Chronic Treatment by L-NAME differently Affects Morris Water Maze Tasks in Ovariectomized and Naïve Female Rats

Mahmoud Hosseini[1,2], Azadeh Feizpour[1,2], Mohsen Rezaeipour[1,2], Atefeh Amani[1,2], Fatima Saffarzadeh[1,2], Esmaeil Farrokhi[1,2]

1. Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Physiology, Mashhad University of Medical Sciences, Mashhad, Iran

A B S T R A C T

Introduction: The role of ovarian hormones and nitric oxide (NO) in learning and memory and their interaction has been widely investigated. The present study carried out to evaluate different effect of L-NAME on spatial learning and memory of ovariectomized (OVX) and sham operated rats.

Methods: 32 rats were divided into 4 groups: 1) Sham; 2) OVX; 3) Sham-LN; and 4) OVX-LN. The animals of groups 3 and 4 were treated by L-NAME (10 mg/kg/day) for 8 weeks while the animals of groups 1 and 2 received saline (1ml/kg/day) instead of L-NAME. The animals of all groups were then tested in Morris water maze during five days. The escape latency and traveled distance were compared between groups.

Results: Distance and time in OVX group was significantly higher than Sham group ($p < 0.01$ and $p < 0.05$). Time and distance in Sham-LN group was higher in comparison with Sham group ($p < 0.05$ and $p < 0.01$). There were no significant differences between OVX-LN and OVX groups in escape latency and traveled distance.

Discussion: The results of present study showed that removal of ovarian hormones could impair Morris water maze tskas includiong time and distance. Administarion of non specific nitricoxide inhibitor, L-NAME, affects Morrsi water maze tasks however, its effect is different in the absence and presence of ovarian hormones but it needs to be more investigated.

1. Introduction

Researches demonstrated that gonadal hormones are affective for the maintenance of cognitive ability. The presence of estrogen receptors (ERα and ERβ) in the hippocampus or the prefrontal cortex confirm this hypothesis (Wallace et al., 2006). Estradiol administration to ovariectomized (OVX) subjects has been shown in many studies to be associated with more rapid acquisition of memory tasks (Wallace et al., 2006). It has also been suggested that estrogen replacement therapy may help to reduce the risk and severity of Alzheimer disease in postmenopausal women (Gibbs, 2000; Savonenko and Markowska, 2003). The effects of estrogen on learning and memory may be due to its interaction with cho-

*** Corresponding Author:**
Mahmoud Hosseini
Dept. of Physiology, Medical School, Mashhad University of Medical sciences, Mashhad, Iran.
Tel:0511-8828564-5
E-mail: hosseinim@mums.ac.ir

linergic, serotonergic and dopaminergic system (Gibbs, 2000; Heikkinen et al., 2002; Savonenko and Markowska, 2003). Another possible mechanism whereby estrogen might affect learning and memory is the modulation of dendritic spines (Heikkinen et al., 2002; Wallace et al., 2006). Ovariectomy has been shown to decrease the density of CA1 hippocampal dendritic spines and synapses (Woolley and McEwen, 1994). In addition loss of ovarian function has been shown to produce significant decrease in the level of nerve growth factor receptor mRNA (Gibbs, 2000).

Nitric oxide is a putative intercellular messenger in the central nervous system which is produced from the enzymatic conversion of L-arginine to L-citrulline by NO synthase (NOS) cooperation (Böhme et al., 1993). NO, as a critical factor in signal transduction, may be involved in certain forms of synaptic plasticity including long-term potentiation (LTP) (Bannerman et al., 1994), learning and memory formation (Zou et al., 1998). It has been well documented that induction of LTP requires the activation of N-methyl-D-aspartate (NMDA) receptors (Bannerman et al., 1994). On the other hand activation of this sort of receptors has been shown to induce NOS activity which then activates soluble guanylate cyclase and leads to formation of cyclic guanosine monophosphate (cGMP) in the brain (Yamada et al., 1995). This cascade of events finally conclude to important cognitive effects while NOS inhibitors, such as NG-nitro-L-arginine methyl ester (L-NAME), can conversely inhibit many NMDA-mediated cognitive effects (Khavandgar et al., 2003). As reported L-NAME impair spatial learning in rats and causes an apparent block of hippocampal LTP in their brain (Böhme et al., 1993).

It has been documented that sex hormones influence the NO system in both peripheral and nervous tissues (Farsetti et al., 2009; Lopez-Jaramillo and Teran, 1999; Nematbakhsh and Khazaei, 2004). Moreover, nitric oxide synthase may co-localize with gonadal hormone receptors (Panzica et al., 2006). In fact, in mice knockout for estrogen receptor alpha, the nitric oxide synthase-expressing population is significantly reduced in specific regions (Panzica et al., 2006). The results of previous studies showed that removal of ovarian hormones impaired memory which was prevented by L-arginine (Hosseini et al., ; Saffarzadeh et al.). It was also reported that L-NAME prevented memory enhancing effect of estradiol in ovariectomized rats(Azizi-Malekabadi et al.). Since there are some interactions between NO system and ovarian hormones, the aim of the this study was to clarify the differences in effect of L-NAME (non-specific inhibitor of nitric oxide synthase) on Morris water maze tasks of OVX (absence of ovarian hormones) and naïve female rats (presence of ovarian hormones).

2. Methods

2.1. Animals and Drugs

Thirty two, 8- week virgin female wistar rats (200±10 g) were used. All rats were housed 4 per standard cage (26.5*42*15 Cm) at room temperature (22± 1 °C) on a 12 h light/dark cycle. Food and water were available ad libitum properly. Rats were given one week to adapt to new environment before any procedures were initiated. Animal handling and all related procedures were approved by the Mashhad Medical University Committee on Animal Research. L-NAME was purchased from Sigma Aldrich (USA) and dissolved in saline. Ketamin and xylazine were purchased from Alfasan Company (Holand).

2.2. Experimental protocol

The animal groups were determined as follows: 1) Sham; 2) Ovariectomy (OVX); 3) Sham - L-NAME (Sham-LN) and 4) Ovariectomy- L-NAME (OVX-LN). The animals in Sham-LN and OVX-LN groups received 10 mg/kg/day of L-NAME for 8 weeks before the five days of behavioral study. The animals of Sham and OVX groups received 1 ml/kg of saline instead of L-NAME for 8 weeks. The volume of the L-NAME injected was equal to the volume of saline. The treatments were carried out from the day after surgery till the first day of behavioral study, in all animals. Finally, all animals were tested in Morris water maze.

2.3. Ovariectomy

The animals were ovariectomized under ketamine (150 mg/kg, i.p.) and xylazine (0.1 mg/kg, i.p.) anesthesia (Hosseini et al., 2009a). Anesthesia was confirmed by reduced respiratory rate and no response to gentle pinching of foot pad. A ventral incision was made through the skin of the rat's flank. Ovaries and ovarian fats were removed. Ovaries were isolated by ligation of the most proximal portion of the oviduct before removal. The same procedure was carried out for sham groups except removing the ovaries.

2.4. Apparatus

To assess behavioral functions, rats were tested by Morris water maze which is a black circular pool with a diameter of 150 cm and a height of 60 cm, filled with 24

± 1°C water to a depth of 30 cm which is divided geographically into four equal quadrants and release points that are designed at each quadrant as N, E, S and W for north, east, south and west respectively. A hidden circular platform (10 cm in diameter), made of Plexiglas, is located in the center of the southeast quadrant, submerged 1.5 cm beneath the surface of the water. It has been previously shown that the Plexiglas is invisible for the rats. Fixed, extra maze visual cues were present at various locations around the maze (i.e. computer, Morris water maze hard wares, and posters) during recordings. 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 motion can be recorded and sent to the computer. A tracking system was used to measure the escape latency and traveled path (Hosseini et al.).

2.5. Behavioral Assessment

Animals received a block of four trials during five daily sessions. During the 5 days, the platform, situated in the center of the southeast quadrant, was submerged 1.5 cm below the surface of water and therefore invisible, for testing spatial learning. The platform position remained stable during 5 days. A trial was started by placing a rat into the pool, facing the wall of the tank. Each of the 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 4th trial the rats were kept warm for an hour and returned to their home cage (Hosseini et al., ; Hosseini et al.). The time latency and traveled distance to reach the platform were compared between groups. All tests were conducted between 16 :00 to 18 :00 o'clock.

2.6. Statistical Analysis

All data were expressed as means ± SEM. The data of all 5 days of different groups was compared using ANOVA test with Tukeys' post hoc. Differences were considered statistically significant when $p<0.05$.

3. Results

3.1. The Effect of L-NAME on Escape Altency

Escape latency in OVX group was significantly higher than the sham group ($p<0.05$) (Fig 1). The animals of the Sham-LN group had significantly higher time latency to reach the platform compared to Sham group ($p<0.05$) (Fig 1). There was no significant difference in time latency between OVX- LN group and OVX group (Figs 1).
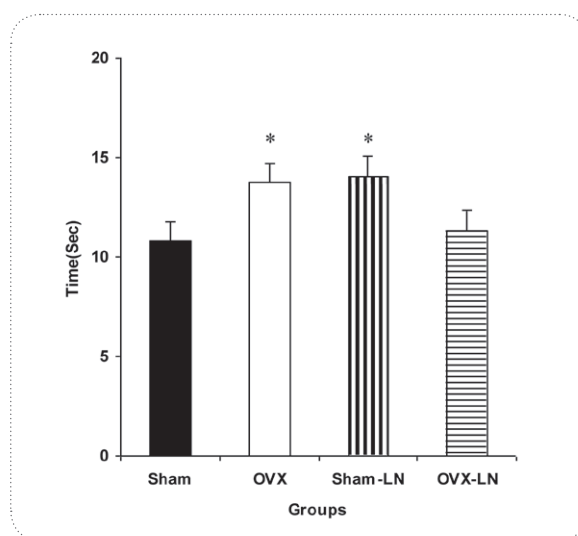
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Figure 1. Comparison of time latency among Sham, OVX, Sham-LN and OVX-LN groups. Data are presented as mean ± SEM. (n = 8 in each group). *P<0.05 compared to Sham group.
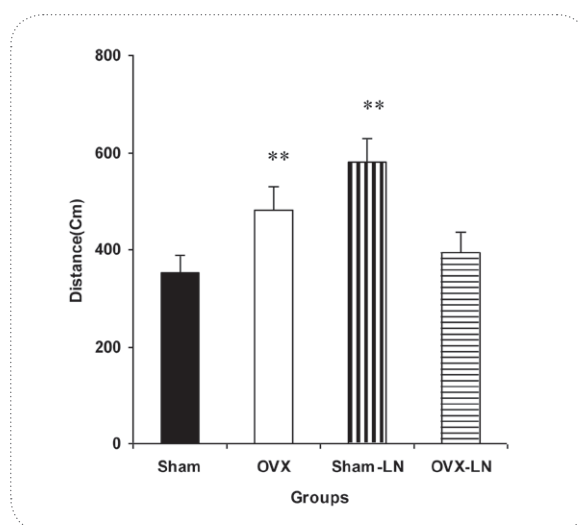
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Figure 2. Comparison of path length among Sham, OVX, Sham-LN and OVX-LN groups. Data are presented as mean ± SEM. (n = 8 in each group). **P<0.01 compared to Sham group.

3.2. The Effect of L-NAME on Traveled Path

The traveled path in OVX group was significantly higher than the sham group (p<0.01) (Fig 2). Traveled path length to reach the platform in Sham-LN group was also longer than Sham group (p<0.01) (Fig 2). There was no significant difference in traveled path length between OVX- LN group and OVX group (Fig 2).

4. Discussion

In this study the effect of chronic administration of L-NAME on Morris water maze tasks either in presence of estradiol or absence of this hormone was investigated respectively in naïve and OVX female rats. The results demonstrated a decrease in performance on memory tasks in OVX group as escape latency and traveled path in the OVX group were significantly higher than sham group. However, the results of present study may only be some behavioral evidences but Morris water maze is an experimental method which is commonly used to evaluate spatial learning and memory in animal models (Azizi-Malekabadi et al., ; Hosseini et al., ; Hosseini et al., ; Morris, 1984; Nunez, 2008; Saffarzadeh et al., 2010). Therefore spatial learning and memory impairement due to elimination of ovarian hormones is suggested.

It has been well documented that estrogen might affect learning and memory by modulation of the dendritic spines (Heikkinen et al., 2002; Wallace et al., 2006; Woolley and McEwen, 1994) or modulation of neurotransmitters such as acetylcholine, glutamate, dopamine and norepinephrine or their receptors (Gibbs, 2000; Heikkinen et al., 2002; Savonenko and Markowska, 2003). Some types of nuclear estrogen receptors known as ERα and ERβ are reported to be present in the hippocampus or prefrontal cortex (Wallace et al., 2006) which confirms estrogen responsibility for the modulation of learning and memory probably by changing dendritic spines' density. The better performance of naïve female rats relative to OVX ones in Morris water maze memory tasks is consistent with some previous studies. Wallace et al showed that both non-spatial memory and spatial memory tasks, declined in OVX as compared to intact females following surgery (Wallace et al., 2006). Singh et al. also found that OVX rats did not perform as well as intact rats in active avoidance behavior (Singh et al., 1994). Our previous studies also demonstrated the spatial learning and memory inmpairements following ovarian hormone deficiency (Hosseini et al., 2010; Saffarzadeh et al., 2010) which was improved after estradiol therapy (Hosseini et al., 2010; Markham et al.,

2002). Such results support the hypothesis which suggest that ovarian hormones contribute to maintenance of memory function in female rats. In contrast, in an study carried out by Herlitz and coworkers, there were no noticeable differences in cognitive performance between premenopausal and postmenopausal women (Herlitz et al., 2007). In this case they didn't even find any associations between blood estrogen levels and cognitive performance. Other researchers also reported negative (Chesler and Juraska, 2000; Fugger et al., 1998) or no effects (Fader et al., 1999; Healy et al., 1999) of estrogen on learning and reference memory. In addition Jennifer et al documented that the high level estradiol impairs but low level estradiol facilitates working memory performance on the radial arm maze (Wide et al., 2004) which is in agreement with Holmes et al's study (Holmes et al., 2002). The controversies in results regarding the role of estradiol on cognition may be due to the differences in estradiol doses, the duration and method of estradiol administration and the starting time of treatment.

Present findings also indicated that the animals of Sham-LN group had significantly higher time latency and traveled path length to reach the platform as compared to Sham group which is in accordance with Bohme et al findings. He reported that systemically administered NOS inhibitor leads to hippocampal LTP blockade and impairment of spatial learning (Böhme et al., 1993). Other investigators reported that NOS inhibitors, such as L-NAME and 7-NI, impaired both reference and working memories (Hölscher et al., 1996; Zou et al., 1998). In contrast with our results, Bannerman et al. documented that intraperitoneally injected L-NAME had no effect on retention of previously learned spatial information (Bannerman et al., 1994). As there was no significant difference in time latency or traveled path length between OVX- LN and OVX group in our present study, an interaction of nitric oxide and estradiol in specific brain functions is suggested. Activation of NMDA receptors has been shown to induce NO synthesis. It activates soluble guanylate cyclase which leads to the formation of cGMP in the brain and induces important cognitive effects (Yamada et al., 1995). Besides, it has been documented that estrogen influences the NO system in both peripheral and nervous tissues (Farsetti et al., 2009; Lopez-Jaramillo and Teran, 1999; Nematbakhsh and Khazaei, 2004). More precisely, protein–protein interactions between neuronal NO synthase (nNOS) and glutamate NMDA receptors in the hypothalamic preoptic region of adult female rats is sensitive to cyclic estrogen fluctuation (d'Anglemont de Tassigny et al., 2007). As estrogen affects the NO system, NO

inversely mediates GnRH release in response to progesterone and 17 beta-estradiol (Gyurko et al., 2002) which is due to its stimulatory effect on the soluble guanylate cyclase activity that leads to the production of cGMP.

On the other hand, the interaction between nitric oxide and estradiol in the cholinergic, serotonergic and dopaminergic system (Gibbs, 2000; Heikkinen et al., 2002; Kiss, 2000; Kiss et al., 2004; Prast and Philippu, 1992; Savonenko and Markowska, 2003) can be considered as a possible mechanism for the results observed in the present study. The results of our previous study also showed that L-arginine, the precursor of nitric oxide, improved learning and memory impairement caused by elimination of ovarian hormones in ovariectomized rats (Saffarzadeh et al.). It was also shown that the improving effects of estradiol on learning and memory was prevented by L-NAME (Azizi-Malekabadi et al.). It has also been previously suggested that nitric oxide contributes in sex dependent differences in behavior (Hosseini et al., ; Hosseini et al., 2009b; Sadeghipour et al., 2007). Regarding these findings and the results of present study it might be concluded that nitric oxide has interaction with estradiol in nervous system to modulate learning and memory.

Acknowledgments

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