



Agmatine Improves Spatial Memory Consolidation: The Role of Nitric Oxide

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Abstract

Objective: The existence of agmatine in the hippocampus suggests a role in memory formation. Endogenous agmatine increases after training in the hippocampus, however the effect of exogenous agmatine on memory is not consistent yet. This work was aimed to assess the differential effect of systemic agmatine on spatial memory consolidation and retrieval. Additionally L-NAME was used to assess if nitric oxide is involved in the effect of agmatine.

Methods: Male Sprague-Dawley rats (250-350 g) were trained in water maze single training session. After 24 h the memory of animals were examined in a probe trial which consisted of a trial without platform. To assess the effect of agmatine (40 mg/kg) on memory consolidation it was administered immediately after training and to assess its effect on memory retrieval it was injected 30 min before probe trial. In order to evaluate the involvement of nitric oxide, L-NAME (3 mg/kg) was co-administered with agmatine.

Results: Post-training injection (to assess consolidation phase) of agmatine improved the performance of animals in probe test, while its pre-probe administration (to assess retrieval phase) had no effect. L-NAME prevented the improving effect of agmatine on consolidation phase of memory.

Conclusion: Agmatine improves spatial memory consolidation while has no effect on memory retrieval. Nitric oxide is involved in the effect of agmatine.

Keywords: Agmatine; Nitric oxide; Spatial memory; Rat; Morris water maze

Introduction

Agmatine is a cationic polyamine synthesized by arginine decarboxylase and is widely distributed in the brain including hippocampus [1]. Considering the critical role of hippocampus in memory, it was questioned if agmatine affects learning and memory processes. Interestingly it was found that during spatial memory in a water maze the level of agmatine increases in hippocampal area [2]. Nonetheless when agmatine is administered exogenously, there is not a consistent report about its impact on memory. The studies report its insufficiency in changing spatial memory (reference memory which is related to hippocampus) [3-5], its improving [6] or its impairing effect [7]. Therefore it remains unanswered if agmatine is increased after learning and memory formation, how exogenously administered agmatine affects memory.

Memory is consisted of different stages including consolidation and retrieval. Sometimes the discrepancies regarding the effect of a drug refer to its unlike effect on separate phase of memory. Then in this study we aimed to assess the differential effect of systemically administered agmatine on memory consolidation and retrieval. For this purpose a 1 day training protocol was designed to give us the capability to determine post training and pre-probe effect of agmatine as the usual protocols consisting different days of training involve repetitive multi-training sessions for several days which make it difficult to differentiate the effect of drug on different memory aspects. In assessing the effect of drugs on different phases, they should be administered in different time. When the consolidation phase is assessed, usually the drug is used immediately after training; while to assess retrieval phase, it is administered before probe trial [8]. Due to some reports about the impact of agmatine on nitric oxide synthesis, when an effect seen after agmatine injection, L-NAME hydrochloride (N-nitro-L-arginine-

methylester) which is a non-selective NOS inhibitor [9] was used to examine if nitric oxide (NO) is involved in that effect.

Materials and Method

Animals

Male Sprague-Dawley rats (250-350 g) were housed four per cage in a constant temperature ($24 \pm 1^\circ\text{C}$), under a 12-12 h light/dark cycle. Food and water were provided ad libitum. The behavioral experiments were done during the light phase. The experimental protocols were approved by the ethics committee of the university and the animal care was according to the NIH Guide for the Care and Use of Laboratory Animals.

Drug administration

Agmatine sulphate and L-NAME were purchased from Sigma, USA. In post-training groups, the drugs were injected intraperitoneally immediately after training. In pre-probe groups, the drugs were administered 30 min before probe trial. The animals were divided into groups of 8 and received saline (ip) as vehicle, agmatine (40 mg/kg), L-NAME (3 mg/kg) and a combination of agmatine (40 mg/kg) and

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Received February 19, 2016; Accepted April 25, 2016; Published April 28, 2016

Citation: Khaless GY, Khajeh A, Moosavi M (2016) Agmatine Improves Spatial Memory Consolidation: The Role of Nitric Oxide. J Pharma Reports 1: 115.

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L-NAME (1 mg/kg). The doses were chosen from previous research works and pilot studies [10,11].

Behavioral testing

Water maze apparatus: The water maze has been described previously [12]. Briefly it was a black circular pool with a diameter of 140 cm and a height of 70 cm, filled with $20 \pm 1^\circ\text{C}$ water to a depth of 25 cm. The maze was divided geographically into four equal quadrants and release points that were designed at each quadrant as N, E, S and W. A hidden circular platform (11 cm in diameter), was located in the center of the southwest quadrant, submerged 1.5 cm beneath the surface of the water. Fixed, extra maze visual cues were present at various locations around the maze (i.e., computer, a door, a window, bookshelves, posters). A CCD camera was mounted above the center of the maze so that the animal motion could be recorded and sent to the computer. The path of animal's swimming was automatically recorded by a computerized system (Noldus EthoVision, 3.1 versions) and then analyzed by computing several parameters, e.g. latency to find the platform, the swimming speed and the time percent which animal spent in target zone was measured.

Procedure: The single training session consisted of eight trials with four different starting positions that were equally distributed around the perimeter of maze [10]. The task requires rats to swim to the hidden platform guided by distal cues. After mounting the platform, the rats were allowed to stay there for 20 s until the start of the next trial. The animals were given a maximum of 90 s to find the platform; if they failed to find the platform in this time, they would be placed by the experimenter on the platform and allowed to stay there for 20 s. After completion of the training, the animals were returned to their home cage until the retention testing (probe trial) 24 h later. The probe trial consisted of a 60 s free swim period without a platform and the time spent in the target quadrant was recorded. After completion of probe test, the hidden platform was removed and a visible platform-covered by a piece of aluminum foil which was not submerged in water-was placed in another position (southeast quadrant) to test rat motivation, visual ability and sensorimotor coordination. The rats were given 4 trials of 60 s to find the visible platform and the escape latency to reach the platform was recorded.

Data analysis

Data were expressed as the mean \pm S.E.M. An analysis of variance (ANOVA) followed by Tukey's multiple comparison tests was used for statistical comparison. In all cases, a P value of less than 0.05 was considered statistically significant.

Experimental protocol

Experiment 1: The aim of experiment 1 was to determine the effect of post-training and pre-probe injection of agmatine on spatial memory. The rats were divided into 3 groups (n=6-8).

Experiment 2: The aim of experiment 2 was to determine the effect of post-training injection of L-NAME and agmatine on spatial memory. The rats were divided into 3 groups (n=6-8).

Results

The effect of agmatine on memory consolidation and retrieval

The aim of this experiment was to explore the effect of post-training and pre-probe administration of agmatine on probe test. One way ANOVA showed significant differences between groups (Figure

1A, $p=0.0016$, $F(2,19)=9.178$). Post hoc test Tukey showed that post-training agmatine improved animals' performance in probe test while its pre-probe injection had no effect. This means that agmatine improves spatial memory consolidation while has no effect on memory retrieval.

Figure 1B shows that all animals learnt the task during training session. One way ANOVA showed that there was no difference between their learning abilities which confirms that the improving effect seen is related to the effect of agmatine ($p=0.7248$, $F(2,19)=0.3273$).

Figure 1C shows the performance of animals in finding the visible platform. As it is depicted there was no difference between groups ($p=0.7497$, $F(2,19)=0.2925$). This test verifies motivation and visuomotor abilities of animals and revealed that there is no change in this capability when agmatine was administered. This suggests that the changes induced by agmatine are not due to its effect on motivation or visuomotor ability.

Table 1 depicts the animals' speed during probe test which shows that agmatine had no effect on animals' motor performance ($p=0.7304$, $F(2,19)=0.3195$), which means that agmatine did not interfere animal's swimming speed.

The effect of L-NAME on agmatine induced memory improvement

The aim of this experiment was to explore the effect of L-NAME on agmatine induced improvement of memory consolidation. One way ANOVA showed significant differences between groups (Figure 2A, $p=0.0007$, $F(3,22)=8.176$). Post hoc test Tukey showed that L-NAME prevents agmatine induced memory improvement. The dose of L-NAME which was used has no effect on memory per se.

Figure 2B shows that all animals learnt the task during training session. One way ANOVA showed that there was no difference between their learning abilities ($p=0.5377$, $F(3,22)=0.7433$).

Figure 2C shows the escape latency to reach the visible platform. As it is depicted there was no difference between groups ($p=0.7173$, $F(3,22)=0.4537$). This test verifies motivation and visuomotor abilities of animals and revealed that there is no change in this capability when L-NAME was administered.

Table 2 illustrates the animals' speed during probe test which shows that L-NAME had no effect on animals' motor performance ($p=0.8114$, $F(3,22)=0.3191$).

Discussion

This study revealed that agmatine potentiates memory consolidation. Consolidation is a phase that starts immediately after training and lets the memory to be stored in the brain. As agmatine administered immediately after training session improved the performance of animals in memory test, 24 h later, it is proposed that agmatine boost memory consolidation process. It is in accordance with previous reports showing that endogenous brain agmatine increases after learning [11-13]. Then taken together it could be suggested that agmatine, both endogenous and exogenous, improve memory consolidation. The finding that agmatine did not affect memory retrieval might be related to the different molecular processes responsible for those stages. For instance Ca^{2+} entry into the post-synaptic cell is assumed a prerequisite for memory consolidation [14]; while Steele and Morris [15] have found that blocking NMDA receptors in the hippocampus has no effect on the retrieval of a previously established

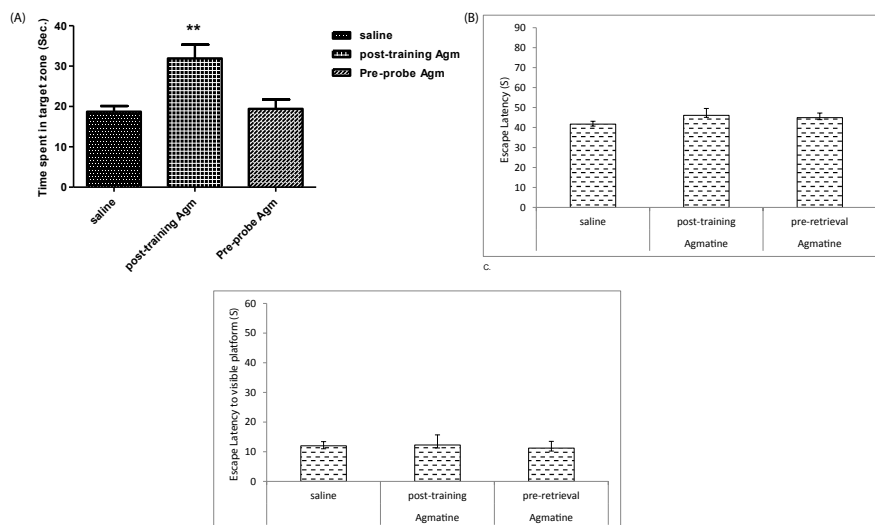


Figure 1: The effect of post-training and pre-retrieval administration of agmatine on spatial memory. Figure 1A shows the time spent in target zone in probe test. This figure shows that post-training agmatine administration improves memory recall while its pre-probe injection has no effect. Figure 1B shows animals' performance in training session which was not different from saline group. Figure 1C illustrates the effect of agmatine on animals' performance in finding the visible platform. ** p<0.01 shows the difference between saline and agmatine treated group.

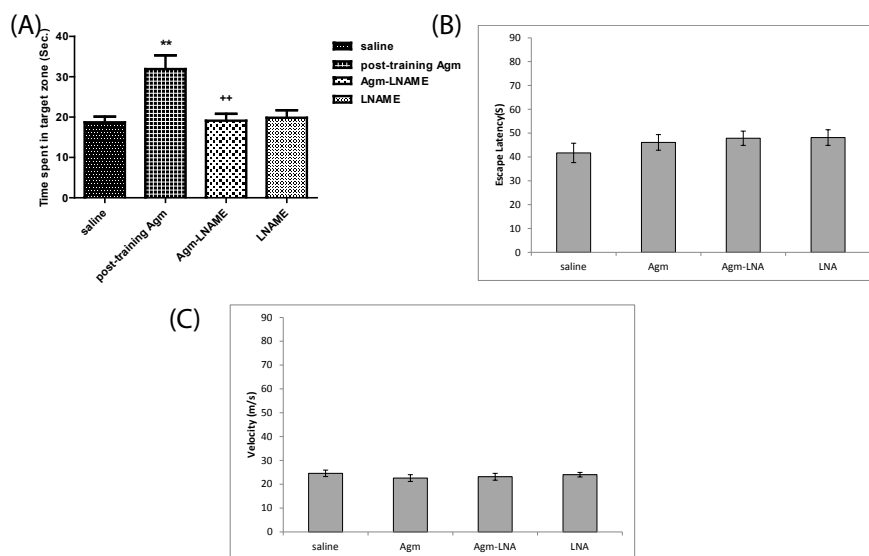


Figure 2: The effect of L-NAME on agmatine mediated memory improvement. Figure 2A shows the time spent in target zone in probe test. This figure shows that L-NAME inhibits agmatine induced memory improvement. Figure 2B shows animals' performance in training session which was not different from saline group. Figure 2C illustrates the performance of animals during visible platform task. ** p<0.01 shows the difference between saline and agmatine treated group and ## p<0.01 shows the difference between agmatine and L-NAME treated animals.

spatial memory. Thus, whereas the NMDA receptor is involved in consolidation, it may not be engaged in retrieving memories.

L-NAME prevented the effect of agmatine on memory consolidation. As it was mentioned, in some tissues like aorta, agmatine is shown to stimulate eNOS and elevate NO and cyclic GMP levels [16]. In addition recently it is shown that nitric oxide is involved in the ameliorating effect of agmatine on the compulsive-like behaviour in mice [17]. NO is found to be an intra- and inter-cellular messenger in the brain and its role in learning and memory is well documented [18]. The findings of this study propose that NO is involved in the boosting effect of

agmatine on memory consolidation. Of the 3 nitric oxide synthase enzyme, nNOS and eNOS are known to be expressed constitutively [19], but iNOS which exists in macrophages [20] or in the glial cells [21] gets activated by inflammatory stimuli [22]. Mice lacking nNOS or eNOS alone shows normal or nearly normal LTP [23,24], while lacking both nNOS and eNOS impairs LTP in CA1 region of the hippocampus [25]. Then probably agmatine stimulates both nNOS and eNOS in the brain to improve memory consolidation. Although eNOS is originally detected in endothelial cells, there are some evidences showing its presence in hippocampal pyramidal cells [26,27]. Then it might be

Group	saline	Agm-post training	Agm-pre retrieval
Mean	24.57	23.8	24.6
SEM	1.36	1.35	1.2

Table 1: The swimming velocity of animals during probe session.

Group	saline	Agm	Agm-LNA	LNA
Mean	24.57	22.6	23.16	24
SEM	1.36	1.43	1.44	0.96

Table 2: The swimming velocity of animals during probe session.

suggested that agmatine improves memory consolidation through both nNOS and eNOS stimulation and NO synthesis.

In conclusion, this study revealed that systemic agmatine administration improves spatial memory consolidation and NO is involved in this boosting effect of agmatine. Knowing the role of new neurotransmitters in memory processes, will help us in fighting memory deficits exist in dementia and Alzheimer's disease.

Acknowledgement

This work was supported by a grant from Shiraz University of Medical Sciences, Shiraz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interests.

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