Agmatine, a metabolite of L-arginine, reverses scopolamine-induced learning and memory impairment in rats

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ABSTRACT

Agmatine (l-amino-4-guanidino-butane), a metabolite of L-arginine through the action of arginine decarboxylase, is a novel neurotransmitter. In the present study, effects of agmatine on cognitive functions have been evaluated by using one trial step-down passive avoidance and three panel runway task. Agmatine (20, 40, 80 mg/kg i.p.) was administered either in the presence or absence of a cholinergic antagonist, scopolamine (1 mg/kg i.p.). Scopolamine significantly impaired learning and memory in both passive avoidance and three panel runway test. Agmatine did not affect emotional learning, working and reference memory but significantly improved scopolamine-induced impairment of learning and memory in a dose dependent manner. Our results indicate that agmatine, as an endogenous substance, may have an important role in modulation of learning and memory functions.

Introduction

Agmatine (l-amino-4-guanidino-butane) is an endogenous polycationic amine synthesized by the decarboxylation of arginine by the enzyme arginine decarboxylase. Agmatine has been proposed to act as a novel neurotransmitter and/or neuromodulator in the mammalian brain (Raasch et al., 1995; Reis and Regunathan, 2000). Previous studies have shown that agmatine binds to imidazoline and alpha2-adrenoceptors and blocks N-methyl-D-aspartate (NMDA) receptor channels (Reis and Regunathan, 1999; Yang and Reis, 1999) and other ligand-gated cationic channels, including nicotinic receptors. Although it was reported that agmatine inhibits all three isoforms of nitric oxide synthase (NOS) (Galea et al., 1996), recently Mun et al. (2010) showed that it stimulates endothelial NOS in the rat brain. Several studies indicated that agmatine has a wide range of effects on central nervous system, such as its roles in morphine analgesia and morphine dependence (Aricioglu-Kartal and Uzbay, 1997; Aricioglu-Kartal and Regunathan, 2002; Aricioglu-Kartal et al., 2003; Aricioglu et al., 2004a, 2004b; Yavanci et al., 2007), and antidepressant, anxiolytic (Aricioglu and Altunbas, 2003; Zomkowski et al., 2002, 2004, 2005), antistress (Aricioglu et al., 2003a, 2003b, 2003c; Aricioglu and Regunathan, 2005), antinoceptive (Aricioglu et al., 2003a, 2003b, 2003c; Kolesnikov et al., 1996; Fairbanks et al., 2000; Santos et al., 2005), anticonvulsive (Aricioglu et al., 2005a, 2003b, 2003c; Demehri et al., 2003; Feng et al., 2005), antiproliferative, and neuroprotective (Gilad et al., 1996; Gilad and Gilad, 2000; Kim et al., 2004; Wang et al., 2006) effects in animal models. In contrast, the role of agmatine in learning and memory has not been extensively investigated. It was shown that agmatine disrupts both the acquisition and early consolidation of conditioned contextual stimuli, suggesting that high levels of agmatine are able to inhibit NMDA receptor activity (Stewart and McKay, 2000). Agmatine was also examined for its roles in water maze place learning, contextual and auditory cued fear learning and conditioned taste aversion learning. It has been found that systemically administered agmatine selectively impairs behavioral inferences of specific types of learning and memory (McKay et al., 2002). Agmatine has a facilitatory effect on memory consolidation in inhibitory avoidance task (Arteni et al., 2002). Agmatine given by intracerebroventricular microinfusion has shown to have an effect on working but not on reference memory in radial arm maze in a dose dependent manner. A study...
demonstrated age-related changes in agmatine levels in memory-associated brain structures (Liu et al., 2008a) and spatial learning-induced region-specific elevation has also been detected in agmatine levels (Liu et al., 2008b). Repeated agmatine treatment produces transient impairments in exploratory and locomotor activity in the open field test in a dose-dependent manner. Agmatine significantly facilitates spatial working memory at a longer delay, but not reference memory, suggesting its differential influence on spatial learning and memory (Liu and Bergin, 2009). The behavioral effects of agmatine are task- and delay-dependent, and agmatine facilitates memory particularly when the task difficulty is increased due to memory trace decay and/or greater interference (Liu and Collie, 2009).

Although there are studies showing that agmatine may have an important role in learning and memory, the question of whether it is able to reverse impaired learning and memory still remains. It is well established that central cholinergic systems play an important role in learning and memory (Palmer, 1996). Regarding the influence of cholinergic system on cognitive performance, scopolamine was applied to induce memory impairment (Blokland, 1995). Taken together, we investigated the effects of agmatine on memory disturbances induced by scopolamine, an antagonist of cholinergic receptors.

2. Materials and methods

2.1. Animals

240 male Wistar Albino rats weighing 230–250 g were housed five per cage and maintained under standard conditions with a temperature of 22 ± 2 °C and 12:12 h light–dark cycle. They were taken on a food deprivation schedule, on condition that they maintain their weight at 80–85% of the free-feeding level. All experiments were carried out according to the guidelines for the care of laboratory animals and ethical approval was granted by the Kocaeli University of Ethics Committee (Number: AEK 141/14, Kocaeli, Turkey). Different rat groups were used in each experiment and there were 10 rats in each group.

2.2. Drugs and treatment

Agmatine sulfate and scopolamine HCl were obtained from Sigma Chemicals (St. Louise, MO) and were dissolved in saline and administered intraperitoneally (i.p). Rats were given 20, 40, 80 mg/kg of agmatine, and 1 mg/kg of scopolamine, respectively. Control rats received saline (1 ml/kg, i.p). Behavioral tests were performed 30 min after agmatine and 20 min after scopolamine treatments. In case of combination same intervals were kept constant.

2.3. Three-panel runway test

The test was used to assess reference and working memory performances of rats according to the method previously described (Furuya et al., 1988; Ohno et al., 1992). The three-panel runway apparatus (175 × 36 × 25 cm) was composed of a start box, a goal box, and four consecutive intervening choice points. Each choice point consists of a gate with three panels (12 × 25 cm). The rats are prohibited from passing through two of three panels by front stoppers and are also prohibited from returning either to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all gates. When rats reached the goal box, they received food pellets as positive reinforcement. At the beginning of the test, all front stoppers were removed so that a rat could pass through any of the three panel gates at each choice point. The rats were forced to run the task repeatedly until the time, elapsed from leaving the start box to reach the goal box, fell consistently below 20 s. Once rats reached this state, they were forced to run the task under the condition that the front stopper of only one of the three panel gates (the correct panel gate) was removed at each choice point.

In the working memory task, six consecutive trials were performed each day at 2 min intervals (one session). They were returned to their home cage between trials and have free access to water. The locations of the correct panel gates were held constant within a session, but were changed from one session to the next. Thus, twelve different patterns of correct panel gate locations were used in this experiment. The criterion of learning was less than 12 errors summed across the six trials of a session in working memory tasks as previously described by Ohno et al. (1992). In the acquisition process, the number of errors in trial 1 remained stable at approximately 4–5, while the number of errors made from trials 2 to 6 markedly declined with repeated training. Approximately 25–30 training sessions were required for the rats to reach the criterion of less than 12 errors summed across the 6 trials of a session. The latency values also declined as the sessions were repeated.

In the reference memory task, six consecutive trials were applied each day at 2 min intervals (one session). The locations of the correct panel gates were held constant both within a session and in succeeding sessions. The number of times an animal pushed an incorrect panel gate (defined as error) and the time required for the animal to take food pellets (defined as latency) were recorded for each rat in each trial of a session. The criterion of learning was less than 6 errors summed across the six trials of a session in reference memory tasks as previously described by Ohno et al. (1992). The number of errors and the latency performance of rats in all 6 trials of a session decreased with repeated training in the reference memory procedure test. After 12–15 training sessions, the rats could run the three panel runway task within the 6 error criterion summed across 6 trials.

In the three panel runway tasks, after three consecutive sessions rats, that failed to reach the learning criterion, were discarded from the study.

2.4. Passive-avoidance (PA) test

Animals were trained in a step-down passive avoidance apparatus (Ugo Basile model 7551, Italy) for evaluating memory based on contextual fear conditioning and instrumental learning as previously described (Kameyama et al., 1986). In this task the animal learns that a specific place should be avoided since it is associated with an aversive event. Decrease in step down latency (SDL) indicates an impairment in memory in the PA task. The training apparatus consisted of two compartments, an illuminated chamber and a dark chamber, each measuring 22 × 21 × 22 cm, connected to each other with a guillotine door (5 × 5 cm). At the beginning of the trial, rats were placed in the illuminated compartment. After 30 s, the door between these two boxes was opened and the animal moved into the dark compartment freely (preacquisition trial). The acquisition (training) trial was carried out 15 min after the preacquisition trial. The illuminated compartment with a 20 W lamp was situated centrally above the grid floor. The dark chamber (i.e. conditioning chamber) with a steel-rod grid floor consists of 36 parallel steel rods, 0.3 cm in diameter with 1.5 cm apart from the neighboring rod. On the first day of training, rats were placed individually into the illuminated compartment and allowed to explore the box without receiving any drug treatment. If the rat completely enters the dark compartment, the sliding door was closed automatically and an electric foot-shock (0.5 mA) of a 3 s duration was delivered to the animal through grid floor immediately. Animals were then removed from the dark chamber and returned to their home cages. Any animal failing to cross from the illuminated to the dark compartment within 300 s was discarded from the experiment. Between each training session, both compartments of the chamber were cleaned to remove any confounding olfactory cues. On the following day, animals were put into the light compartment. Animals which completely entered into the dark compartment
within 300 s (without foot-shock) was recorded. If the animal had not entered into the dark compartment, it was returned to its cage and a maximum latency of 300 s was recorded. This latency served as a measure of retention performance of the step-down avoidance response (retention latency). In this study, rats received agmatine and scopolamine 30 min and 20 min before foot-shock training, respectively.

2.5. Data analysis

The number of errors and latency were summed across all six trials of a session for the reference memory task. They were summed from the first and second to the sixth trial of a session for the working memory. The presence of a significant difference between the group was determined by a two way analysis of variance (ANOVA) that was followed by Bonferroni’s Multiple Comparison Test when F ratios reached significance (p<0.05).

3. Results

3.1. Effects of agmatine on the reference memory performance in three panel runway test

Scopolamine (1 mg/kg, i.p.) significantly increased the number of latency [F(1,72) = 108.02, p<0.0001, two way ANOVA, effect of drug] and agmatine treatment dose-dependently reversed the effect of scopolamine [F(3,72) = 7.25, p=0.0003, two way ANOVA, effect of treatment] (Fig. 1A) in reference memory trials of rats. Further analysis also revealed a significant drug×treatment interaction effect suggested that rats treated with agmatine (20, 40 and 80 mg/kg, i.p.) alone did not change the latency and number of errors in working trials of rats (p>0.05, Bonferroni’s test) (Fig. 2A, B).

In the working memory, scopolamine at 1 mg/kg produced a significant increase in the total latency in trial 1 F(1,72)=207.70 (two way ANOVA, effect of drug), p<0.0001 (Fig. 2A), and in error responses F(1,72) = 6.86, p = 0.0107 (two way ANOVA, effect of drug). Agmatine reversed the effects of scopolamine on the latency [two way ANOVA, effect of treatment F(3,72) = 61.25, p<0.0001] and number of errors [two way ANOVA, effect of treatment F(3,72) = 9.17, p<0.0001] performance of rats in the three panel runway task (Fig. 2A and B). Further analysis also revealed a significant drug×treatment interaction effect suggested that agmatine blocked the effects of scopolamine [two way ANOVA, drug×treatment F(3,72) = 63.07, p≤0.0001; F(3,72) = 3.78, p = 0.0140, respectively]. Additionally, post hoc comparisons showed that rats treated with agmatine (20, 40 and 80 mg/kg, i.p.) alone did not change the latency and number of errors in working trials of rats (p>0.05, Bonferroni’s test) (Fig. 2A, B).

In addition, scopolamine (1 mg/kg, i.p.) significantly increased the latency [two way ANOVA, effect of drug, F(1,72) = 112.45, p<0.001] and error responses in trials 2–6 (two way ANOVA, effect of drug F(1,72) = 145.59, p<0.0001 (Fig. 3A and B). Agmatine reversed the effects of scopolamine on the latency [two way ANOVA, effect of treatment F(3,72) = 32.91, p<0.0001] performance of rats in the three panel runway task (Fig. 3A and B). Further analysis also revealed a significant drug×treatment interaction effect suggested that agmatine blocked the effects of scopolamine [two way ANOVA, drug×treatment F(3,72) = 10.38, p = 0.0001; F(3,72) = 22.30, p<0.0001]. Additionally, post hoc comparisons also showed that rats treated with agmatine (20, 40 and 80 mg/kg, i.p.) alone did
not change the latency and number of errors in working trials of rats (p > 0.05, Bonferroni’s test) (Fig. 3A and B).

3.3. Effects of agmatine on passive avoidance test

During the training (acquisition) session (on day 1) of step-down passive avoidance task, vehicle treated animals and agmatine-[F(3,72) = 0.99, p = 0.4046], scopolamine- [F(1,72) = 0.25, p = 0.6152] and agmatine + scopolamine [F(3,72) = 0.25, p = 0.8621] treated rats showed a similar SDL (two way ANOVA) (data not shown). However, on day 2 (retention), scopolamine-treated rats showed a significantly lower SDL compared to that of control group [F(1,72) = 30.47, p < 0.0001, two way ANOVA, effect of drug] (Fig. 4). Decrease in SDL indicates an impairment in learning in the passive avoidance task. In addition, a nearly significant effect of agmatine (20, 40 80 mg/kg) pretreatment was demonstrated [two way ANOVA, effect of treatment F(3,72) = 7.61, p = 0.002]. Further analysis also revealed a significant pretreatment × treatment interaction effect suggested that agmatine
AGM40 AGM20 AGM40 AGM20 +SCOP AGM40 +SCOP AGM80 +SCOP

Fig. 4. Effects of agmatine (20, 40 and 80 mg/kg) on retention test of the step-down type of an inhibitory passive avoidance task in rats. Each value represents the mean ± SEM of the parameters recorded, and the statistical analysis by Bonferroni’s test following two way ANOVA. + indicates a significant difference with control and * from the scopolamine group.

4. Discussion

In the present study, scopolamine, a cholinergic antagonist, significantly impaired learning and memory in three panel runway and step-down passive avoidance tests. The main findings of the present study are: i) agmatine alone did not affect cognitive function, ii) agmatine pretreatment reversed the impairment of learning and memory induced by scopolamine in passive avoidance test, and iii) agmatine also improved impairment in reference and working memory in three-panel runway test. Therefore, we have shown for the first time that agmatine treatment significantly and dose dependently reduced the scopolamine-induced learning and memory deficits.

Three panel runway test is a useful method for studying learning and memory in rats, particularly because it provides the possibility to compare working and reference memory conditions. Working memory allows animals to remember information that is useful for a single session of an experiment, but not for subsequent sessions, whereas reference memory is defined as to hold information that is considered as a continued value across all sessions (Ohno et al., 1992). Also the inhibitory avoidance test is a classic behavioral test model, with a strong aversive component, utilized for evaluating learning and memory in rats (Kameyama et al., 1986). A brief explanation of these tests with regards to learning and memory would be useful.

Recently, a dose-dependent acquisition deficit in young rats has been demonstrated when agmatine was administered prior to acquisition of contextual fear training. There was a time-dependent impairment of consolidation in the same task with a post-training agmatine administration (Stewart and McKay, 2000). It has been also demonstrated that, agmatine impaired contextual fear learning in a dose-dependent manner, but had no significant effects on water maze place learning and preference for the training quadrant during probe trials in young adult rats. Agmatine (1, 5, 10 or 50 mg/kg) was examined for its role in water maze place learning, contextual and auditory-cued fear learning and conditioned taste aversion learning, when administered systemically. Agmatine did not affect latencies to find the hidden platform or preference for the training quadrant during probe trials. In contextual or auditory-cued fear-conditioning sessions, agmatine evoked a linear dose-dependent impairment in the magnitude of learned fear to the contextual stimuli. It had no effect on the magnitude of learned fear to the auditory stimulus. These studies indicate that systemically administered agmatine selectively impairs behavioral inferences of specific types of learning and memory (Stewart and McKay, 2000). The discrepancy may be due to the different behavioral tasks used in animal models and/or the systemic route of the treatment related to central site of action coupled with limited or varied access from blood to brain (Nguyen et al., 2003). By contrast, Arteni et al. (2002) investigated cognitive effect in inhibitory avoidance task in rats, suggesting that agmatine has been found to facilitate memory consolidation through activation of locus coeruleus. Similarly, in the present study agmatine alone did not change emotional learning in passive avoidance task, but improved scopolamine-induced learning impairment.

It has been shown that, agmatine levels can significantly increase in the prefrontal, entorhinal, and perirhinal cortices. It is also found, that L-citrulline levels increased in the DG subregion of the hippocampus and the prefrontal cortex in the T-maze, which is considered to be a spatial working memory task (Barnes et al., 1980). It is most likely, that increased agmatine and L-citrulline levels seen in the T-maze training group are learning and memory specific. Learning and memory are associated with changes in the efficacy of synaptic neurotransmission (Kandel, 2001), which is related to NO. NO is involved in synaptic plasticity, learning and memory (Feil and Kleppisch, 2008). Since L-citrulline is co-produced with NO, it is likely that increased levels of L-citrulline reflect the up-regulation of NOS activity and/or NOS containing neurons. Rats with agmatine treatment prior to each daily test made markedly fewer errors in a working memory, but not in the reference memory. It has been well documented that working memory has limited capacity and is susceptible to interference (Olton and Paras, 1979; Jonides et al., 2008). Recently, it has been reported that agmatine treatment (20 and 40 mg/kg i.p.) attenuates impaired prepulse inhibition induced by the psychotomimetic drug phencyclidine (Palsson et al., 2008). An experimental assessment of pre-attentive information processing refers to the early phases of information processing. This processing is important for efficient processing of sensory information and for coherent cognitive operations (Ellenbroek, 2004). In the latest study, agmatine was investigated in several commonly used behavioral tasks. Agmatine (1 and 40 mg/kg) improved animals’ performance in the water maze probe test, but did not affect animals’ performance in the open field and the place navigation probe tests. Agmatine treated group was found to be less anxious compared to the controls, with no performance changes in the open field. In the water maze task, post-training agmatine treatment did not affect place and cued navigation, but significantly improved animals’ performance in the probe test conducted 24 h after training and the reversal test. In the working memory version of the task, it took significantly less time for agmatine treated rats and generated markedly shorter path length to reach the platform. In the object recognition task, rats with pre-test agmatine treatment spent significantly more time to explore displaced objects, but not novel object, as compared to the controls (Liu and Collie, 2009). This finding is consistent with the results of the current study demonstrated that agmatine did not affect reference memory, but improved impaired reference memory in three panel runway test.

In a simplified reference memory version of the water maze task, rats given high dose of agmatine displayed a transient impairment in searching a hidden platform, whereas the low dose group reduced latency in the first probe test. In the object recognition task, all groups...
could detect the novel object, but rats in the agmatine low dose group spent significantly more time to explore displaced objects compared to the saline controls. In the radial arm maze task, agmatine (10 μg)-treated rats made significantly fewer errors compared to the saline controls in the working, but not the reference memory version of the task (Liu and Bergin, 2009). As a result, findings of the study demonstrated age-related changes in agmatine levels in memory-associated brain structures (Liu et al., 2009). Rats taken agmatine treatment prior to each daily test made markedly fewer errors in the working memory, but not in the reference memory version of the task compared to the saline controls in radial arm maze (Liu et al., 2008a; 2008b). In the present study, agmatine even in lowest dose (20 mg/kg) significantly improved working memory, which resulted in less number of errors in working memory. Besides when working memory was disturbed with scopolamine, agmatine reversed this effect.

Previous studies have proposed the NMDA and NOS mechanisms based on the negative influence of agmatine on the NMDA receptors and the neuronal and inducible isoforms of NOS (Lavinsky et al., 2003; Li et al., 1994; Zomkowski et al., 2002). Recent evidence, however, suggests the differential effect of agmatine on endothelial nitric oxide synthease (eNOS), agmatine stimulates eNOS and elevates NO and cyclic GMP levels to produce vasodilatation of blood vessels (Santhanam et al., 2007). It has been documented that the basal level of eNOS-derived NO is vital to stabilise the vascular microenvironment (de la Torre and Stefano, 2000) and is important for synaptic plasticity, learning and memory (Blackshaw et al., 2003; Dinerman et al., 1994; Hooper and Garthwaite, 2006). Agmatine can be metabolised by agmatinase with the production of polyamine putrescine (Halaris and Piletz, 2007). While polyamines have been known to play an important role in maintaining cellular functions (Oredsson, 2003; Wallace, 2000; Williams, 1997), recent evidence suggests a novel function of putrescine in adult neurogenesis in the dentate gyrus of the hippocampus (Matarterre et al., 2004). It has been reported that i.c.v. infusion of agmatine increased the firing rate of locus coerulesc neurons in a dose-related manner (Ruiz-Duranté et al., 2002). The exact mechanisms underlying the behavioral effects of agmatine are not fully understood; however, according to the results of the present study agmatine itself had no effect on learning and memory performance.

In conclusion, agmatine reversed scopolamine-induced impairment in working and reference memory, tested in three panel runway task and in emotional learning, tested in passive avoidance task. This result revealed that agmatine improved cognitive performance in memory-impaired rats. Our findings are consistent with a previous study reported that agmatine may provide protection against amyloid-beta-induced learning and memory deficits (Bergin and Liu, 2010). Overall, previous studies and findings from the current study demonstrated that agmatine may have an important role in both normal and pathological situations. Further studies will help to understand the physiological and pharmacological roles of agmatine in learning and memory which will provide new therapy strategies in cognitive disorders.

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