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Agmatine, A Metabolite of Arginine, Improves Learning and Memory in Streptozotocin-Induced Alzheimer's Disease Model in Rats

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ABSTRACT:

Agmatine, a metabolite of arginine, improves learning and memory in streptozotocin-induced Alzheimer's disease model in rats

Objective: Agmatine, the decarboxylation product of arginine produced by arginine decarboxylase, is a novel neurotransmitter and exists in the mammalian brain. Agmatine has been reported to modulate cognitive functions, including learning and memory.

Methods: In the present study, we evaluated the effects of agmatine on cognitive performance and oxidative damage in intracerebroventricular (i.c.v.) streptozotocin (STZ) model of Alzheimer's disease (AD). Adult male Sprague-Dawley rats were injected STZ (3mg/kg, i.c.v, bilaterally) on days 1 and 3. The learning and memory patterns were assessed by using passive avoidance, Morris water maze, and closed field activity tests. Also, malondialdehyde (MDA), glutathione (GSH) levels and myeloperoxidase (MPO) activity have been determined as the parameters of oxidative damage. The behavioral tests were performed after 14 days from the first injection of STZ. Rats with impaired learning and memory performance were treated with intraperitoneal (i.p.) agmatine (40 mg/kg) twice daily for 7 days. After agmatine treatment, rats were subjected to the aforementioned behavioral tests again. Immediately after decapitation of the rats, the brains were collected and analyzed for oxidative damage parameters.

Results: Agmatine improved the STZ-induced both spatial and emotinal memory impairment and oxidative damage. Findings of the study demonstrated the effectiveness of agmatine in reversing the cognitive deficits as well as the oxidative damage caused by i.c.v STZ.

Conclusion: Taken together, our results have provided experimental evidence suggesting a possible therapeutic potential of agmatine as a regulator in etiopathogenesis of neurodegenerative diseases such as Alzheimer's disease.

Keywords: agmatine, streptozotocine, Alzheimer's disease, passive avoidance, Morris water maze

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia affecting people older than 65[1] and becoming an important health problem as a consequence of the world's aging population[2]. AD is characterized by irreversible, progressive loss of memory followed by complete dementia. The cognitive decline is accompanied by impaired performance of daily activities, behavior, speech and visuospatial perception[1]. The two major neuropathological hallmarks of AD are extracellular amyloid-ß (Aß) plaques and intracellular neurofibrillary tangles[3].

Various hypotheses such as involvement of oxidative stress[4,5], inflammation[6], and glutamate excitotoxicity[7] have been proposed for the pathogenesis of AD. Novel treatment approaches aiming to target these mechanisms that are thought to be involved in the pathogenesis of AD are still investigated.

Agmatine is a putative neurotransmitter and interacts with a number of receptor subtypes, including N-methyl-D-aspartate (NMDA) receptors. Agmatine is a competitive inhibitor of both neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS)[8]. However, it conversely stimulates eNOS in the rat brain after cerebral ischemia[9]. Agmatine has a variety of pharmacological effects in the CNS such as anticonvulsant[10,11], neuroprotective[12-15], anti-stress, anxiolytic, and antidepressant activity potentials[16-18] and also preventing tolerance and withdrawal signs in morphine dependence, providing analgesia[19-23] and reducing thermal and mechanic hyperalgesia in neuropathic pain model[24,25]. Recent research suggests that endogenous agmatine may directly participate in the processes of learning and memory as a neurotransmitter[26-28] and that aging affects agmatine levels in memory-related structures dramatically in a region-specific manner[28]. In spite of the given comprehensive pharmacological effects of agmatine, less is known about its role in cognition. However, today there is accumulating evidence that agmatine has noticable effects on learning and memory. Agmatine was shown to protect neurons against ischemia and excitotoxicity[13].

Furthermore, endogenous agmatine production was found 20 fold higher after ischemic injury[29]. Agmatine protects aganist ischemia-like injury induced by oxygen-glucose deprivation in primary cultured cortical cells. A study demonstrated neuroprotection by agmatine against oxygen glucose deprivation in primary cortical culture and experimental stroke in the adult brain[30]. Recently, a number of studies have reported age-related cognitive decline and agmatine might be benefical to aged rats[29,31]. Latest findings suggest that agmatine modulates and/ or participates in the processes of learning and memory under normal and pathological conditions. Therefore, in this study, we aimed to examine the effects of agmatine on learning and memory in streptozotocin (STZ)-induced model of sporadic Alzheimer's disease in male rats.

MATERIALS AND METHODS

Subjects

Adult male Sprague-Dawley rats aging 6–8 weeks old and weighing 200–230 g were used in the present study. The animals were supplied from Marmara University Animal Center (DEHAMER, Istanbul, Turkey). Rats were housed in groups of four per cage prior to STZ administration and one per cage after STZ administration. Animals were maintained in standard environmental conditions (temperature 21±3°C, 12 h light/dark cycle; lights on at 8.00 am) with free access to food and tap water (ad libitum). All behavioral tests performed under standard conditions (22–24°C, during the light cycle of the day). All experimental procedures were carried out in accordance with the approval of the Animal Research Ethics Committee of Marmara University.

Experimental Procedure

Intracerebroventricular (i.c.v.) injection of STZ

The study rats were anesthetized with a mixture of ketamine hydrochloride and chlorpromazine (50 mg/kg and 1 mg/kg, respectively, i.p.). The head was positioned in a stereotactic frame and a midline sagittal incision was made in the scalp. Burr holes were drilled through the skull on both sides over the lateral ventricles using the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, 3.6 mm beneath the surface of brain[32]. Coordinates for placement of cannulae were determined by using the atlas of Paxinos and Watson[33]. The i.c.v. cannula placements were evaluated after each experiment by 200 µl methylene blue. Only those rats with

proper i.c.v. placements were included in the data analysis. Following the surgery, special care was undertaken for 3–4 days. Rats were given a bilateral i.c.v. injection of 3 mg/kg STZ at a 10 µl volume by using Hamilton syringe on days 1 and 3. behavioral tests were performed after 14 days from the first injection.

Agmatine administration

The study rats were subjected to spontaneous locomotor activity, passive avoidance, and Morris water maze tests, respectively. The rats that showed learning and memory impairment were treated with intraperitoneal (i.p.) agmatine (40 mg/kg) twice daily for 7 days. After agmatine treatment, the rats were subjected to those behavioral tests again while agmatine was administered half an hour before behavioral tests.

Experimental design

The study rats were randomly divided into 3 groups and each group comprised of 8 rats according sample size calculation.

Sham operated group: The rats were administered serum physiological through stereotaxically placed cannulae at the same volume of STZ on days 1 and 3. During the experiment serum physiological was administered instead of agmatine.

STZ group: The rats were administered 3 mg/kg STZ through stereotaxically placed cannulae at a volume of 10 µl on days 1 and 3. During the experiment serum physiological was administered instead of agmatine.

STZ+Agmatine group: The rats were administered 3 mg/kg STZ through stereotaxically placed cannulae at a volume of 10 µl on days 1 and 3. The rats that showed learning and memory impairment were treated with i.p. agmatine (40 mg/kg) twice daily for 7 days. 30 min after the last agmatine administration on day 7; the rats were subjected to aforementioned behavioral tests. The rats were sacrificed after all the behavioral tests were performed. The brain tissues and blood samples were collected to be used for biochemical analyses.

Behavioral tests

Passive avoidance test

On the day 15 and 16 of stereotaxic lesioning, the rats were tested for memory retention deficits by using passive avoidance apparatus. Passive avoidance apparatus (Ugo Basile model 7551, Italy) was utilized for the assessment of emotional memory based on contextual fear conditioning, as described in a previous trial[34]. Briefly, rats learn to avoid a specific place associated with an aversive event. The reduction of latency to avoid was used as learning. A guillotine door separated two-compartment containing (the light and dark chamber) apparatus was used. The rats were placed in the light chamber after 20 s, the guillotine door separating was opened, and the initial latency to enter the dark chamber was recorded. As the rat entered the dark chamber, it was given a footshock of 0.5 mA for 3 s through the grid floor of the dark compartment. Then the rats were returned to their home cage. 24 h later, the retention latency time was measured in the same way as in the acquisition trial but foot shock was not delivered. Cut-off time was limited with 300 sec.

Morris water maze

A water tank (150 cm in diameter), was used to measure spatial memory as previously described[35]. The platform was put in the center of the Southwest quadrant and submerged 1.5 cm below the surface of water, and small black plastic ball were placed on the water surface. The platform was not changed during the first four days, and latency to find the platform was determined. A randomly ordered trials, each of three starting positions (North, East, and West) were used. Each trial was terminated as soon as the rat had climbed onto the escape platform or at the end of 60 s. Each rat was allowed to stay on the platform for 20 s. In case rats could not find the platform within 60 s were put on the platform and were allowed to stay there for 20 s. A 'probe trial', was used to assess the rat's spatial retention of the location of the hidden platform on day 5. During this trial, the platform was removed from the maze and the rat was allowed to search

the pool for 60 s in order to spent time in the quadrant that previously contained the hidden platform, so called target quadrant. During this time, animals that have learned the task were expected to spend more time searching in target quadrant than in the other quadrants.

Biochemical Analysis

Measurement of blood glucose

Blood glucose levels were determined using a commercial glucometer and glucosesensitive dipstics (Accutrend-Alpha glucometer, Boehringer, Mannheim, Germany).

Measurement of glutathione (GSH) and malondialdehyde (MDA) in the brain tissue

Brain tissue samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and GSH levels. GSH measurements were performed using a modification of the Ellman procedure[36]. Briefly, after centrifugation at 3000 rpm for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4.2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36×10^5 M^{-1} cm^{-1}. Results were expressed in µmol GSH/g tissue. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously[37]. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and results are expressed as nmol MDA/g tissue.

Measurement of myeloperoxidase (MPO) activities in the brain tissue

MPO is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear (PMN) leukocytes. Tissue MPO activity is frequently utilized to estimate tissue PMN accumulation in inflammed tissues and correlates significantly with the number of PMN determined histochemically in tissues. MPO activity was measured in tissues in a procedure similar to that documented by Hillegass et al.[38]. Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0), and centrifuged at 41,400 g (10min); pellets were suspended in 50mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41,400 g for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, o-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Statistical Analysis

Results are presented as mean±S.E.M. Data were analyzed by one-way or two way analysis of variance (ANOVA) followed by Post-hoc Bonferroni's test by using GraphPad Prism 4.0. P values of lower than 0.05 were considered statistically significant.

RESULTS

Behavioral assessment

Increased locomotor activity may produce behavioral disinhibition and can affect learning and memory processes. To exclude this possibility, the locomotor activity of animals was also assessed by measuring the number of movements over a 5 min period. Statistical analysis of the data showed that STZ, agmatine or STZ+agmatine treatments do not modify the number of movements in the locomotor activity test (data not shown).

Passive avoidance test

During the training session (on day 1) of the like-dark type passive avoidance task, there were no significant differences between any groups [$F(2,21)=0.1121$, $p=0.8945$, one-way ANOVA, Fig. 1a]. However, there was a significant difference

Figure 1a



Figure 1b

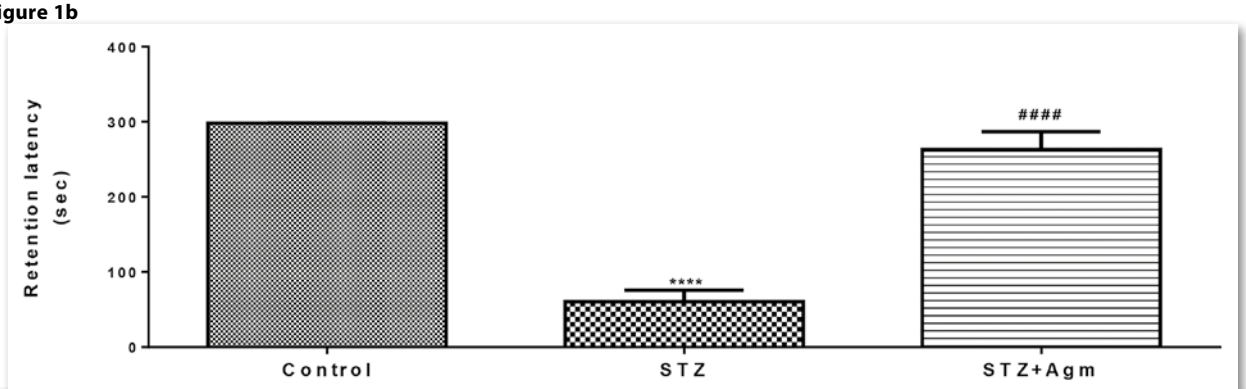


Figure 1: Effects of agmatine treatment on the passive avoidance task in rats. Effects of saline alone (control), STZ, STZ+agmatine (STZ+Agm) (40 mg/kg,i.p.) on a) acquisition b) retention test. Each value represents the mean±SEM of the parameters recorded and the statistical analysis by Bonferroni's test following one way ANOVA. * a significant difference compared with the saline and ### STZ and STZ+Agm group where p<0.001. The number of animals was 8 in each group.**

between the groups in the retention test [F(2,21)=60.28 p<0.0001, one-way ANOVA, Bonferroni's test; Fig 1b]. STZ administered rats showed significantly lower latency compared to control rats during the retention test, which was performed 24 h after the training test (p<0.001; Fig. 1b). The reduced retention latency indicates impaired retention of the passive avoidance task. The effect of STZ was reversed by 40 mg/kg of agmatine (p>0.05, vs. control; Fig. 1b).

Morris water maze test

We found that subjecting animals to the STZ protocol for 7 days resulted in performance deficits in the water maze tasks. As displayed in Figure 2, statistical analysis showed a significant effect of day in the data set (two way ANOVA, effect of day, F(3,84)=14.41, p<0.0001). In addition, an extremely significant effect of treatment was demonstrated (two way ANOVA, effect of treatment, F(3,84)=65.09, p<0.0001). Further analysis also revealed that day x treatment interacton was considered not significant (two way ANOVA, day x treatment, F(6,84)=0.05, p=0.9995). Post-hoc comparison showed that STZ caused a significant disruption of learning and memory, indicated by an increase in the escape latency compared to the control animals (two way ANOVA, Bonferroni's test, effect of treatment, p<0.001, p<0.001, p<0.001, p<0.001, respectively, Fig. 2a). Bonferroni's test suggesting that Agmatine (40 mg/kg/day, i.c.v), administration after STZ, reversed STZ-induced impairment of the escape latency in the task of water maze (two way ANOVA, effect of treatment, p<0.001, p<0.001, p<0.001, p<0.001, respectively, Fig. 2a).

Figure 2a

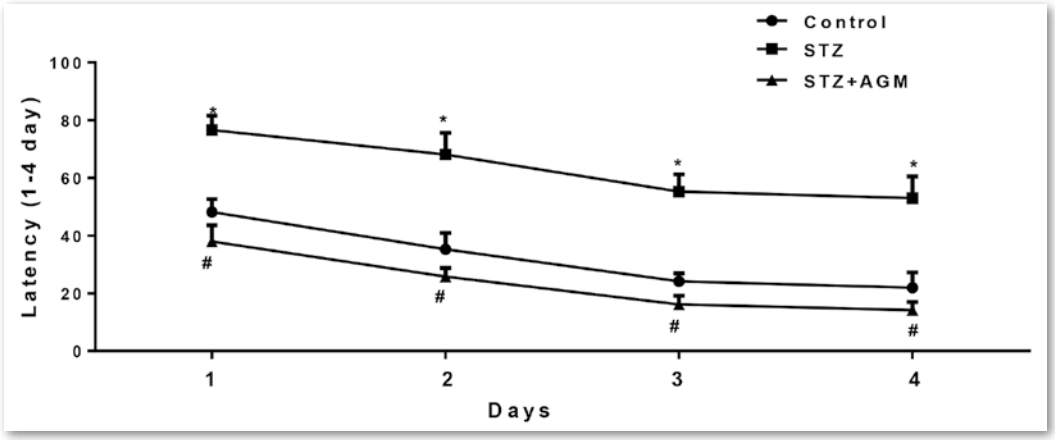


Figure 2b

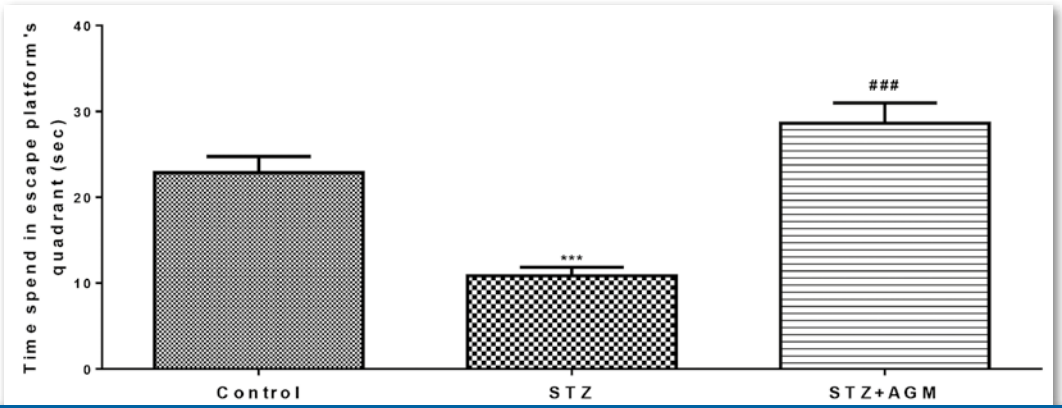


Figure 2: Effects of agmatine treatment on Morris water maze test. Effects of saline alone (control), STZ, STZ+agmatine (STZ+Agm) (40 mg/kg,i.p.) on a) acquisition (day 1-4) and b) probe test (day 5). Each value represents the mean±SEM of the parameters recorded and the statistical analysis by Bonferroni's test following two and one way ANOVA. * a significant difference compared with the saline and ### STZ and STZ+Agm group where p<0.001. The number of animals was 8 in each group.**

Table 1: Blood glucose levels

Groups	n	Blood glucose levels (mg/dl)	
		Day 0	Day 14
Sham operated control	8	145.9±9.2	156.6±10.4
STZ	8	126.1±8.7	131.1±9.7
STZ-Agmatine (40 mg/kg)	8	138.3±7.6	150.4±9.8

There was a significant difference between STZ-treated and control groups in terms of time spent in the escape platform quadrant during the probe trial of the Morris water maze test (one-way ANOVA, Bonferroni's test, $F(2,21)=24.54$, $p<0.001$). Post-hoc comparisons also showed that 40 mg/kg agmatine prolonged the time spent in the escape platform quadrant, suggesting that agmatine administration reversed the reduction in the time spent in escape platform's quadrant of STZ treated-rats ($p<0.001$, Bonferroni's test, Fig. 2b).

Biochemical estimation

Blood glucose level estimation

Blood glucose levels were measured in all groups one day before the surgery (day 0) and 14 days after the surgery. There was no significant difference in blood glucose levels among groups or no significant time-dependent changes (Table 1).

Oxidative damage estimation

Glutathione levels

Glutathione (GSH) level was 2.54±0.09 in sham operated control group, 1.63±0.05 in i.c.v. STZ group, 2.25±0.05 in i.c.v. STZ+ Agmatine group. A significant decrease in the level of GSH was observed in the STZ group. There was a significant increase in the level of GSH in the i.c.v. STZ + Agmatine group (2.25±0.05, $p<0.01$) compared to the i.c.v. STZ group.

Malondialdehyde (MDA) levels

Malondialdehyde (MDA) level was 28.75±2.91 in sham operated control group, 70.27±3.34 in i.c.v. STZ group, 53.73±2.20 in i.c.v. STZ + Agmatine group. A significant increase in the level of MDA was observed in the STZ group as compared to sham operated control group ($p<0.001$). Agmatine treatment (40 mg/kg) significantly decreased MDA level as compared to i.c.v. STZ group ($p<0.05$).

Activity of Myeloperoxidase

Myeloperoxidase (MPO) level was 4.87±0.49 in sham operated control group, 12.43±1.55 in i.c.v. STZ group, 8.96±0.32 in i.c.v. STZ + Agmatine group. A significant increase in the level of MPO was observed in the STZ group as compared to sham operated control group ($p<0.001$). The increase in MPO level was significantly supressed by agmatine treatment ($p<0.001$).

DISCUSSION

The i.c.v. STZ model has been described as an appropriate animal model for sporadic Alzheimer type dementia as both are characterized by progressive deterioration in memory and learning with the presence of oxidative stress[39,40].

In the present study, the results showed that agmatine was effective in improving impaired functions associated with cognition and oxidative damage induced by STZ model.

Many studies aiming to clarify the effects of agmatine on cognitive functions have been issued to date. From the beginning of 2000s, the effect of exogenous/ endogenous agmatine on learning and memory has been investigated in a variety of tasks, such as fear conditioning (contextual and cued conditioning), water maze, inhibitory avoidance, and object recognition[26-28,41].

First study investigating the role of agmatine in learning and memory was published in 2000. In this study, systemic administration of agmatine has been found to have an amnesic effect in a contextual fear conditioning task. Findings of a later study supported the results of the previous one in the same task, but found no significant effect on Morris water maze[42]. On the contrary, another study reported a facilitatory effect of agmatine in the inhibitory avoidance task[42]. Since central cholinergic activity has an essential role in learning and memory; scopolamine, a muscarinic receptor antagonist, has been recently used in studies concentrating on the effect of agmatine on hippocampus-related cognitive deficits. In this manner, a recent study from our group investigating the effect of agmatine on scopolamine-induced cognitive impairment in passive avoidance task revealed that agmatine dose-dependently reduced scopolamine-induced cognitive deficits in passive avoidance task. The results also showed that agmatine did not affect learning and memory when given alone[41]. In another study, agmatine pre-treatment prevented both scopolamine-induced deficits in water maze performance and inactivation of hippocampal molecular signaling pathways involved in learning and memory, such as extracellular regulated kinases (ERK) and Akt, a serine/ threonine kinase[42]. These findings are consistent with our results demonstrating that agmatine improves STZ-induced learning and memory impairment in passive avoidance and Moris water maze tasks.

There are a growing number of studies investigating the effect of agmatine on learning and memory in different task other than passive avoidance and Moris water maze tasks. Liu et al. demonstrated that i.c.v. administration of agmatine at a relatively low dose of 10 microgram improved the animals' performance in the standard radial arm maze by providing fewer

errors in the working, but not in the reference memory[28]. Additionally, rats significantly spent more time to explore displaced objects in object recognition memory task[43]. In a different study, low (1 mg/kg) and high (40 mg/kg) doses of agmatine were evaluated in water maze, T-maze and object recognition tasks. It was shown that low and high doses of agmatine significantly reduced the time to reach the platform location in water maze without affecting the motor activity. Besides, agmatine (40 mg/kg) treatment increased the percentage of time spent in the target quadrant with the longer retention time. On the other hand, agmatine treatment did not improve memory involved in object recognition, but facilitated the "object/place" memory, which is related to the medial temporal lobe structures, perirhinal cortex, and hippocampus[44]. Since aging itself is a major process causing cognitive impairments and nitric oxide (NO) plays crucial role in that process, agmatine was examined in aged rats from the aspects of evaluating cognitive functions and age-related changes in NOS activity. It was found that agmatine treatment (40 mg/kg, i.p.) significantly improved spatial working memory in water maze task and object recognition memory in aged rats, while exploratory activity and spatial reference memory were not affected. Moreover, agmatine attenuated the total NOS activity induced by aging process and restored eNOS protein to its normal level[45].

Another findings from a recent study investigating the effect of chronic administration of agmatine on behavioral function as well as neurochemistry in aged rats revealed that aged rats treated with saline, displayed an impairment in spatial learning and memory in the water maze and object recognition memory relative to younger rats. Whereas, chronic agmatine treatment improved animals' performance in the water maze, T-maze, and object recognition memory tests, and significantly suppressed age-related elevation in NOS activity in the dentate gyrus of the hippocampus and prefrontal cortex. However; this prolonged supplementation was unable to improve spatial reference learning and memory in aged rats, consistent with the previous studies[43].

A study, aiming to explain the impact of aging on endogenous agmatine levels in different hippocampal regions and dentate gyrus, showed that the levels of agmatine were significantly decreased in the CA1, but they were increased in the CA2/3 and dentate gyrus, in aged and middle-aged rats. They also demonstrated significantly increased levels of agmatine in entorhinal and perirhinal cortices in aged rats as compared with middle-aged and younger ones and in postrhinal and temporal cortices in aged and middle-aged rats compared with young rats. A dramatic decrease in the levels of agmatine has been detected in prefrontal cortex of aged rats relative to middle-aged and younger ones[43,46].

All of these findings mentioned above pointed out to the fact that endogenous agmatine might play a significant role in aging process, and changes in agmatine levels in memory-associated areas are "region-specific" and "age-related". Furthermore, not only changes in endogenous agmatine levels are related with aging process but also pharmacological interventions of agmatine could stand for novel treatment approaches in terms of improving cognitive functions via at least part of NOS pathway. Another study investigating the role of endogenous agmatine in learning and memory processes, compared agmatine levels in the hippocampus, parahippocampal region, cerebellum, and vestibular nucleus of rats that were trained in the water-maze task, forced to swim in the pool without a platform, or kept in the holding-box. In water maze group, agmatine levels were significantly increased in the CA1 and dentate gyrus subregions of the hippocampal formation, the entorhinal cortex and the vestibular nucleus when compared with the non-training groups. Results demonstrated "spatial learning-induced", "region-specific" elevation in agmatine levels[25,47].

In a recent study, agmatine was shown to be able to significantly decrease $A\beta_{25-35}$-induced spatial learning and memory impairment in different tasks including water maze, radial arm maze, and the object recognition tests suggesting that agmatine might have a considerable

neuroprotective effect in the pathology of Alzheimer's disease since $A\beta_{25-35}$ is the neurotoxic component of the full length $A\beta_{1-42}$, which has an essential role in the pathogenesis of the disease[43].

The improving effects of agmatine on cognitive dysfunctions were also examined in STZ-induced memory deficits in diabetic rats using Morris water maze and object recognition paradigms. It was shown that chronic treatment with agmatine (5–10 mg/kg, i.p. for 30 days) improved cognitive performance, which was shown to be impaired thirty days after diabetes induction in diabetic groups, and additionally lowered hyperglycemia, oxidative stress, and choline esterase activity[44]. Our results from the present study are highly compatible with these findings from previous studies.

There are number of studies endeavoring to explain mechanisms of neuroprotective effects of agmatine. Intrathecal or systemic administration of agmatine has been reported to attenuate the extent of neuronal loss due to ischemia and excitotoxicity. It was thought that this neuroprotective effect may be mediated through voltage-gated calcium channels blockade, NMDA receptor blockade and generation of inducible NOS inhibition[8,9]. It is suggested that this neuroprotective effect might be due to neuronal and inducible NOS by using middle artery occlusion model. In this model it was shown that agmatine decreased the extent of neuronal loss before or during occlusion[15]. The study investigating the effects of agmatine on voltage-gated ion channels in cultured rat hippocampal neurons found that agmatine reversibly blocked voltage-gated calcium channels but had no effect on potassium and sodium channels. Cultured cells from neonatal rat cortex incubated with NMDA, staurosporine (protein kinase inhibitor) and calcimycin (calcium ionophore) in the presence and absence of agmatine provided the knowledge that agmatine had a protective effect against NMDA excitotoxicity in neurons and PC12 cells, but had no effect on the cell death induced by protein kinase blockade or increase in cellular calcium[50]. Neuronal loss due to excitotoxicity is one of the mechanisms responsible for Alzheimer's disease. In the present study, finding that improving effect of agmatine on cognitive functions is likely due to the attenuation of the neuronal damage by blocking of NMDA receptors, therefore, mediation to the influx of calcium into the neurons and attenuating nNOS and iNOS activities. This hypothesis is consistent with a previous study reporting that agmatine administration significantly improved memory in aged rats and suppressed age-related elevation in total NOS activity. Thus, agmatine may have ameliorated the cognitive impairment induced by aging via regulating total NOS expression[46].

A study investigating the protective effects of agmatine against tumor necrosis factor (TNF)-α-induced apoptosis revealed that agmatine had neuroprotective effects against TNF-α-induced apoptosis in retinal ganglion cells in vitro. In a complementary way, agmatine was shown to decrease hippocampal caspase-3 activation, an indicator of neuronal apoptosis, induced by lipopolysaccharide (LPS). In the same study, this neuroprotective effect was also accompanied with an improving effect of agmatine on LPS-induced spatial memory impairment in water maze task[49]. In ischemia-like model, agmatine showed neuroprotective effect against cell damage induced by oxygen-glucose deprivation in primary cultured cortical cells. It was suggested that agmatine reduced ischemic injury of neurons primarily through inhibition of nNOS, while another study indicated that, this effect might have been due to agmatine regulation on the activity and translocation of nuclear factor kappa B[50,51]. There are also some findings proposing that agmatine prevents the ischemic renal injury probably via imidazoline receptors and alpha 2 receptors[50,52]. While the levels of endogenous agmatine was found 20 times higher after ischemic injury, exogenous agmatine provided a protection against ischemia like injury induced by oxygen-glucose deprivation in primary cultured cortical cells. A study demonstrated that exogenously administered agmatine (50mg/kg) had neuroprotective effects against repeated restraint-

induced structural changes in the medial prefrontal cortex and hippocampus. The parallel increase in endogenous brain agmatine levels triggered by a repeated immobilization has been shown[51,53].

It is well-known that NO interacts with the glutamatergic neurotransmission. NMDA receptor activation stimulates intracellular calcium influx in the neurons, leading to calcium-dependent NOS activation. This process contributes to the NO production and release, which spreads in a retrograde manner to the presynaptic neurons, enhancing glutamatergic activity. An interesting feature of NMDAR-mediated increase in intracellular calcium is that different levels of calcium inflow elicit opposite responses. For instance, smaller increases in intracellular calcium influx can promote neuronal survival, which also includes preventing from neurodegenerative diseases such as AD, whereas larger increases can lead to excitotoxicity decreases cell survival and triggers the stress responses such as ER stress and oxidative stress within the neurons resulting with the neurodegeneration as in Huntington's disease. Furthermore the excessive amounts of NO may cause nitrosative stress which also results in neurodegeneration. Inhibiting the NOS activities, agmatine might have a role in reducing these indirect effects of NO. Small amounts of NO and its relation to NMDA receptor retrograde activation is also important for learning and memory functions of the brain. By making a better discusssion including the relationship between agmatine and oxidative/ nitrosative stress might strengthen the hypothesis even if the NOS or NO levels have not been measured in this study. Furthermore nNOS and iNOS might be more important in this action and agmatine might balance this NO production at a level to improve the learning and memory related functions and to protect from the excessive amounts of NO related to neurodegeneration. Furthermore, we also measured the levels of MDA, GSH, and MPO activity which might also explain the relationship between agmatine and neuroprotective effects, since GSH levels reflect the oxidative reactions related to OH radical, and MDA reflects whether the membrane lipids have been peroxidized or not, leading to neurodegeneration. Our group has a smilar preliminary study on agmatine's effect on inflammatory part of STZ-induced Alzheimer's model. Agmatine lowers the MDA levels protecting the lipids of the neural membranes from peroxidation in both studies. In addition, MPO activity is a sign to reflect the PMN leukocytes migration to the stressed regions which is related to inflammation responses of the cell and which might also be related by the authors again with AD, since the neurodegeneration of the disease is also related to inflammation process. Additionally, the results show agmatine's effect on iNOS activity where the MPO levels increase the PMN leukocytes migration triggers the iNOS activation even in the microglia surrounding these cells. When iNOS is activated it usually results in excessive amounts of NO production which might also cause nitrosative stress[54-57].

It was also shown that, agmatine (50 mg/kg/day) has neuroprotective effects against structural alterations caused by glucocorticoids (21-day treatment) in vivo. The parallel alterations in the endogenous agmatine levels and ADC expression in the brain after treatment with glucocorticoids were also shown in aforementioned study. All of these data suggest that, the increase in the levels of endogenous agmatine can be considered as a defense mechanism against neuronal loss. On the other hand, the administration of exogenous agmatine provides an additional protection[58].

When considering the impact of agmatine over NOS activity, which was established by numerous studies mentioned above, it would be noteworthy that assessing NOS activity in our study provided a strong basis for the agmatine's improving effect on cognitive function and oxidative damage in the model of Alzheimer's disease.

CONCLUSIONS

This is the first study suggesting that agmatine reverses learning and memory disruptions and it attenuates oxidative damage seen in STZ-induced model of Alzheimer's disease. The present study

has certain limitations, especially by not including any parameters revealing the relationship between this cognitive and neuroprotective effects. However; as discussed above, there are numerous studies addressing this issue by proposing the neuroprotective effect of agmatine could be due to the inhibition of nNOS and/ or iNOS, suppressing inflammatory cytokines or blocking NMDA receptors accordingly decreasing the influx of calcium to the neurons. Further studies are required to elucidate the precise mechanisms by which agmatine improves cognitive functions in relevance with the pathology of Alzheimer's disease.

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