is possibly a result of the activation of cholinergic and aminergic systems. However, the exact mechanism is still unknown.[9] It has been well documented that estrogen influences the NO system in both peripheral and nervous tissues.[10-12] Additionally, estrogen increases eNOS activity and expression[13,14] as well as the production of nitric oxide in endothelial cells.[15] Estrogen alters nNOS mRNA regulation, the number of nNOS-expressing neurons and NO production in the brain.[16,17]

Regarding the possible interaction between estradiol and NO systems, the aim of the present study was to elucidate the effects of L-arginine (the precursor of nitric oxide) and estradiol in learning and memory processes in OVX and intact female rats using a Morris water maze test.

MATERIAL AND METHODS

Animals and Drugs

Forty-eight 8-week-old female Wistar rats (200 ± 10 g) were obtained from the Razi vaccine and serum research institute of Razi Institute for Vaccine and Serum Production (Mashhad, Iran). All rats were housed four per standard cage at room temperature (23 ± 1 ℃) on a 12 h light/dark cycle with free access to water and food *ad libitum*. Rats were given one week to adapt to this new environment before any procedure was initiated. Animal handling and all related procedures were approved by the Mashhad University of Medical Sciences, Ethical Committee Acts (Mashhad, Iran). L-arginine was purchased from Sigma Aldrich (USA) and dissolved in saline. Ketamine and estradiol valerate were provided by Darou Pakhsh Pharma Cem. Co. (Tehran, Iran).

Ovariectomy

The animals were ovariectomized under anesthesia with ketamine (150 mg/kg, i.p.) and rompun (0.1 mg/kg, i.p.).[18] Anesthesia was confirmed by a reduced respiratory rate and lack of response to gentle pinching of the foot pad. A ventral incision was made through the skin of the flank of the rat, and the ovaries and ovarian fat were removed. The ovaries were isolated by ligation of the most proximal portion of the oviduct before removal. The same procedure was carried out for the sham groups except for the removal of the ovaries.

Groups and Treatments

After a one-week recovery period, OVX and sham-operated rats were randomly divided into the following groups: (1) sham, (2) OVX, (3) sham-Est, (4) OVX-Est, (5) sham-Est-LA, and (6) OVX-Est-LA. The animals of the sham-Est and OVX-Est groups received a weekly injection of estradiol valerate (2mg/kg; i.m.).[11] The sham-Est-LA and OVX-Est-LA groups were treated with estradiol valerate in the same manner as groups 3 and 4 and additionally received a daily injection of L-arginine (200 mg/kg; i.p.). The animals of the sham and OVX groups received saline instead of L-arginine and estradiol valerate.[11]

Behavioral Assessment

For assessment of behavioral function, the rats were tested in a Morris water maze (MWM). The MWM was a black circular pool with a diameter of 136 cm and a height of 60 cm, filled with 20 ± 1℃ water to a depth of 30 cm. The maze was divided geographically into four equal quadrants and included release points in each quadrant (marked as N, E, S and W). Fixed, extra-maze visual cues were present at various locations around the maze (i.e., computer, MWM hardware, posters). An infrared camera was mounted above the center of the maze. An infrared LED was attached to each rat as a probe so that the animal's motion could be recorded and sent to the computer. A tracking system was used to measure the escape latency, traveled path and swimming speed.[9,20]

Animals performed a block of four trials during five daily sessions. During the five daily sessions, a Plexiglas platform situated in the center of the southeast quadrant was submerged 1.5 cm below the surface of water (thereby made invisible) for testing of spatial learning. The platform position remained stable during the five days of the assessment.

Each trial was started by placing a rat into the pool, facing the wall of the tank. Each of four starting positions (north, east, south and west) was used once in a series of four trials; their order was randomized. Each trial was terminated as soon as the rat had climbed onto the escape platform or when 60 s had elapsed. A rat was allowed to stay on the platform for 15 s. Then, it was taken from the platform and the next trial was started after 20 s. Rats that did not find the platform within 60 s were put on the platform by the experimenter and were allowed to stay there for 15 s. After completion of the fourth trial, each rat was kept warm for an hour and returned to its home cage. The time latency and path length to reach the platform was compared between groups. All tests were conducted between 16:00 and 18:00.

Statistical Analysis

All data were expressed as the means ± SEM. The data of different groups over the five days were compared using repeated measured ANOVA tests with Tukey's post hoc analysis between groups. Differences were considered statistically significant when $P<0.05$.

RESULTS

Time latency and path length in the OVX group were significantly higher than in the sham group ($P<0.05$; fig 1). The animals of the OVX-Est group had significantly shorter time latency and path length traveled compared with the OVX group ($P<0.001$; fig 2). Time latency and path length in the sham-Est group were significantly higher than in the sham group ($P<0.001$; fig 3). Time latency and path length in the OVX-Est-LA group were significantly higher than in the OVX-Est group ($P<0.01$ and $P<0.05$; fig 2). There was no significant difference between the OVX-Est-LA group and the OVX group (fig 2). There was no significant difference between the sham-Est and sham-Est-LA groups in traveled path length or time latency (Fig 3).

DISCUSSION

In the present study, we investigated the chronic effects of estradiol valerate alone and in combination with L-arginine (the precursor of nitric oxide) on the learning and spatial memory of OVX and sham-operated female rats. OVX rats have been frequently used as a model of hormone deprivation to study post-menopausal changes in adult females.[21,20] With this in mind, we used this model to evaluate the effects of estradiol and L-arginine on the learning and memory of OVX rats. The results of the present study indicate that estrogen therapy in OVX rats improves the efficiency of spatial memory retention; the time latency and traveled path length to find the hidden platform in animals of OVX- Est group was significantly lower than OVX group. Although the results of the present study may only serve adequately as behavioral evidence, the Morris water maze is an experimental method commonly used to evaluate spatial learning and memory in animal models.[22-24] This result was in agreement with Ping et al., who showed that treatment with estrogen significantly improved spatial learning performance in low-estrogen (OVX) rats.[25] However, Herlitz and co-workers showed that there were no considerable differences in cognitive performance between premenopausal and post-menopausal women.[26] Other researchers have also reported that estrogen has negative effects [27,28] or no effect [29,30] on learning and memory.

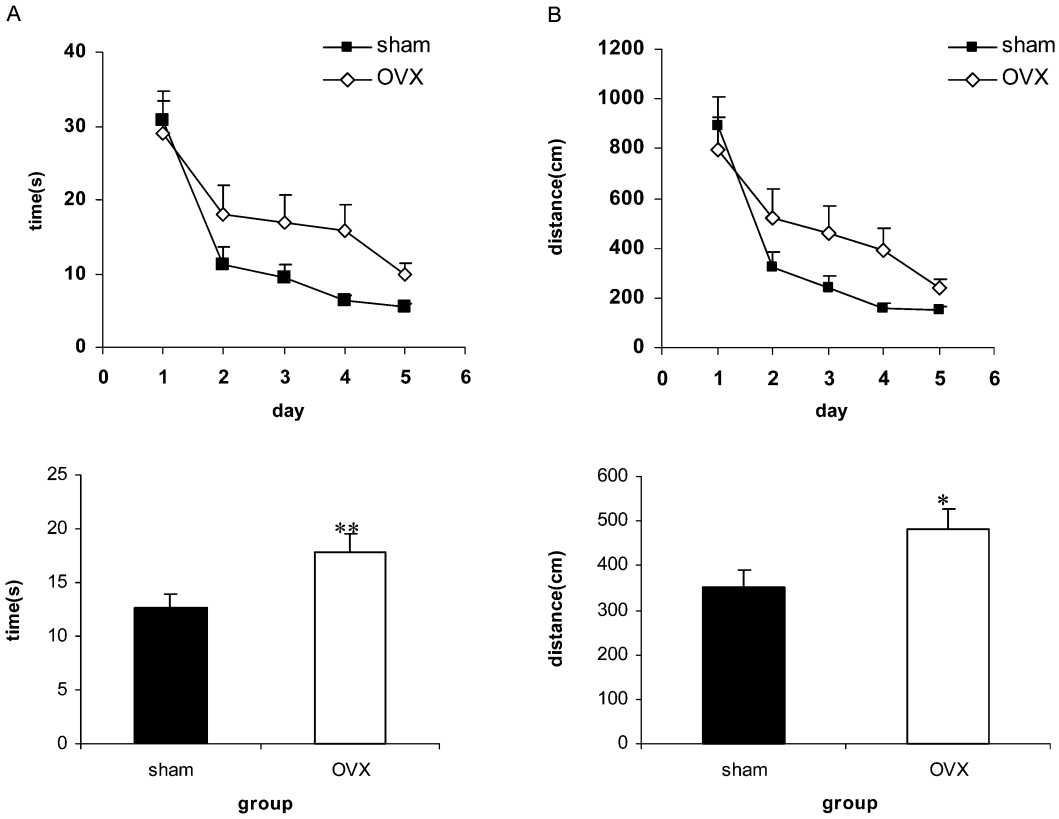

Figure 1 - Comparison of time latency (A) and traveled path length (B) between sham and OVX groups. Data are presented as mean ± SEM. (n = 8)*$P<0.05$, **$P<0.01$ compared to OVX group. The upper portion of the figure compares groups' total time latency or traveled path length over five days, and the lower portion of each figure is the comparison of time latency or traveled path length each day between groups.

The exact mechanisms by which estrogen regulates the functions of spatial memory have been widely investigated. Reports indicate that elevated levels of circulating estrogen in female rats result in increased spine and synaptic density as well as parallel increases in NMDA receptor binding in area CA1 of the hippocampus.[31,32] The increase in spine density is associated with the increased sensitivity of CA1 pyramidal cells to NMDA receptor-mediated synaptic input,[33] suggesting that the new spines and synapses induced by estrogen are rich in NMDA-receptors.[34] There is also evidence showing that ovariectomy decreases NMDA binding density in the hippocampal CA1 region (the dentate gyrus) and that estradiol restores and increases NMDA binding density in the CA1 region.[35] Estrogen also influences cholinergic neurochemistry in the basal forebrain and hippocampus, and it has been previously suggested that the ability of estrogen to alter NMDA receptor binding in CA1 is related to its ability to alter cholinergic systems.[36] Although the results of the current study indicate that estradiol treatment of sham-operated rats impedes the learning procedure, the time latency in the sham-Est group was greater than in the sham group. The time latency and traveled path length to reach the hidden platform in the OVX-Est group was also lower than in the sham-Est group. Some studies have indicated that medium doses of estradiol have a greater effect on memory function than lower or higher doses.[37,38] In this study, a high dose of estrogen was used based the dosages described in a previously published paper. [11] However, the results with low or medium doses of estrogen were not comparable. On the other hand, it has been suggested that high-estrogen phases of the menstrual cycle are associated with improved performance in verbal tasks and reduced performance in visuospatial tasks.[38] However, Ping et al. showed that treatment with estrogen improved spatial learning performance in both normal-estrogen (sham-operated) and ovariectomized rats.[25]

It has been well documented that activation of NMDA receptors induces NO synthesis. Consequently, NO takes part in mechanisms of synaptic plasticity, including long-term potentiation (LTP) in the hippocampus.[39] It has been widely reported that estrogen affects nNOS mRNA regulation, the number of nNOS-expressing neurons and NO production in the brain.[16,17] Previous studies imply that estrogen in the central nervous system participates in increased nitric oxide production.[10] It is believed that administration of L-arginine improves memory consolidation and learning,[40] but the results of the present study show that chronic administration of L-arginine in combination with estradiol attenuated the estradiol-mediated improvement of learning in OVX rats. The time latency of the animals treated with both estradiol and L-arginine was significantly higher than that in the estradiol-treated group. Intrahippocampal injection of L-arginine did not affect escape latencies, traveled distance, quadrant entries or swimming speeds when administered alone. However, it blocked the deleterious effects of N (G)-nitro-L- arginine methyl ester (L-NAME).[7] It is suggested that hippocampal NO is not critically involved in place learning in rats.[41] Other researchers examined the acute effects of L-arginine on learning and memory, but in the present study the

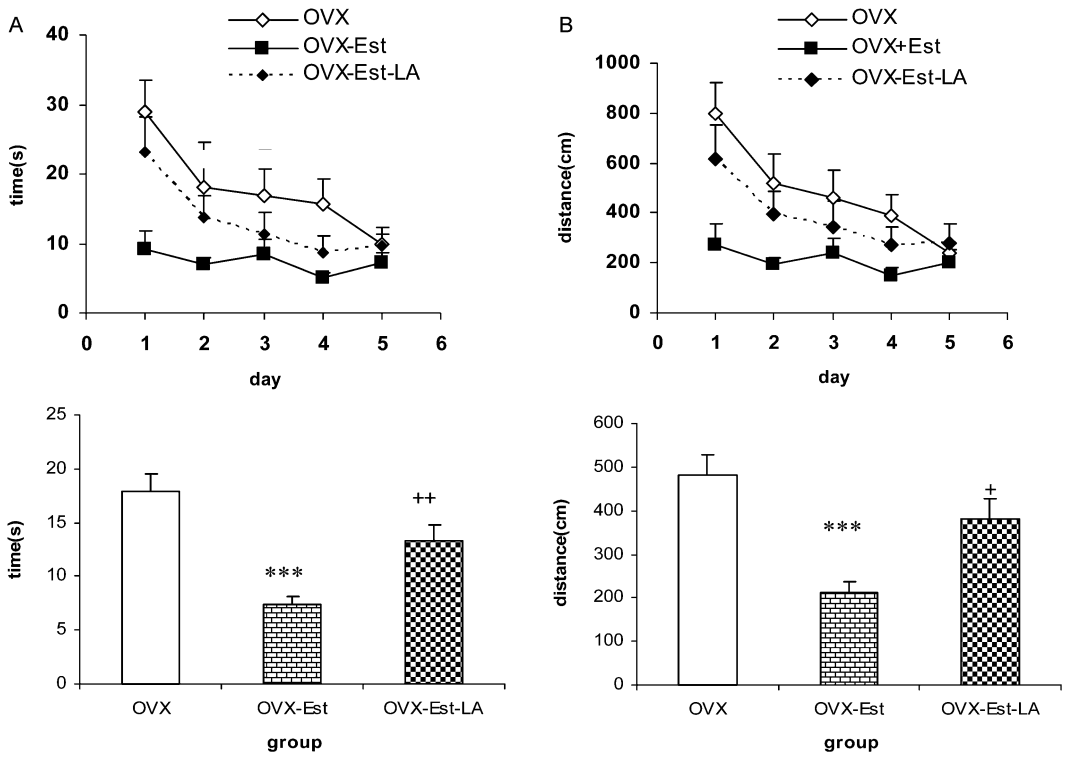

Figure 2 - Comparison of time latency (A) and path length (B) among OVX, OVX-Est and OVX-Est-LA groups. Data are presented as mean ± SEM. (n = 8 in each group). ***$P<0.001$ compared to OVX group, $^{+}P<0.05$, $^{++}P<0.01$ compared to OVX-Est group. The upper portion of the figure compares groups' total time latency or traveled path length over five days, and the lower portion of each figure is the comparison of time latency or traveled path length each day between groups.

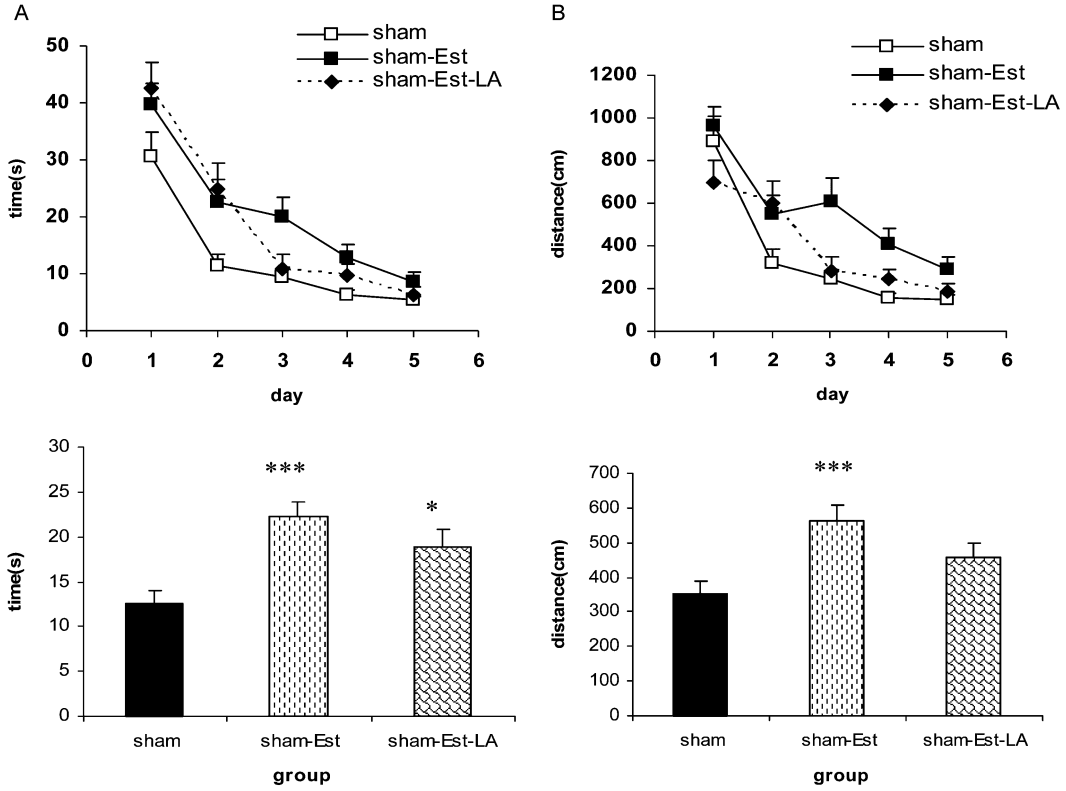

Figure 3 - Comparison of time latency (A) and path length (B) among sham, sham-Est and sham-Est-LA groups. Data are presented as mean ± SEM. (n = 8 in each group). ***$P<0.001$ compared to sham group. The upper portion of the figure compares groups' total time latency or traveled path length over five days, and the lower portion of each figure is the comparison of time latency or traveled path length each day between groups.

animals were chronically treated for eight weeks. Our results show that estradiol treatment in addition to administration of L-arginine in OVX rats impaired MWM tasks. Although the precise mechanism underlying this effect is not clear, it is possibly related to the conversion of exogenous L-arginine to agmatine by arginine decarboxylase enzyme.[42,43] Agmatine is known to inhibit nNOS and other NOS isoforms.[44]

The results confirmed the functional role of estrogen in reversing memory impairment following ovariectomy. It is also likely the nitric oxide system takes part in memory formation in combination with the gonadal hormones of the endocrine system, among which estrogen plays a remarkable role.

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