Pharmacology, Biochemistry and Behavior 97 (2010) 256–261

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Agmatine inhibits morphine-induced memory impairment in the mouse step-down inhibitory avoidance task

Wei Lu, Hua-Jin Dong, Zheng-hua Gong, Rui-Bin Su *, Jin Li *

Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Beijing 100850, China

ARTICLE INFO

Article history:
Received 6 April 2010
Received in revised form 14 August 2010
Accepted 18 August 2010
Available online 27 August 2010

Keywords:
Agmatine
Morphine
Step-down inhibitory avoidance test
Memory
Imidazoline receptor
Idazoxan

ABSTRACT

The effect of agmatine on memory formation in morphine-treated mice on the step-down inhibitory avoidance test was examined. Pre-training and pre-test administration of agmatine (5, 10 and 20 mg/kg, s.c.) facilitated memory formation and retrieval while post-training administration of agmatine (5, 10 and 20 mg/kg, s.c.) had no effect on memory consolidation. Idazoxan (5 mg/kg, i.p.) inhibited the effect of agmatine on memory formation and retrieval. Pre-training administration of morphine (1.25, 2.5 and 5 mg/kg, s.c.) impaired memory formation while post-training and pre-test administration of morphine (1.25, 2.5 and 5 mg/kg, s.c.) had no effect on memory consolidation and retrieval. Pre-training agmatine treatment reversed the impairment of morphine on memory formation. Moreover, pre-test administration of agmatine inhibited morphine-induced amnesia. Pre-training and pre-test idazoxan (5 mg/kg, i.p.) treatment inhibited the effect of agmatine on morphine induced memory impairment. In conclusion, agmatine inhibited morphine-induced memory impairment on the mice step-down inhibitory avoidance test. The mechanism was exerted, at least in part, through activation of imidazoline receptors.

1. Introduction

Morphine has been widely used in the clinical management of pain for about 200 years, but its strong dependent potential is a serious obstacle to its clinical practice. More and more attention has been paid to the mechanisms of opioid dependence. Several lines of evidence suggest that the opioid system is involved in the acquisition and storage of memory related to dependence (Introini et al., 1985; Saha et al., 1991; Ragozzino and Gold, 1994; Vaccarino et al., 1999). That means addiction is a malfunction of learning and memory induced by long-term adaptation in specific neural systems. For example, chronic morphine treatment impaired the formation and retrieval of spatial memory (Ma et al., 2007; Pu et al., 2002). Opioid receptor agonists have been shown to influence learning and memory in inhibitory avoidance tasks (Itoh et al., 1994; Stone et al., 1991; Walker et al., 1991).

Agmatine, an amine formed by the decarboxylation of L-arginine by the enzyme arginine decarboxylase (ADC), exerts a wide range of biological activities on several systems, including the CNS. It has been shown to modulate the release of neurotransmitters or hormone (Li et al., 1994; Kalra et al., 1995), and possibly act as a neurotransmitter/modulator in the brain (Regunathan et al., 1995; Reis and Regunathan, 2000). In various disease models, agmatine attenuates thermal, tactile allodynia and has an anti-inflammatory effect (Fairbanks et al., 2000; Regunathan and Piletz, 2003; Onal et al., 2004; Karadag et al., 2003). It has a weak analgesic effect in tail flick test and enhances morphine-induced antinociception (Kolesnikov et al., 1996; Yesilyurt and Uzbay, 2001). Furthermore, it inhibits tolerance to morphine (Fairbanks and Wilcox, 1997) and attenuates morphine dependence *in vitro* and *in vivo* (Aricioglu-Kartal and Uzbay, 1997; Li et al., 1998; Aricioglu et al., 2003 a, b). Moreover, agmatine binds with high affinity to α2-adrenergic and imidazoline receptors of all subclasses (Li et al., 1994; Piletz et al., 1995). Agmatine also antagonizes NMDA receptors, inhibits NOS and blocks calcium channels (Yang and Reis, 1999; Galea et al., 1996; Zheng et al., 2004), which all play important roles in learning and memory. These results suggest that agmatine also influences memory and the effect of agmatine on morphine dependence is related to its effect on learning and memory. Recently, some evidence suggested that agmatine facilitates memory consolidation in inhibitory avoidance tasks (Arteni et al., 2002). However, other researchers suggested that systemically administered agmatine selectively impairs behavioral inferences in contextual fear learning, but had no effect on other memory models (McKay et al., 2002).

The present study is intended to find out not only whether agmatine has any effect on memory on the single-trial step down inhibitory avoidance task in saline or morphine treated mice, but also whether the effect of agmatine on memory is related to imidazoline receptors.

* Corresponding authors. Su is to be contacted at Tel.: +86 10 66828341; fax: +86 10 68211656. Li, Tel.: +86 10 66931603; fax: +86 10 68211656.
E-mail addresses: ruibinsu@126.com (R.-B. Su), Lijin@nic.bmi.ac.cn (J. Li).

0091-3057/$ – see front matter.
doi:10.1016/j.pbb.2010.08.007

2. Materials and methods

2.1. Animals

Male Kunming mice weighing 18–22 g were used. The mice were acclimated to the housing conditions and handled for 3–4 days before the experiments to minimize handling stress during the test. These animals were maintained on a 12 h light/dark cycle (lights on between 7:00 A.M. and 7:00 P.M.) and kept on *ad libitum* access to food and water. All experimental procedures were conducted in accordance with the guidelines for the use of experimental animals and approved by the Institutional Review Committee on Animal Care and Use.

2.2. Drugs

Morphine hydrochloride was purchased from Qinhai Pharmaceutical Factory, China. Agmatine sulfate was synthesized by Beijing Institute of Pharmacology and Toxicology, China. Idazoxan was obtained from Sigma Chemical Company (Sigma, St. Louis, MO, USA). All the drugs were dissolved in 0.9% saline to the final concentrations and injected in a volume of 10 ml/kg. Agmatine and morphine were given subcutaneously (s.c.). Idazoxan was administered intraperitoneally (i.p.).

2.3. Apparatus

The step down inhibitory avoidance apparatus consisted of a Plexiglas box ($12\times12\times18$ cm high) with a steel-rod floor ($d=4.2$ mm). A Plexiglas platform ($d=4.8$ cm, $h=4.5$ cm) was set in the corner of the grid floor. Electric shocks (0.15 mA) were delivered to the grid floor.

2.4. Procedures of the single-trial step down inhibitory avoidance task

During the 5 min training session, the mice were gently placed on the platform facing the rear left corner. The mice that stepped down the platform and touched the grid received a 0.15 mA electric shock. Mice that stayed on the platform for more than 300 s or couldn't step up the platform were not used in the experiment. 24 h after the training session, the mice were tested in the same condition without electric shock. The latency for the mice to step down the platform was recorded automatically with an end-point of 300 s (Arteni et al., 2002).

2.4.1. Effect of agmatine on the formation, consolidation and retrieval of memory

To study the effect of agmatine on memory formation, mice were trained 30 min after agmatine (0, 5, 10 and 20 mg/kg, s.c., Arteni et al., 2002; Aricioglu et al., 2004) administration and were tested 24 h later. To study the effect of agmatine on memory consolidation, agmatine (0, 5, 10 and 20 mg/kg, s.c.) was administrated 30 min after the training session and the mice were tested 24 h later. To study the effect of agmatine on memory retrieval, agmatine (0, 5, 10 and 20 mg/kg, s.c.) was administrated 30 min before the test 24 h after the training session.

To study whether the effect of agmatine on memory formation was related to activation of imidazoline receptors, idazoxan (5 mg/kg, i.p., Wei et al., 2005) was co-administrated with agmatine (10 mg/kg, s.c.) 30 min before the training. The mice were tested 24 h later.

To study whether the effect of agmatine on memory retrieval was related to activation of imidazoline receptors, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) 30 min before the test.

2.4.2. Effect of morphine on the formation, consolidation and retrieval of memory

To study the effect of morphine on memory formation, the mice were trained 30 min after morphine (0, 1.25, 2.5 and 5 mg/kg, s.c., Jafari-Sabet M et al., 2005; Jafari MR et al., 2006) administration and were tested 24 h later. To study the effect of morphine on memory consolidation, morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) was administrated 30 min after the training session and the mice were tested 24 h later. To study the effect of morphine on memory retrieval, morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) was administrated 30 min before the test 24 h after the training session.

2.4.3. Effect of pre-training agmatine administration on morphine-induced memory impairment

To study the effect of pre-training agmatine treatment on morphine-induced memory impairment, mice in the control group received saline plus saline (10 ml/kg) while the mice in the other four groups received agmatine (0, 5, 10 or 20 mg/kg, s.c.) 30 min before morphine (5 mg/kg, s.c.) administration. 30 min after morphine injection, the mice were trained in the step-down inhibitory avoidance task. The mice were tested 24 h later.

To study whether the effect of agmatine on morphine-induced memory impairment was related to activation of imidazoline receptors, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) and morphine (5 mg/kg, s.c.) 30 min before the training. The mice were tested 24 h later.

2.4.4. Effect of pre-test agmatine administration on morphine-induced memory impairment

To study the effect of pre-test agmatine treatment on morphine-induced memory impairment, the mice in the control group received saline (10 ml/kg) while the mice in the other four groups received morphine (5 mg/kg, s.c.) 30 min before the training session. On the test day, the mice pre-treated with morphine were injected with agmatine (0, 5, 10, 20 mg/kg, s.c.) 30 min before the test. The mice were tested 24 h later.

To study whether the effect of agmatine on morphine-induced memory impairment was related to the activation of imidazoline receptors, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) 30 min before the test. The mice were tested 24 h later.

2.5. Data analysis

The mice's latency of stepping down the platform was expressed as mean ± S.D. The data were analyzed using the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) followed by the Mann–Whitney *U* test. Holmes Sequential Bonferroni Correction Test was used when two independent groups were compared. A *P* value of less than 0.05 was the critical criterion for statistical significance.

3. Results

3.1. Agmatine enhanced memory formation and retrieval, but not memory consolidation

The latency for the mice to step down the platform pre-treated with agmatine (0, 5, 10 and 20 mg/kg, s.c.) before the training session was shown in Fig. 1A, indicating that agmatine facilitated memory formation. The latency of the mice in the control group was 207.60 s, compared with 290.27 s in the agmatine (10 mg/kg) pre-treated groups, was significantly prolonged as compared to that in the control ($u=1.98$, $P<0.05$).

The latency for the mice to step down the platform treated with agmatine (0, 5, 10 and 20 mg/kg, s.c.) immediately after the training session was shown in Fig. 1B, indicating that agmatine did not affect memory consolidation. The latency of the mice was 217.50 s in the control group and 204.73–246.11 s in the agmatine (5, 10 and 20 mg/kg) treated groups without significant difference.

In Fig. 1C, the latency for the mice to step down the platform treated with agmatine (0, 5, 10 and 20 mg/kg, s.c.) 30 min before the test session was shown, indicating that agmatine facilitated memory

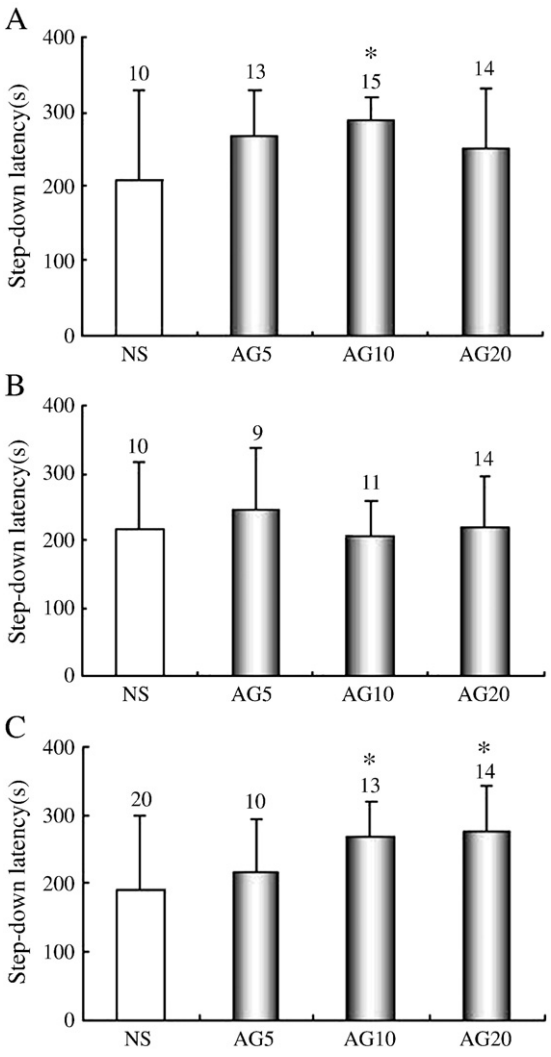

Fig. 1. A. Effect of acute agmatine treatment on memory formation. To study the effect of agmatine on memory formation, the mice were trained 30 min after agmatine (0, 5, 10 and 20 mg/kg, s.c.) administration and were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 10–15$, $^{*}P<0.05$ compared to control. B. Effect of acute agmatine treatment on memory consolidation. To study the effect of agmatine on memory consolidation, agmatine (0, 5, 10 and 20 mg/kg, s.c.) was administrated 30 min after the training session and the mice were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 9–14$. C. Effect of acute agmatine treatment on memory retrieval. To study the effect of agmatine on memory retrieval, agmatine (0, 5, 10 and 20 mg/kg, s.c.) was administrated 30 min before test 24 h after the training session. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 10–20$, $^{*}P<0.05$ compared to the control.

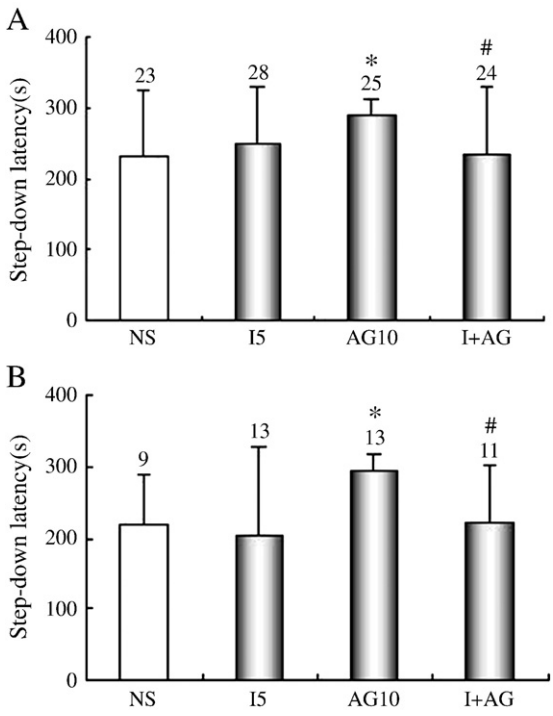

Fig. 2. A. The relationship between idazoxan and agmatine on memory formation. To study whether the effect of agmatine on memory formation was related to activation of imidazoline receptor, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) 30 min before the training. The mice were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 23–28$, $^{*}P<0.05$ compared to the control; $^{\#}P<0.05$ compared to the agmatine treated group. B. The relationship between idazoxan and agmatine on memory formation and memory retrieval. To study whether the effect of agmatine on memory retrieval was related to imidazoline receptor, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) 30 min before the test. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 9–13$, $^{*}P<0.05$ compared to control; $^{\#}P<0.05$ compared to the agmatine treated group.

retrieval. The latency of the mice in the control group was 190.90 s while that of the mice in the agmatine (10 and 20 mg/kg) treated groups was 269.23–277.14 s, which was significantly prolonged compared with that of the control ($u = 2.24$; $P<0.05$).

3.2. Idazoxan inhibited the enhancement of agmatine on memory formation and retrieval

Fig. 2A shows the latencies for the mice to step down the platform pre-treated with idazoxan (5 mg/kg, i.p.) and agmatine (10 mg/kg, s.c.) before the training session, indicating that idazoxan inhibited the enhancement of agmatine on memory formation. The latency of the mice in the control group was 232.48 s while that in the agmatine 10 mg/kg treated groups was 288.88 s, which was significantly prolonged as compared to that in the control ($u = 2.03$; $P<0.05$). Idazoxan (5 mg/kg, i.p.). Pre-treatment significantly inhibited the effect of agmatine, for the latency decreased from 288.88 to 234.04 s ($u = 1.99$; $P<0.05$).

Fig. 2B shows the latency for the mice to step down the platform after pre-test idazoxan (5 mg/kg, i.p.) and agmatine (10 mg/kg, s.c.) treatments, indicating that idazoxan inhibited the enhancement of agmatine on memory retrieval. The latency of the mice in the control group was 219.89 s. Idazoxan (5 mg/kg) alone had no effect on memory retrieval compared to control, the latency is 202.69 s. Agmatine significantly prolonged the latency to 293.69 s ($u = 2.77$; $P<0.01$). Idazoxan (5 mg/kg, i.p.) pre-treatment significantly inhibited the effect of agmatine, as the latency was decreased from 293.69 s to 221.45 ($u = 2.18$; $P<0.05$).

3.3. Morphine impaired memory formation but not memory consolidation and retrieval

The latency for the mice to step down the platform pre-treated with morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) before the training session was shown in Fig. 3A, indicating that morphine impaired memory formation. The latency of the mice in the control group was 240.60 s, while that of the mice in the morphine (2.5 and 5 mg/kg) treated groups was 132.50–100.14 s, significantly different from that of the control ($u = 2.20$; $P<0.05$).

The latency for the mice to step down the platform pre-treated with morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) after the training session was shown in Fig. 3B, indicating that morphine did not influence memory consolidation. The latency of the mice was 258.13 s in the control group and 217.22–274.62 s in the morphine (1.25, 2.5 and 5 mg/kg) treated groups without significant difference.

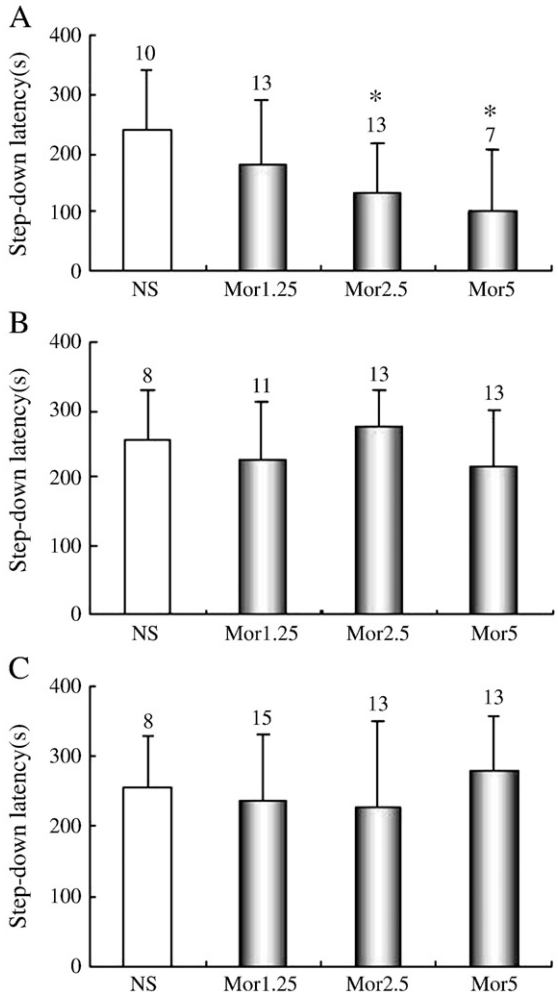

Fig. 3. A. Effect of acute morphine treatment on the formation of memory. To study the effect of morphine on memory formation, the mice were trained 30 min after morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) administration and were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 7–13$, $^{*}P<0.05$ compared to the control. B. Effect of acute morphine treatment on the consolidation of memory. To study the effect of morphine on memory consolidation, morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) was administrated 30 min after the training session and the mice were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 8–13$. C. Effect of acute morphine treatment on the retrieval of memory. To study the effect of morphine on memory retrieval, morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) was administrated 30 min before the test 24 h after the training session. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 8–15$.

Fig. 3C shows the latency for the mice to step down the platform pre-treated with morphine (0, 1.25, 2.5 and 5 mg/kg, s.c.) before the test, indicating that morphine did not influence memory retrieval. The latency of the mice was 258.13 s, in the control group but 229.00–278.77 s in the morphine (1.25, 2.5 and 5 mg/kg) treated groups without much discrepancy.

3.4. Pre-training agmatine administration inhibits morphine-induced memory impairment

The latency for the mice to step down the platform pre-treated with agmatine (5, 10 and 20 mg/kg, s.c.) and morphine (5 mg/kg, s.c.) before the training session was shown in Fig. 4A, indicating that pre-training agmatine administration inhibited morphine-induced memory formation impairment. The latency of the mice in the control group was 203.72 s, compared with 91.42 s in the morphine (5 mg/kg) treated group, which was significantly shorter than that of the control ($u = 2.86$; $P<0.05$). The latency of the mice in agmatine (10 and 20 mg/kg) and morphine (5 mg/kg) treated group was 184.54–196.84 s, significantly different from that of the morphine treated groups ($u = 2.01$; $P<0.05$).

Pre-training idazoxan treatment inhibited the effect of agmatine on morphine-induced memory formation impairment. The latency of the mice in the control group was 258.90 s and that of morphine (5 mg/kg) treated mice was147.00 ± 115.74 s, which was significantly shorter than that of the control ($u = 2.49$; $P<0.05$). The latency of the agmatine (10 mg/kg) and morphine (5 mg/kg) treated mice was 235.00 s, which was significantly different from that of the morphine treated group ($u = 2.00$; $P<0.05$). Pre-training idazoxan (5 mg/kg) treatment did not influence the memory impairment induced by morphine 10 mg/kg, but significantly inhibited the effect of pre-training agmatine treatment on morphine induced memory impairment. The latency of the mice treated with idazoxan + agmatine + morphine was 102.00 s, which was different from that of the agmatine + morphine treated group ($u = 3.01$; $P<0.01$). See Fig. 4B.

3.5. Pre-test agmatine administration inhibits morphine-induced memory impairment

The mice pre-treated with morphine (5 mg/kg, s.c.) before the training session were injected with agmatine (5, 10 and 20 mg/kg, s.c.) 30 min before the test. The result indicated that pre-test agmatine treatment inhibited morphine-induced memory formation impairment. The latency of the mice in the control group was 195.29 s as against 84.50 s in the morphine (5 mg/kg) treated group, which was

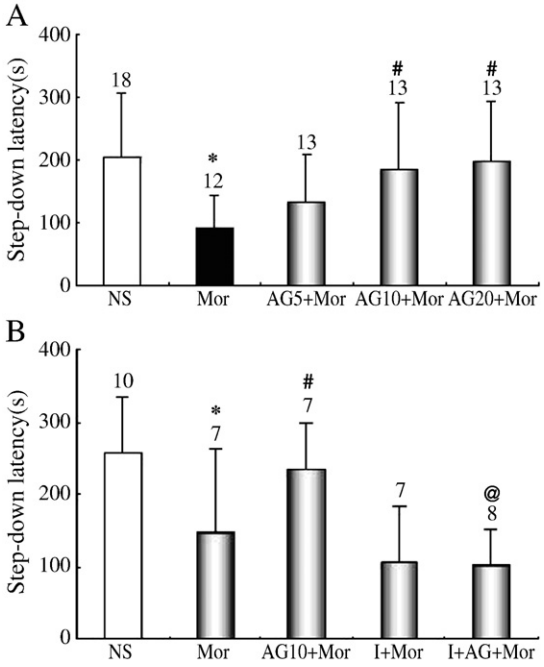

Fig. 4. A. Effect of pre-training agmatine treatment on morphine-induced memory formation impairment. To study the effect of pre-training agmatine treatment on morphine-induced memory impairment, the mice in the control group received saline plus saline (10 ml/kg), the mice in the other four groups received agmatine (0, 5, 10 or 20 mg/kg, s.c.) 30 min before morphine (5 mg/kg, s.c.) administration. 30 min after morphine injection, the mice were trained in the step-down inhibitory avoidance task. The mice were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 12–18$, $^{*}P<0.01$ compared to control; $^{\#}P<0.05$ compared to morphine. B. Effect of idazoxan on the pre-training agmatine treated mice. To study whether the effect of agmatine on morphine-induced memory impairment was related to imidazoline receptor, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) and morphine (5 mg/kg, s.c.) 30 min before the training. The mice were tested 24 h later. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n = 7–10$, $^{*}P<0.01$ compared to the control; $^{\#}P<0.05$ compared to morphine; $^{@}P<0.01$ compared to the agmatine + morphine treated group.

significantly different from that of the control ($u=2.88$; $P<0.05$). The latency of the mice in agmatine (10 and 20 mg/kg) + morphine (5 mg/kg) pre-treated group was 125.46–169.23 s, which was significantly different from that of the morphine treated group ($u=2.04$; $P<0.01$). See Fig. 5A.

Pre-test idazoxan treatment inhibited the effect of agmatine on morphine-induced memory formation impairment. The latency of the mice in the control group was 244.80 s compared to 90.43 s of the morphine (5 mg/kg) treated mice, which was significantly different from that of the control ($u=2.93$; $P<0.01$). The latency of the morphine (5 mg/kg) + agmatine (10 mg/kg) treated mice was 177.33 s, which was significantly different from that of the morphine treated group ($u=2.20$; $P<0.05$). Pre-test idazoxan (5 mg/kg) did not influence the impairment induced by morphine 10 mg/kg but significantly inhibited the effect of pre-test agmatine treatment on morphine induced memory impairment. The latency of the mice treated with morphine + idazoxan + agmatine was 95.14 s, which was different from that of the morphine + agmatine treated group ($u=2.08$; $P<0.05$). See Fig. 5B.

4. Discussion

There is growing evidence that agmatine inhibits tolerance to morphine (Fairbanks and Wilcox, 1997) and attenuates behavioral signs of morphine abstinence syndrome *in vitro* and *in vivo*. This study provided evidence for the modulation of agmatine on morphine-induced memory impairment and shed light on the possible mechanisms. The results suggested that acute agmatine (5, 10 and 20 mg/kg) treatment facilitated the formation and retrieval of memory which could be antagonized by idazoxan. As in other studies, acute morphine (5 mg/kg) treatment impaired the formation of memory. Pre-training and pre-test agmatine treatment inhibited the memory impairment induced by morphine, and these effects were also antagonized by idazoxan.

As a neurotransmitter and/or modulator, agmatine plays an important role in CNS. Systemic agmatine treatment was found to facilitate memory consolidation in inhibitory avoidance tasks (Arteni et al., 2002). The mechanism involved the activation of imidazoline receptors in the tractus solitarii (NST)–nucleus paragigantocellularis (PGi)–locus coeruleus (LC) pathway. However, the effect of agmatine on memory was not consistent. McKay found that agmatine (1, 5, 10 and 50 mg/kg, i.p.) did not affect latencies for the mice to find the hidden platform or preference for the training quadrant during probe trials. When administered 20 min prior to contextual or auditory-cued fear-conditioning sessions, agmatine evoked a dose-dependent impairment in the magnitude of learned fear to the contextual stimuli when assessed during motor activity. Agmatine (50 mg/kg) along with malaise-evoking agent following presentation to a novel sucrose solution abolished learned taste aversions, which suggested that agmatine selectively impairs behavioral inferences of specific types of learning and memory related to specific brain regions (Mckay et al., 2002). Unlike previous studies, we found that acute agmatine treatment facilitated the formation and retrieval of memory; but had no effect on the consolidation of memory in the present study. Such discrepancy could be due to the different animal model used, different experimental conditions and different drug treatment in the studies.

Ferry and McGaugh (2008) found that consolidation of inhibitory avoidance memory depends critically on prolonged activation of the noradrenergic system in the basolateral nucleus of the amygdala and indicated that this modulatory effect is mediated, in part, by pre-synaptic α2-adrenoceptors. Namely, α2-adrenoceptors facilitate consolidation of memory. However, many studies failed to demonstrate any physiological effect of agmatine by binding to α2-adrenoceptors (Pineda, 1996; Pinthong, 1995). Other investigators have documented memory enhancement of NMDA receptor agonists in different experimental models (Flood et al., 1990; Myhrer and Paulsen, 1992). On the other hand, it has been reported that both the competitive and non-competitive NMDA receptor antagonists impair learning and memory in various behavioral tasks (Ward et al., 1990; Parada et al., 1992; Maurice et al., 1994). To explain the cellular action of NMDA receptor antagonists, it was suggested that the memory impairment induced by NMDA receptor antagonists was due to their influence on NO and subsequent cGMP production in the brain (Yamada et al., 1996a, b). Agmatine was found to antagonize NMDA receptors, inhibit NOS, and block calcium channels, which all play an important role in learning and memory. Based on the above analysis, it can be asserted that agmatine might also impair learning and memory. However, our results indicated that agmatine facilitated memory formation. Therefore, the effect of agmatine on learning and memory might be mediated by other targets, such as imidazoline receptors. It was once proposed that drug addiction is an aberrant form of learning, mediated by maladaptive recruitment of certain memory systems in the brain (Robbins and Everitt, 1999). In agreement with previous reports (Valili et al., 2004; Zarrindast et al., 2006), we found that pre-training administration of morphine impaired formation of memory. Many studies have reported the effects of agmatine in inhibiting morphine withdrawal symptoms (Aricioglu-Kartal, 1997; Li et al., 1998; Aricioglu et al., 2003a, b), drug discrimination (Su et al., 2008), conditioned place preference (Wei et al., 2005), and drug self-administration (Su et al., 2009), but the underlying mechanisms are unclear. Since agmatine binds to many targets that play important roles in learning and memory, the effect of agmatine in inhibiting morphine dependence might be associated with

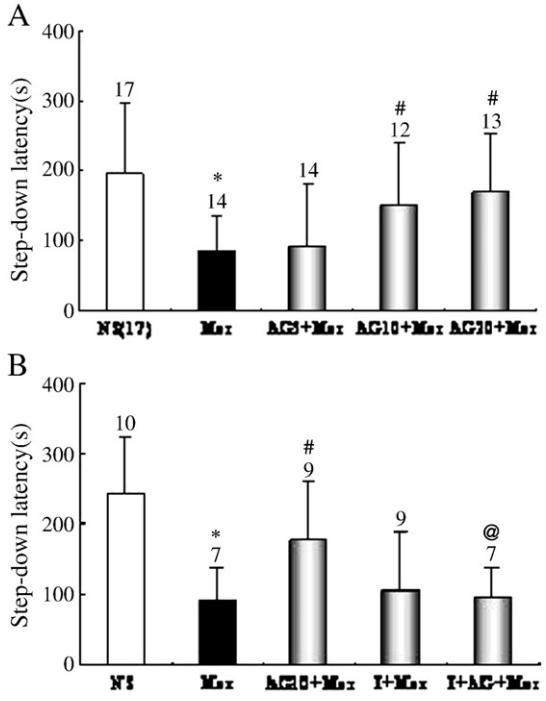

Fig. 5. A. Effect of pre-test agmatine administration on morphine-induced memory impairment. To study the effect of pre-test agmatine treatment on morphine-induced memory impairment, mice in the control group received saline (10 ml/kg), the mice in the other four groups received morphine (5 mg/kg, s.c.) 30 min before the training session. On the test day, the mice pre-treated with morphine were injected with agmatine (0, 5, 10, 20 mg/kg, s.c.) 30 min before the test. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n=12$–17, $^{*}P<0.01$ compared to the control; $^{\#}P<0.05$ compared to morphine. B. Effect of idazoxan on the pre-test agmatine treated mice. To study whether the effect of agmatine on morphine-induced memory impairment was related to imidazoline receptors, idazoxan (5 mg/kg, i.p.) was co-administrated with agmatine (10 mg/kg, s.c.) 30 min before the test. The latencies for the mice to step down the platform were expressed as mean ± S.D. $n=7$–10, $^{*}P<0.01$ compared to the control; $^{\#}P<0.05$ compared to morphine; $^{@}P<0.05$ compared to the morphine + agmatine treated group.

modulation on learning and memory. In our study, agmatine facilitated the formation and retrieval of memory in morphine-treated mice, and imidazoline receptor antagonist idazoxan inhibited the effect of agmatine. Thus, we can make the hypothesis that agmatine modulates memory formation impairment induced by morphine and that the mechanism is related to activation of imidazoline receptors. Agmatine can increase the concentration of beta-endorphin by activation of imidazoline I (2A) receptors (Chang et al., 2010), which plays an important role in morphine treated animals (Izquierdo, 1985). However, the effect of agmatine on the memory of chronic morphine-treated mice remains unclear and needs to be further studied.

In conclusion, agmatine facilitated memory formation and retrieval in the step-down inhibitory avoidance test. Acute morphine treatment impaired memory formation in the same model. Pre-training and pre-test agmatine treatment reversed the impairment of morphine on memory formation through activation of imidazoline receptors.

Acknowledgements

This project was supported by the National Basic Research Program of China (Nos. 2003CB515400 and 2009CB522000), National Natural Science Foundation of China (No. 30930040/C140209) and National Key Technology R&D Program of China (No. 2007BAI07B02).

References

Aricioglu-Kartal F, Uzbay IT. Inhibitory effect of agmatine on naloxone-precipitated abstinence syndrome. Life Sci 1997;61:1775–81.
Aricioglu F, Ercil E, Dulger G. Agmatine inhibits naloxone induced contractions in morphine-dependent guinea pig ileum. Ann NY Acad Sci 2003;1009:147–51.
Aricioglu F, Korcegez E, Bozkurt A. Effect of agmatine on acute and mononeuropathic pain. Ann NY Acad Sci 2003;1009:106–15.
Aricioglu F, Means A, Regunathan S. Effect of agmatine on the development of morphine dependence in rats: potential role of cAMP system. Eur J Pharmacol 2004;504: 191–7.
Arteni NS, Lavinsky D, Rodrigues AL, et al. Agmatine facilitates memory of an inhibitory avoidance task in adult rats. Neurobiol Learn Mem 2002;78:465–9.
Chang CH, Wu HT, Cheng KC, et al. Increase of beta-endorphin secretion by agmatine is induced by activation of imidazoline I (2A) receptors in adrenal gland of rats. Neurosci Lett 2010;468:297–9.
Fairbanks CA, Schreiber KL, Brewer KL, et al. Agmatine reverses pain induced by inflammation, neuropathy, and spinal cord injury. Proc Natl Acad Sci 2000;97: 10584–9.
Fairbanks CA, Wilcox GL. Acute tolerance to spinally administered morphine compares mechanistically with chronically induced morphine tolerance. J Pharmacol Exp Ther 1997;282:1408–17.
Ferry B, McGaugh JL. Involvement of basolateral amygdala alpha2-adrenoceptors in modulating consolidation of inhibitory avoidance memory. Learn Mem 2008;15: 238–43.
Flood JF, Baker ML, Davis JL. Modulation of memory processing by glutamic acid receptor agonists and antagonists. Bain Res. 1990;521:197–202.
Galea E, Regunathan S, Eliopoulos V, et al. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem J 1996;316:247–9.
Introini IB, McGaugh JL, Baratti CM. Pharmacological evidence of a central effect of naltrexone, morphine, and beta-endorphin and a peripheral effect of met- and leu-enkephalin on consolidation of an inhibitory response in mice. Behav Neural Biol 1985;44:434–46.
Itoh J, Ukai M, Kameyama T. Dynorphin A-(1–13) potently improves the impairment of spontaneous alternation performance induced by the mselective opioid receptor agonist DAMGO in mice. J Pharmacol ExpTher 1994;269:15–21.
Izquierdo I, Netto CA. The brain beta-endorphin system and behavior: the modulation of consecutively and simultaneously processed memories. Behav Neural Biol 1985;44:249–65.
Jafari MR, Zarrindast MR, Djahanguiri B. Influence of cholinergic system modulators on morphine state-dependent memory of passive avoidance in mice. Physiol Behav 2006;88:146–51.
Jafari-Sabet M, Zarrindast MR, Rezayat M, et al. The influence of NMDA receptor agonist and antagonist on morphine state-dependent memory of passive avoidance in mice. Life Sci 2005;78:157–63.
Kalra SP, Pearson E, Sahu A, et al. Agmatine, a novel hypothalamic amine, stimulates pituitary luteinizing hormone release in vivo and hypothalamic luteinizing hormone-releasing hormone release in vitro. Neurosci Lett 1995;194:165–8.
Karadag HC, Ulugol A, Tamer M, et al. Systemic agmatine attenuates tactile allodynia in two experimental neuropathic pain models in rats. Neurosci. Lett 2003;339:88–90.
Kolesnikov Y, Jain S, Pasternak GW. Modulation of opioid analgesia by agmatine. Eur J Pharmacol 1996;296:17–22.
Li G, Regunathan S, Barrow CJ, et al. Agmatine: an endogenous clonidine-displacing substance in the brain. Science 1994;263:966–9.
Li J, Li X, Pei G, et al. Agmatine inhibited tolerance to and dependence on morphine in guinea pig ileum in vitro. Zhongguo Yao Li Xue Bao 1998;19:564–8.
Ma MX, Chen YM, He J, et al. Effects of morphine and its withdrawal on Y-maze spatial recognition memory in mice. Neuroscience 2007;147:1059–65.
Maurice T, Hiramatsu M, Itoh J, et al. Behavioral evidence for a modulating role of sigma ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-induced amnesia. Brain Res 1994;647:44–56.
McKay BE, Lado WE, Martin LJ, et al. Learning and memory in agmatine-treated rats. Pharmacol Biochem Behav 2002;72:551–7.
Myhrer T, Paulsen RE. Memory dysfunction following disruption of glutamergic systems in the temporal region of the rat: effects of agonistic amino acids. Bain Res 1992;599:345–52.
Onal A, Delen Y, Ulker S, et al. Agmatine attenuates neuropathic pain in rats: possible mediation of nitric oxide and noradrenergic activity in the brainstem and cerebellum. Life Sci 2004;73:413–28.
Parada J, Czuezwar SJ, Turski WA. NBQX dose not affect learning and memory tasks in mice: a comparison with D-CPPene and ifenprodil. Brain Res Cogn Brain Res 1992;1: 67–71.
Piletz JE, Chikkala DN, Ernsberger P. Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and alpha-2 adrenergic receptors. J Pharmacol Exp Ther 1995;272:581–7.
Pineda J, Ruiz-Ortega JA, Martin-Ruiz R, et al. Agmatine does not have activity at α2-adrenoceptors which modulate the firing rate of locus coeruleus neurons: an electrophysiological study in rat. Neurosci Lett 1996;219:103–6.
Pinthong D, Wright IK, Hanmer C, et al. Agmatine recognizes α2-adrenoceptor binding sites but neither activates nor inhibits α2-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 1995;351:10–6.
Pu L, Bao GB, Xu NJ, et al. Hippocampal long-term potentiation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates. J Neurosci 2002;22:1914–21.
Ragozzino ME, Gold PE. Task-dependent effects of intra-amygdala morphine injections: attenuation by intra-amygdala glucose injections. J Neurosci 1994;14:7478–85.
Reis DJ, Regunathan S. Is agmatine a novel neurotransmitter in brain? Trends Pharmacol Sci 2000;21:187–93.
Regunathan S, Feinstein DL, Raasch W, et al. Agmatine (decarboxylated arginine) is synthesized and stored in astrocytes. Neuroreport. 1995;6:1897–900.
Regunathan S, Piletz JE. Regulation of inducible nitric oxide synthase and agmatine synthesis in macrophages and astrocytes. Ann N Y Acad Sci 2003;1009:20–9.
Robbins TW, Everitt BJ. Drug addiction: bad habits add up. Nature. 1999;398:567–70.
Saha N, Datta H, Sharma PL. Effects of morphine on memory: interactions with naloxone, propranolol and haloperidol. Pharmacology 1991;42:10–4.
Stone WS, Walser B, Gold SD, et al. Scopolamine- and morphine-induced impairments of spontaneous alternation performance in mice: reversal with glucose and with cholinergic and adrenergic agonists. Behav Neurosci 1991;105:264–71.
Su RB, Wang WP, Lu XQ, et al. Agmatine blocks acquisition and re-acquisition of intravenous morphine self-administration in rats. Pharmacol Biochem Behav 2009;92(4):676–82.
Su RB, Ren YH, Liu Y, et al. Effect of agmatine on morphine induced discrimination in rats. Eur J Pharmacol 2008;593(1–3):62–7.
Vaccarino AL, Olson GA, Olson RD, et al. Endogenous opiates. Peptides 1998;20: 1527–74 1999.
Valili A, Tayebi K, Jafari MR, et al. Effect of ethanol on morphine state-dependent learning in the mouse: involvement of GABAergic, opioidergic and cholinergic systems. Alcohol Alcohol 2004;39:427–32.
Walker DL, McGlynn T, Grey C, et al. Naloxone modulates the behavioral effects of cholinergic agonists and antagonists. Psychopharmacology (Berl). 1991;105: 57–62.
Ward L, Manson SE, Abraham WC. Effects of the NMDA antagonists CPP and MK-801 on radial arm maze performance in rats. Pharmacol Biochem Behav. 1990;35:785–90.
Wei XL, Su RB, Yuan BL, et al. Inhibition by agmatine on morphine-induced conditioned place preference in rats. Eur J Pharmacol 2005;515(1–3):99-106.
Yamada K, Noda Y, Hasegawa T, et al. The role of nitric oxide in dizocilpine-induced impairment of spontaneous alternation behavior in mice. J Pharmacol Exp Ther 1996a;276:460–6.
Yamada K, Hiramatsu M, Noda Y, et al. Role of nitric oxide and cyclic GMP in the dizocilpine-induced impairment of spontaneous alternation behavior in mice. Neuroscience 1996b;74:365–74.
Yang XC, Reis DJ. Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J Pharmacol Exp Ther 1999:288.
Yesilyurt O, Uzbay IT. Agmatine potentiates the analgesic effect of morphine by an alpha(2)-adrenoceptor-mediated mechanism in mice. Neuropsychopharmacology 2001;25:98-103.
Zarrindast MR, Fazli-Tabaei S, Ahmadi S, et al. Effect of lithium on morphine state-dependent memory of inhibitory avoidance in mice. Physiol Behav 2006;87: 409–15.
Zheng JQ, Weng XC, Gai XD, et al. Mechanism underlying blockade of voltage-gated calcium channels by agmatine in cultured rat hippocampal neurons. Acta Pharmacol Sin 2004;25:281–5.