BRAIN RESEARCH 1429 (2012) 61–71

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Research Report

Involvement of nitric oxide in granisetron improving effect on scopolamine-induced memory impairment in mice

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ARTICLE INFO

Article history:
Accepted 3 August 2011
Available online 9 August 2011

Keywords:
Granisetron
Spatial recognition memory
Fear memory
Nitric oxide
Mice

ABSTRACT

Granisetron, a serotonin 5-HT_3 receptor antagonist, widely used as an antiemetic drug following chemotherapy, has been found to improve learning and memory. In this study, effects of granisetron on spatial recognition memory and fear memory and the involvement of nitric oxide (NO) have been determined in a Y-maze and passive avoidance test. Granisetron (3, 10 mg/kg, intraperitoneally) was administered to scopolamine-induced memory-impaired mice prior to acquisition, consolidation and retrieval phases, either in the presence or in the absence of a non-specific NO synthase inhibitor, L-NAME (3, 10 mg/kg, intraperitoneally); a specific inducible NO synthase (iNOS) inhibitor, aminoguanidine (100 mg/kg); and a NO precursor, L-arginine (750 mg/kg). It is demonstrated that granisetron improved memory acquisition in a dose-dependent manner, but it was ineffective on consolidation and retrieval phases of memory. The beneficial effect of granisetron (10 mg/kg) on memory acquisition was significantly reversed by L-NAME (10 mg/kg) and aminoguanidine (100 mg/kg); however, L-arginine (750 mg/kg) did not potentiate the effect of sub-effective dose of granisetron (3 mg/kg) in memory acquisition phase. It is concluded that nitric oxide is probably involved in improvement of memory acquisition by granisetron in both spatial recognition memory and fear memory.

This article is part of a Special Issue entitled The Cognitive Neuroscience.

1. Introduction

Granisetron which serves as a 5-HT_3 antagonist of serotonin receptors is of use in prevention of chemotherapy-induced nausea and vomiting (Bermudez et al., 1988; Carmichael et al., 1988; Gralla, 1993). On the other hand, 5-HT_3 antagonists has been found to obviously improve learning and memory (Buhot et al., 1999; Roman and Marchetti, 1998) and to antagonize the effects of anticholinergic (Hodges et al., 1995) or age-induced (Pitsikas and Borsini, 1996, 1997) memory loss; moreover, the

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doi:10.1016/j.brainres.2011.08.006

correlation between cholinergic neurons degeneration and the degree of cognitive impairment (Bowen et al., 1976; Whitehouse, 1992) which is seen in the central nervous system (CNS) of patients with Alzheimer disease (Nakahiro et al., 1988) has been proven. Following these studies, scientists have shown that 5-HT_3 receptors are involved directly and indirectly in reduction of cholinergic release (Morales et al., 1996; Ramírez et al., 1996) and 5-HT_3 antagonists efficiently compensate cholinergic dysfunction (Barnes et al., 1990; Buhot et al., 1991; Chugh et al., 1991). It has been found that 5-HT3 agonist administration enhanced hippocampal cholinergic function and this effect was eliminated by 5-HT3 receptor antagonists (Meneses, 1999; Richter-Levin and Segal, 1996). On the other hand, several behavioral studies have demonstrated the interference of nitric oxide (NO) in particular forms of memory formation and disturbance of learning by NO synthase (NOS) inhibitors (Böhme et al., 1993; Chapman et al., 1992; Meyer et al., 1998; Yamada et al., 1996). NO, a solvable, short lived and freely dispersible gas, acts as an intracellular retrograde messenger in the brain (Garthwaite, 1991; Moncada and Palmer, 1991) and participates in synaptic plasticity, including long-term potentiation of the hippocampus (Haley et al., 1992; O'Dell et al., 1991). NO apparently plays an important role in performance during the memory acquisition (Yamada et al., 1995); additionally, NO donors were able to reverse NOS inhibitor-induced learning impairment (Huang and Lee, 1995; Meyer et al., 1998). Presumably, the nitric oxide (NO)-pathway is involved in serotonin-mediated vasodilatation and also in serotonergic neurotransmission (Bruning et al., 1993; Straub et al., 2007); in addition, NO releases from cholinergic fibers and exists in the cholinergic cells contacting the cortex (Mariño and Cudeiro, 2006). Moreover, it has been proposed that nitric oxide is involved in the 5-HT_3 receptors' proconvulsive action (Gholipour et al., 2010), chloride secretory response to 5-HT_3 activation in distal colon (King et al., 2004) and also neurogenic relaxations of guinea pig proximal colon (Sevcík et al., 1998); in addition, it has been suggested that NO is involved in the pressor response elicited by 5-HT_3 stimulation in the nucleus tractus solitarii (Sévoz-Couche et al., 2002) and 5-HT_3 induced release of substance P in rat spinal cord (Inoue et al., 1997) . However, the role of NO pathway is still unclear in the granisetron mechanism of action as a $5HT_3$ antagonist on learning procedure.

Scopolamine-induced memory impairment is a generally used model to evaluate the effects of drugs on learning ability and cognition status in experimental animals (Kopelman and Corn, 1988; Stevens, 1981); scopolamine disarranges short-term and working memory (Stevens, 1981); consequently, different memory phases are affected (Abel and Lattal, 2001) in Y-maze and passive avoidance task.

According to our knowledge, the present study is conducted for the first time to investigate the granisetron mechanism of action during acute administration on different phases of memory (acquisition, consolidation and retrieval) in amnesic mice; furthermore, we investigated the potential involvement of NO pathway in positive effects of granisetron on memory performance in memory impaired mice, using a NOS inhibitor, L-NAME, a specific iNOS inhibitor, aminoguanidine and a precursor of nitric oxide, L-arginine.

2. Results

2.1. Y-maze

2.1.1. Effect of granisetron on acquisition of spatial recognition memory

2.1.1.1. Effect of granisetron on memory acquisition. The effect of granisetron (3 and 10 mg/kg) was evaluated in mice with normal memory status. Total exploration time (factor granisetron, $F_{(1,\ 50)}=0.802$, $P>0.05$) and number (%) of arm entries (factor granisetron, $F_{(1,\ 50)}=2.051$, $P>0.05$) were not affected by granisetron in saline-treated mice in comparison with control group (data not shown); moreover, the entire number of arm entries was similar in both experimental and control groups, illustrating that general locomotor activity was not influenced by granisetron ($F_{(1,\ 15)}=3.810$, $P>0.05$; data not shown).

2.1.1.2. Effect of granisetron on acquisition of memory induced by scopolamine. The effect of granisetron on exploratory behavior was examined in scopolamine-induced memory impaired mice. As mentioned in our previous study (Allami et al., 2011), scopolamine-treated mice were not able to discriminate novel arm versus familiar arms in exploration time ($F_{(2,\ 17)}=1.721$, $P>0.05$; Fig. 1A) and number (%) of arm entries ($F_{(2,\ 17)}=0.943$, $P>0.05$; Fig. 1B) in acquisition trial. Memory impairment induced by scopolamine has also been confirmed by significant reduction in exploration time and number (%) of novel arm entries in scopolamine-treated mice versus vehicle-treated group, as shown in Fig. 1 ($F_{(5,\ 48)}=5.879$, $P<0.05$; Fig. 1A; $F_{(5,\ 48)}=6.074$, $P<0.001$; Fig. 1B). Granisetron significantly improved the compromised parameters in scopolamine-treated group, respectively ($F_{(11,\ 104)}=6.789$, $P<0.001$; Fig. 1A; $F_{(11,\ 104)}=3.029$, $P<0.01$; Fig. 1B) in a dose-dependent manner. In addition, duration and number (%) of novel arm exploration was significantly increased by granisetron (10 mg/kg) in comparison with the start and other arms ($F_{(2,\ 35)}=30.770$, $P<0.001$; Fig. 1A; $F_{(2,\ 35)}=6.906$, $P<0.01$; Fig. 1B). However, granisetron did not improve exploration parameters in the novel arm at the dose of 3 mg/kg ($F_{(2,\ 17)}=1.270$, $P>0.05$; Fig. 1A; $F_{(2,\ 17)}=2.288$, $P>0.05$; Fig. 1B).

Besides, the number of total arm entries was similar in all experimental groups, demonstrating that general locomotor activity was affected by granisetron neither in scopolamine- nor in saline-treated groups ($F_{(3,\ 34)}=1.678$, $P>0.05$; data not shown).

2.1.2. Effect of granisetron on consolidation and retrieval of spatial recognition memory

2.1.2.1. Effect of granisetron on consolidation of memory impaired by scopolamine. Fig. 2 demonstrates the effect of granisetron on consolidation stage of spatial recognition memory. In scopolamine-treated memory-impaired group, exploration time (factor granisetron, $F_{(11,\ 86)}=2.119$, $P>0.05$; Fig. 2A) and number (%) of arm entries (factor granisetron, $F_{(11,\ 86)}=0.403$, $P>0.05$; Fig. 2B) did not show any significant change in granisetron-treated group compared with vehicle-treated control mice.

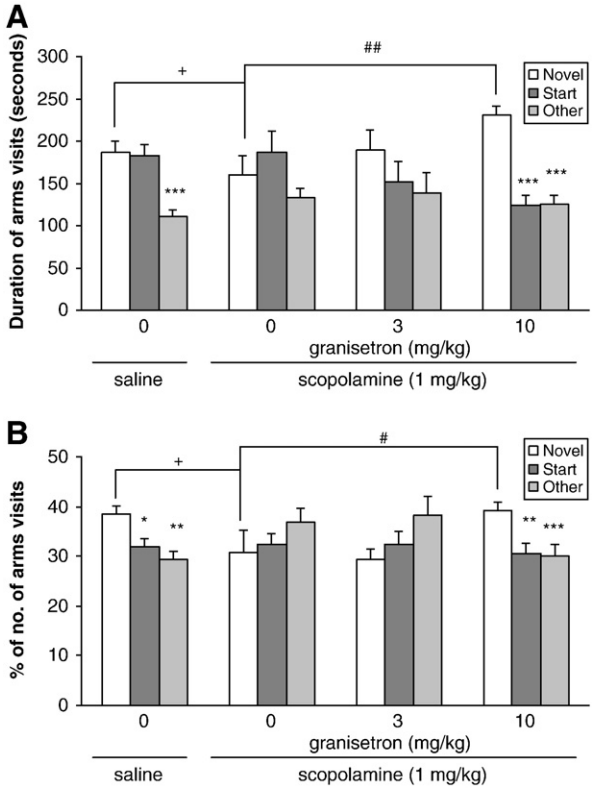


Fig. 1 – Effect of granisetron on acquisition of spatial memory in Y-maze task. Granisetron (3, 10 mg/kg), or vehicle (same volume of saline) was administered 30 min prior training sessions. Scopolamine (1 mg/kg) 30 min prior to training session, impaired memory acquisition. Exploration time in each arm (A) and the percentage of number of arm entries (B) were measured. Values are expressed as the mean ± SEM from 6 to 8 animals and were analyzed using a multivariate ANOVA for exploration time and the percentage of number of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post test. +P < 0.05 versus vehicle control group; *P < 0.05, **P < 0.01, ***P < 0.001 versus corresponding novel group; #P < 0.05, ##P < 0.01 versus scopolamine-treated group.

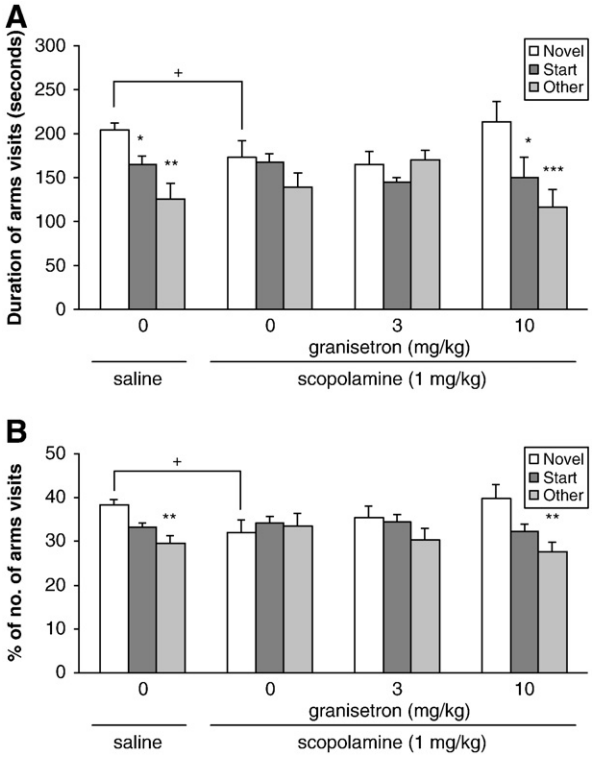


Fig. 2 – Effect of granisetron on spatial memory consolidation in Y-maze task. Granisetron (3, 10 mg/kg) or saline was administered immediately after training sessions. Memory consolidation was impaired by scopolamine (1 mg/kg) administered immediately after training session. Exploration time in e-arm (A) and the percentage of number of arm entries (B) were measured. Data represent means ± SEM from 6 to 8 animals and were analyzed using a multivariate ANOVA for exploration time and the percentage of number of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post test. +P < 0.05 versus vehicle control group; **P < 0.01, ***P < 0.001 versus corresponding novel group.

2.1.2.2. *Effect of granisetron on retrieval of memory impaired by scopolamine.* The effect of granisetron on retrieval stage of spatial recognition memory is demonstrated in Fig. 3. Exploration time (factor granisetron, $F_{(8, 65)} = 0.605$, $P > 0.05$; Fig. 3A) and number (%) of arm entries (factor granisetron, $F_{(8, 65)} = 1.404$, $P > 0.05$; Fig. 3B) did not change significantly in granisetron-treated group compared with vehicle-treated control in scopolamine-induced memory-impaired mice.

2.1.3. *Interaction of nitric oxide with the effect of granisetron on acquisition of spatial recognition memory*

2.1.3.1. *Effects of L-NAME in antagonizing granisetron memory improvement.* According to our previous study (Allami et al., 2011), administration of L-NAME (3 and 10 mg/kg) 30 min before training session, had no effect on memory acquisition and spontaneous locomotor activity either in saline- or in scopolamine-treated groups; however, it nullified the beneficial effect of granisetron (10 mg/kg) on this memory stage, as demonstrated in Fig. 4. L-NAME (10 mg/kg) significantly declined novel arm exploration time and number (%) of novel arm entries in granisetron-treated mice ($F_{(8, 71)} = 5.694$, $P < 0.001$; Fig. 4A; $F_{(8, 71)} = 2.423$, $P < 0.05$; Fig. 4B). In addition, no arm discrimination was seen in experimental group treated with L-NAME (10 mg/kg) and granisetron showing a decrease in general exploratory activity ($F_{(2, 17)} = 1.011$, $P > 0.05$; Fig. 4A; $F_{(2, 17)} = 0.681$, $P > 0.05$; Fig. 4B). Moreover, locomotor activity was not influenced by L-NAME in granisetron-treated mice ($F_{(2, 23)} = 1.796$, $P > 0.05$; data not shown).

2.1.3.2. *Effects of aminoguanidine in antagonizing granisetron memory improvement.* Despite aminoguanidine (100 mg/kg) had no effect on acquisition phase of memory by itself (Javadi-Paydar et al., 2011), it nullified the positive effect of granisetron (10 mg/kg) on this phase. Aminoguanidine, was administered 45 min before training session and significantly decreased the novel arm exploration time and number (%) of novel arm entries in granisetron treated mice ($F_{(5, 53)} = 19.398$, $P < 0.001$;

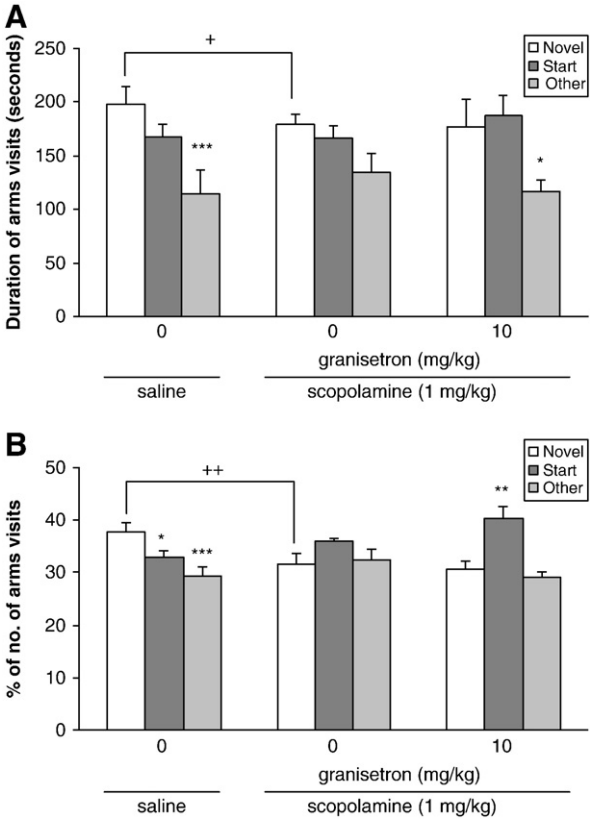


Fig. 3 – Effect of granisetron on spatial memory retrieval in Y-maze task. Granisetron (10 mg/kg) or saline was administered immediately after training sessions. Memory retrieval was impaired by scopolamine (1 mg/kg) administered 30 min before the retention test. Exploration time in each arm (A) and the percentage of number of arm entries (B) were measured. Data represent means ± SEM from 6 to 8 animals and were analyzed using a multivariate ANOVA for exploration time and the percentage of number of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post test. +$P<0.05$, ++$P<0.01$ versus vehicle control group; *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus corresponding novel group.

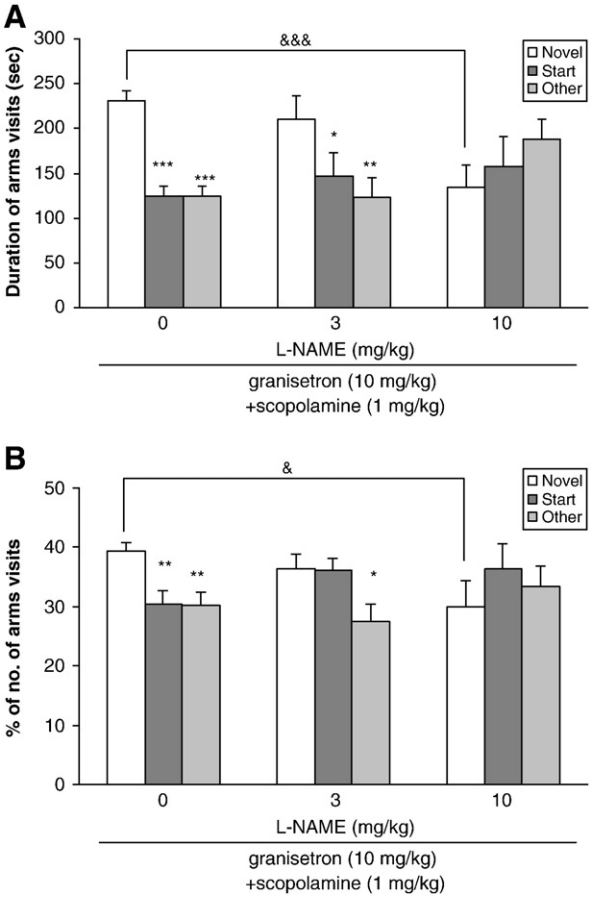


Fig. 4 – Effect of L-NAME on granisetron memory enhancement in spatial memory acquisition. Granisetron (10 mg/kg) or vehicle (same volume of saline) was administered 30 min before training sessions. Memory acquisition was impaired by scopolamine (1 mg/kg), administered 30 min before training session; L-NAME (3, 10 mg/kg) was administered simultaneously with scopolamine. Exploration time in each arm (A) and the percentage of number of arm entries (B) were measured. Values are expressed as the mean ± SEM from 6 to 8 animals and were analyzed using a multivariate ANOVA for exploration time and the percentage of number of arm entries and one-way ANOVA for locomotor activity, followed by Tukey's post test. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus corresponding novel group; &$P<0.05$, &&&$P<0.001$ versus scopolamine-session treated group.

Fig. 5A; $F_{(5, 53)}=5.418$, $P<0.01$; Fig. 5B). Also, locomotor activity was not influenced by aminoguanidine in granisetron treated mice ($F_{(1, 17)}=1.007$, $P>0.05$; data not shown).

2.1.3.3. Effects of L-arginine in agonizing granisetron memory improvement. According to our previous study (Javadi-Paydar et al., 2011), L-arginine (750 mg/kg) administered 30 min before training session, did not change the exploration time and the percentage of frequency of arm entries in memory acquisition neither in saline-treated nor in scopolamine-treated groups. Additionally, L-arginine did not potentiate the effect of granisetron (3 mg/kg) on memory acquisition; as L-arginine administration altered neither exploration time ($F_{(5, 38)}=0.943$, $P>0.05$; Fig. 6A) nor the percentage of frequency of arm entries ($F_{(5, 38)}=1.604$, $P>0.05$; Fig. 6B) in the novel arm in granisetron-treated mice. Moreover, locomotor activity was not changed by L-arginine (750 mg/kg) in granisetron-treated ($F_{(1, 12)}=3.101$, $P>0.05$; data not shown) compared with vehicle-treated mice.

2.2. Passive avoidance

2.2.1. Effect of granisetron on acquisition of long-term memory

2.2.1.1. Effect of granisetron on memory impairment induced by scopolamine. In step-through latency (STL) using the passive avoidance paradigm, granisetron administered 30 min prior to training session, significantly increased latency time in mice with impaired memory by scopolamine in a dose dependent manner; while the same dose of drug did not show significant difference in latency time in mice with normal memory status (factor granisetron, $F_{(2, 21)}=2.705$, $P<0.01$; Fig. 7).

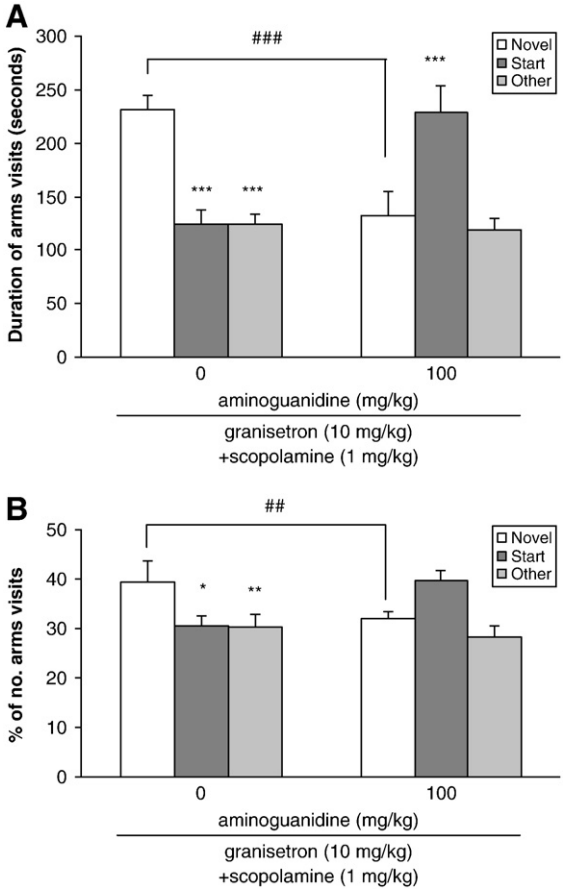


Fig. 5 – Effect of aminoguanidine on memory improvement by granisetron in spatial memory acquisition. Granisetron (10 mg/kg) or vehicle (same volume of saline) was administered 45 min before training session. Memory acquisition was impaired by scopolamine (1 mg/kg), administered 30 min prior to training sessions; aminoguanidine (100 mg/kg) was administered 45 min before training session. Exploration time in each arm (A) and the percentage of number of arm entries (B) were measured. Values are expressed as the mean ± SEM from 6 to 8 animals and were analyzed using a multivariate ANOVA for exploration time and the percentage of number of arm entries and one-way ANOVA for locomotor activity, followed by Tukey's post test. *P < 0.05, **P < 0.01, ***P < 0.001 versus corresponding novel group; ##P < 0.01, ###P < 0.001 versus scopolamine-treated group.

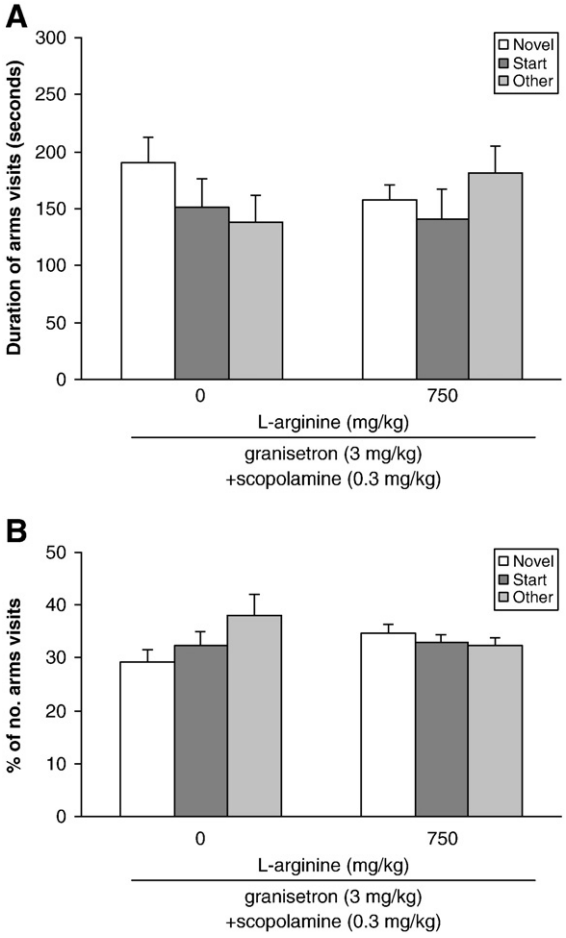


Fig. 6 – Interaction of effect of L-arginine with granisetron in spatial memory acquisition. Granisetron (3 mg/kg) or vehicle (same volume of saline) was administered 30 min before training sessions. Memory acquisition was impaired by scopolamine (0.3 mg/kg), administered 30 min prior training session; L-arginine (750 mg/kg) was administered simultaneously with scopolamine. Exploration time in each arm (A) and the percentage of number of arm entries (B) were measured. Values are expressed as the mean ± SEM from 6 to 8 animals and were analyzed using a multivariate ANOVA for exploration time and the percentage of number of arm entries and one-way ANOVA for locomotor activity, followed by Tukey's post test.

2.2.1.2. Effects of L-NAME in antagonizing granisetron memory improvement. As shown in Fig. 8A, L-NAME administered 30 min before training session, reduced latency time in passive avoidance test, in a dose dependent manner ($F_{(2,\ 24)} = 3.737$, $P < 0.05$; Fig. 8A); moreover, L-NAME (10 mg/kg) had no effect on latency time in acquisition phase by itself; however, it reversed the beneficial effect of granisetron (10 mg/kg) on STL, as demonstrated in Fig. 8B ($F_{(1,\ 12)} = 11.623$, $P < 0.01$; Fig. 8B).

2.2.1.3. Effects of aminoguanidine in antagonizing granisetron memory improvement. Despite aminoguanidine (100 mg/kg) did not change latency time in passive avoidance test (Fig. 9A), it reversed the beneficial effect of granisetron (10 mg/kg) on latency time in acquisition phase (Fig. 9B). Aminoguanidine, administered 45 min before training session significantly decreased the STL in granisetron-treated mice ($F_{(1,\ 9)} = 14.318$, $P < 0.01$; Fig. 9B).

2.2.1.4. Effects of L-arginine in agonizing granisetron memory improvement. As shown in Fig. 10A, L-arginine (750 mg/kg) had no effect on latency time in acquisition phase of fear memory by itself; also, it did not potentiate the effect of sub-effective dose of granisetron (3 mg/kg) in memory-impaired mice ($F_{(1,\ 12)} = 2.232$, $P > 0.05$; Fig. 10B).

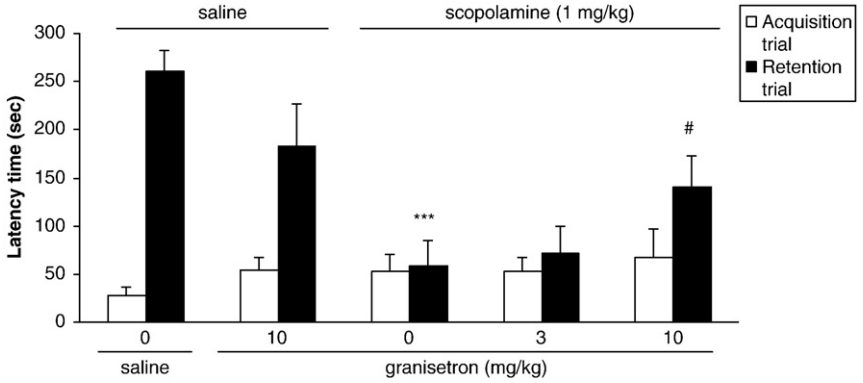


Fig. 7 – Effect of granisetron on intact-memory and scopolamine-induced step-through latency impairment in passive-avoidance task. Granisetron (3, 10 mg/kg) was administered 30 min before training sessions. The latency time to enter the dark compartment was determined. Values are expressed as the mean ± SEM from 6 to 8 animals and was analyzed using a non-parametric ANOVA using a medians test. ***P < 0.001 versus vehicle control group; #P < 0.05 versus scopolamine-treated group.

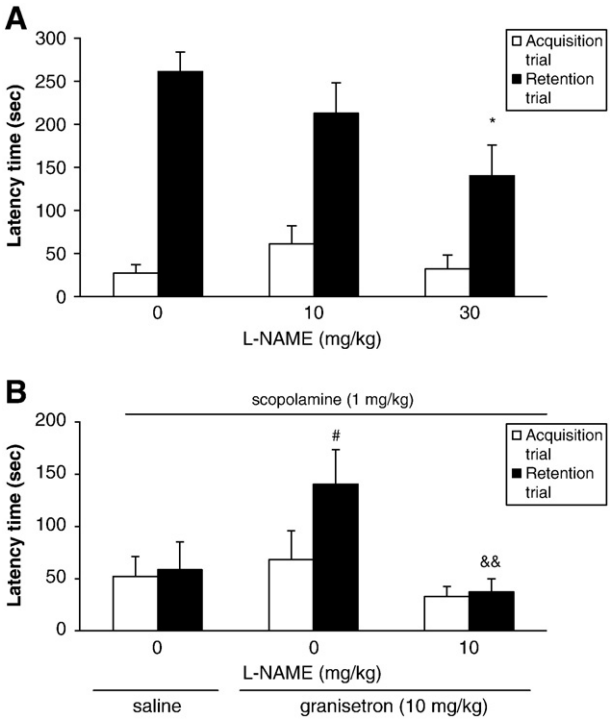


Fig. 8 – Effect of L-NAME on intact-memory (A) and memory acquisition improvement by granisetron (B) in step-through latency impairment in passive-avoidance task. Granisetron (10 mg/kg) was administered 30 min prior training sessions. Memory acquisition was impaired by scopolamine (1 mg/kg) administered 30 min before training session; L-NAME (3, 10 mg/kg) was administered simultaneously with scopolamine. The latency time to enter the dark compartment was determined. Values are expressed as the mean ± SEM from 6 to 8 animals and was analyzed using a non-parametric ANOVA using a medians test. *P < 0.05 versus corresponding novel group; #P < 0.05 versus scopolamine-treated group; &&P < 0.01 versus scopolamine–granisetron-treated group.

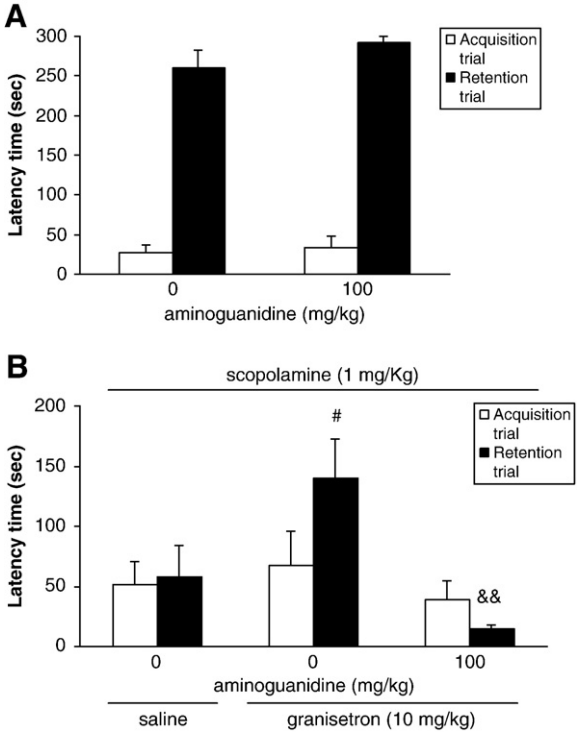


Fig. 9 – Effect of aminoguanidine on intact-memory (A) and aminoguanidine effect on memory improvement by granisetron in step-through latency impairment (B) in passive-avoidance task. Granisetron (10 mg/kg) or saline was administered 30 min before training sessions. Memory acquisition was impaired by scopolamine, (1 mg/kg) administered 30 min before training session; aminoguanidine (100 mg/kg) was administered 45 min prior training session. The latency time to enter the dark compartment was determined. Values are expressed as the mean ± SEM from 6 to 8 animals and was analyzed using a non-parametric ANOVA using a medians test. #P < 0.05 versus scopolamine-treated group; &&P < 0.01 versus scopolamine–granisetron-treated group.

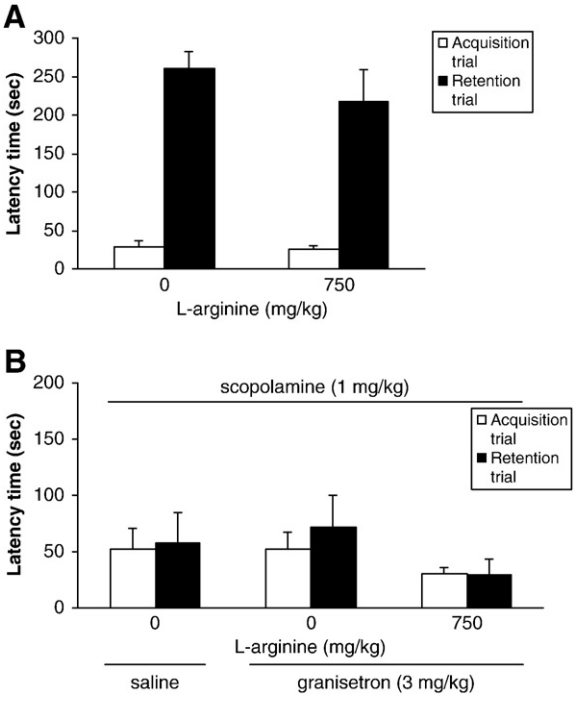


Fig. 10 – Effect of L-arginine on intact-memory (A) and its interaction with granisetron (B) in acquisition of memory in step-through latency. Granisetron (3 mg/kg) or saline was administered 30 min before training session. Memory acquisition was impaired by scopolamine (1 mg/kg), administered 30 min before training session; L-arginine (750 mg/kg) was administered simultaneously with scopolamine. The latency time to enter the dark compartment was determined. Values are expressed as the mean ± SEM from 6 to 8 animals and was analyzed using a non-parametric ANOVA using a medians test.

3. Discussion

The result of the present study determined for the first time that NO pathway is involved in granisetron improving effect on memory performance in scopolamine-induced memory-impaired mice. Our findings revealed that administration of granisetron improved both spatial recognition memory and fear memory in scopolamine-induced memory-impaired mice, this effect is not attributable to animal's locomotor activity and it is reversed due to administration of nitric oxide synthase inhibitors; while determining the consolidation and retrieval phase of memory, granisetron did not enhance the impaired-memory performance in Y-maze task. Previous reports showed that granisetron, a serotonin 5-HT_3 antagonist, improved acquisition, retention and retrieval of memory in passive avoidance test (Chugh et al., 1991); however, others have found that 5-HT_3 receptor antagonists have no effect on learning in spatial discrimination (Buhot, 1997; Hodges et al., 1995). Some evidences show that these antagonists attenuate age-associated memory impairment (Wolf, 2000) and even unimpaired-memory (Chugh et al., 1991); In contrast, other reports suggest the impairment of memory in water maze task by intrahippocampal injection of granisetron (Naghdi and Harooni, 2005). This discrepancy might be explained by the different cognitive task tests used in their study and/or the different rout of drug administration. It can also be attributed to the existence of different 5-HT_3 receptor splice variants on hippocampal CA1 that provide different sites of action for 5-HT_3 receptor antagonists. On the other hand, it has been found that 5-HT_3 receptor agonists caused a substantial increase in cholinergic release in cortical tissue and this effect was reversed by 5-HT_3 receptor antagonists. This could be another explanation for this discrepancy that the modulation of acetylcholine by 5-HT_3 receptor antagonists cannot be ruled out as their mechanism of enhancement of memory and cognitive function (Chugh et al., 1991). However, the exact mechanism of action involved in effects of granisetron on memory performance has not been evaluated extensively.

5-HT_3 receptors, the targets of granisetron, are widely distributed in the rat brain and spinal cord, mainly in hippocampus region (Mössner et al., 2004). These receptors, which are a ligand-gated Na^+ and K^+ cation channel, play a prominent role in the formation of learning and memory (Buhot et al., 1991; Morales et al., 1996). 5-HT_3 antagonists potently improve basal performance in rodent and primate tests of cognition and inhibit the impairments in performance caused by cholinergic deficits (Barnes et al., 1990). In consistent with our results, it has been shown that the 5-HT_3 antagonists prevent scopolamine-induced memory deficits in various animal models (Barnes et al., 1989; 1990; Chugh et al., 1991; Costall et al., 1978; Fontana et al., 1995; Reznic and Staubli, 1997); however, contradiction is also seen with other studies on animals (Naghdi and Harooni, 2005) or humans (Benline and French, 1997).

As it is previously determined, NO is an important neurotransmitter involved in learning and memory through synaptic plasticity in various brain areas such as cerebellum and hippocampus (Susswein et al., 2004). It seems that NO/cGMP pathway plays a prominent role in processing within the brain and it may be an important therapeutic target in preventing and treating mild cognitive impairment (Austin et al., 2010; Chu and Heistad, 2010). It has also been reported that hippocampal NO facilitates the inhibitory avoidance learning task in chicks and rats (Tan, 2007). Considering the role of NO in learning and memory, it is shown that systemic administration of NOS inhibitors like L-NAME impairs spatial memory; however, administration of low doses of L-NAME did not alter the animal behavior in Y-maze task (Tanaka et al., 2009) and in passive avoidance test (Yildirim and Marangoz, 2004); besides, it has been reported that intraperitoneal administration of another NOS inhibitor (N^{ω}-nitro-L-arginine) impairs animal performance in a radial-arm maze (Böhme et al., 1993).

The NO/cGMP pathway is presumed to be involved in acetylcholine- and serotonin-mediated vasodilatation (Bruning et al., 1993). Others have also suggested that the role of 5-HT_3 receptor in neurogenic relaxations of guinea pig proximal colon (Sevcík et al., 1998) and on human esophageal motility (Willis et al., 1994) is at least partly mediated via release of NO from nerve endings. The involvement of nitric oxide has also been proposed as one of the possible pathways for the activation of soluble guanylate cyclase by serotonin in mouse neuroblastoma and rat glioma hybrid cells (Reiser, 1990). In addition, it seems that NO is involved in proconvulsive action of granisetron

(Gholipour et al., 2010). As a result, we have proposed in this study, the involvement of nitric oxide pathway in granisetron beneficial effect on memory performance. Our results support the idea that the beneficial effect of granisetron on spatial memory acquisition and fear memory which is reversed by administration of both iNOS specific (aminoguanidine) and NOS non-specific (L-NAME) inhibitors might be mediated through the involvement of a NO-dependent pathway; although L-arginine, a NO precursor, did not potentiate the effect of sub-effective dose of granisetron.

In conclusion, our study demonstrates that granisetron improved acquisition of scopolamine-induced short-term memory and also fear memory impairment probably through the involvement of nitrergic system in amnesic mice. L-NAME, a non-selective NOS inhibitor, and aminoguanidine, a selective iNOS inhibitor have been shown to possess the ability to reverse the improving effect of granisetron on acquisition of spatial memory and fear memory, but L-arginine, a NO precursor do not potentiate the effect of granisetron on acquisition stage. Our data, thus, reinforce the idea that granisetron can be used as an adjunct therapy in treatment of memory impairment, though further clinical investigation are needed to assess its therapeutic potential.

4. Experimental procedures

4.1. *Housing and handling of animals*

The animals were handled according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals (NIH US publication 86–23 revised 1985). NMRI male mice (Tehran University of Medical Science, Tehran, Iran), 6–8 weeks of age, were kept in a controlled environment (23±2 °C, 50±5% humidity) under a 12 h light/dark cycle (light on 08.00–20.00) and had free access to standard pellet chow and tap water throughout the study. Each mouse was used once and each treatment group comprised of 6–12 animals.

4.2. *Chemicals and drugs*

The L-NAME, scopolamine and aminoguanidine used in this study were purchased from Sigma (St. Louis, USA), L-arginine and granisetron were respectively purchased from Merck (Darmstadt, Germany) and Aburaihan Pharmaceutical Company (Tehran, Iran). All drugs were freshly diluted in physiological saline and administrated intraperitoneally.

4.3. *Y-maze task*

The Y-maze is a simple two-trial recognition test for evaluating spatial recognition memory. It is based on the innate tendency of rodents to explore novel environments (Allami et al. 2011; Dellu et al., 1992). It consists of three horizontal arms (40 cm long, 3 cm wide walls and 12 cm high) symmetrically disposed at 120° to each other. Each arm had different clues for distinction from each other. The Y-maze test comprised two trials separated by a 1 h inter-trial interval. In the first trial (training), which lasted 10 min, each mouse was placed at the end of the start arm and was allowed to explore only two arms (including the start arm), with the third arm (novel arm) being blocked. After 1 h, in the second trial (retention), mouse was placed back in the maze, at the same start arm with free access to all three arms, being allowed to move freely during an 8 min period. The number of arm entries and exploration time in each arm were recorded manually using a stopwatch for each mouse over an 8 min period. The total number of arm entries was measured as an index of locomotor activity to rule out the interference of changes in motility with the parameters of learning and memory. To avoid the presence of olfactory trials, maze arms were thoroughly cleaned between tests.

Recognition of the novel arm from the other two familiar arms is considered as a memory improvement effect. Mice which distinguish the unfamiliar arm show exploratory behavior, so they spend more time and enter more frequently to the novel arm in comparison with the other familiar ones.

4.4. *Passive avoidance task*

A step-through passive avoidance task was used to evaluate the effect of granisetron on long-term memory (Kim et al., 2008). Passive avoidance apparatus was consisted of a two identical compartments (20×20×20 cm), illuminated and non-illuminated boxes (Borj Sanat Company, Tehran, Iran), separated by a guillotine door. The illuminated compartment contained a 40 W bulb, and the floor of non-illuminated compartment was composed of 2 mm stainless steel rods spaced 1 cm apart. During the training trial, each mouse was placed in the lighted compartment, and when the mouse entered the dark compartment the door closed and the mouse received an inescapable electric shock (0.5 mA, 1 s). The test trial was done 24 h after the training trial; in this step, the mouse was again placed in the lighted compartment and the latency time to enter the dark compartment was measured. If the mouse did not enter the dark chamber within the cut-off time (300 s), it was assigned a latency value of 300 s.

4.5. *Experimental design*

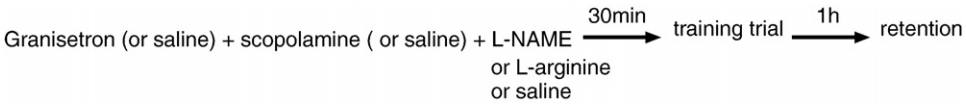
4.5.1. *Y-maze task*

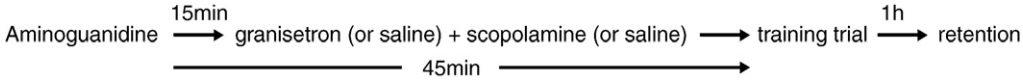
In all experimental groups, memory impairment was induced by scopolamine (1 mg/kg) prior to each experimental trial. Granisetron dose and the optimum time interval of granisetron administration was determined according to our pilot study. Corresponding vehicle controls were used in all experiments.

4.5.1.1. *Acquisition trial.* Mice were administered granisetron (3 and 10 mg/kg) and scopolamine (1 mg/kg) 30 min before training trials. Retention was performed according to the following schema in the presence and absence of scopolamine.

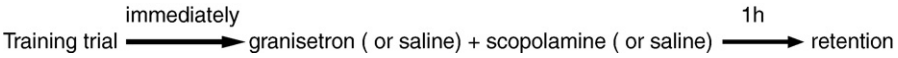
In two other sets of experiments, L-NAME (5, 10 mg/kg) (Allami et al., 2011) or L-arginine (750 mg/kg) (Javadi-Paydar et al., 2011) were administered 30 min before training sessions; in the third set of experiment aminoguanidine (100 mg/kg)

(Javadi-Paydar et al., 2011) was administered 45 min before training session. Granisetron and scopolamine were administered as same as described above.

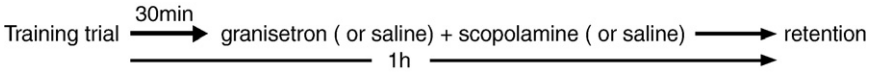




4.5.1.2. *Consolidation trial.* Mice were administered granisetron (3 and 10 mg/kg) and scopolamine immediately after training trials. Retention was performed according to the following schema in the presence and absence of scopolamine.



4.5.1.3. *Retrieval trial.* According to the following schema, granisetron (3 and 10 mg/kg) and scopolamine were respectively administered immediately after training trial and in 30 min before retention test. The retention test was performed in an hour in the presence and absence of scopolamine.

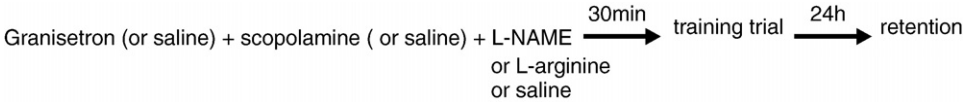


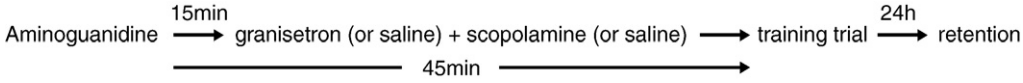
4.5.2. *Passive avoidance*

Memory impairment was induced by scopolamine (1 mg/kg), 30 min before the training trial. In experimental group, granisetron (3 and 10 mg/kg) was administered 30 min prior to training trial. Control animals were administered corresponding vehicle.

4.5.2.1. *Acquisition trial.* Mice were administered granisetron (3 and 10 mg/kg) and scopolamine 30 min before training trials. Retention was performed according to the following schema in the presence and absence of scopolamine.

In two other sets of experiments, L-NAME (5, 10 mg/kg) (Allami et al., 2011) or L-Arginine (750 mg/kg) (Javadi-Paydar et al., 2011) were administered 30 min before training session; in the third set of experiment aminoguanidine (100 mg/kg) (Javadi-Paydar et al., 2011) was administered 45 min before training session. Granisetron and scopolamine were administered as same as described above.





4.6. *Statistical analysis*

The results of each experiment were expressed as: In Y-maze: 1) exploration time (seconds) for each arm visits; 2) percentage of number of arm entries during an 8 min session; 3) total number of arm entries reflected as locomotor activity index. In passive avoidance test, the latency time (seconds) to enter the dark compartment was measured in both acquisition and retention trials. Data were expressed as mean ± SEM and analyzed using SPSS statistical software package. Differences among treatment groups were considered as between-group factor, whereas differences in arm entries and exploration time for each special treatment were considered as within-group factor. Each of between group differences was assessed with multivariate analysis of variance

(ANOVA) and Tukey's post test. One-way ANOVA was used to determine the within-group differences in locomotors activity and $P<0.05$ was considered statistically significant in all experiments.

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