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Agmatine Potentiates Morphine-Induced Conditioned Place Preference in Mice: Modulation by Alpha(2)-Adrenoceptors

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The effects of agmatine, an endogenous polyamine metabolite formed by decarboxylation of L-arginine, and its combination with morphine on conditioned place preference (CPP) has been investigated in male mice. Our data show that subcutaneous administration of morphine (1–7.5 mg/kg) significantly increases the time spent in the drug-paired compartment in a dose-dependent manner. Intraperitoneal administration of agmatine (1–40 mg/kg) alone does not induce either CPP or conditioned place aversion, while combination of agmatine and subeffective doses of morphine leads to potent rewarding effects. Lower doses of morphine (0.1, 0.05, and 0.01 mg/kg) are able to induce CPP in mice pretreated with agmatine 1, 5, and 10 mg/kg, respectively. Concomitant intraperitoneal administration of UK 14 304 (0.5 mg/kg), a highly selective α_2-agonist, with *per se* noneffective dose of morphine (0.5 mg/kg) and also its combination with noneffective doses of agmatine (1 mg/kg) plus morphine (0.05 mg/kg) produces significant CPP. UK 14 304 (0.05, 0.5 mg/kg) alone, or in combination with agmatine (1, 5 mg/kg) have had no effect. We have further investigated the possible involvement of the α_2-adrenoceptors in the potentiating effect of agmatine on morphine-induced place preference. Selective α_2-antagonists, yohimbine (0.005 mg/kg) and RX821002 (0.1, 0.5 mg/kg), block the CPP induced by concomitant administration of agmatine (5 mg/kg) and morphine (0.05 mg/kg). Yohimbine (0.001–0.05 mg/kg) or RX821002 (0.05–0.5 mg/kg) alone or in combination with morphine (0.05 mg/kg) or agmatine (5 mg/kg) fail to show any significant place preference or aversion. Our results indicate that pretreatment of animals with agmatine enhances the rewarding properties of morphine via a mechanism which may involve α_2-adrenergic receptors.
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INTRODUCTION

Agmatine, a polycationic amine synthesized via decarboxylation of L-arginine by arginine decarboxylase, while long recognized to be synthesized and stored in plants, bacteria, and invertebrates (Tabor and Tabor, 1984), has recently been described as a putative neurotransmitter in mammals (Reis and Regunathan, 1998, 2000). It has been suggested that agmatine acts as an endogenous agonist at imidazoline receptors and as a noncatecholamine ligand at α_2-adrenergic receptors (Li *et al*, 1994; Piletz *et al*, 1995). Agmatine and α_2-adrenoceptors have important functional interactions, including the potentiating effect on morphine-induced analgesia (Fairbanks *et al*, 2000; Yesilyurt and Uzbay, 2001), agonistic activity in prejunctional rat tail artery (Gonzalez *et al*, 1996), and multiple effects on sympathetic neurotransmission in rat vas deferens (Jurkiewicz *et al*, 1996). Involvement of α_2-adrenoceptors in anticonvulsant properties of agmatine has been recently shown (Demehri *et al*, 2003).

Several studies have shown that α-adrenergic and opioid systems can interact in a complex manner. The noradrenergic system has been shown to be involved in the development and expression of opioid dependence (Maldoado, 1997). Clonidine attenuates some of the signs of morphine withdrawal in rats (Kosten, 1994) as well as signs of morphine withdrawal in humans (Gold *et al*, 1987). Acute administration of α_2-adrenoceptor antagonist yohimbine increases the physical effects of morphine withdrawal in rats, suggesting that α_2-adrenoceptors are involved in the development of physical dependence to opioids (Dwoskin *et al*, 1983; Iglesias *et al*, 1992). It has also been reported that the acquisition of conditioned opioid withdrawal in rats may be blocked by clonidine (Schulteis *et al*, 1998). In the

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light of these reports, one can expect that agmatine, a ligand that can bind to imidazoline/α_2-adrenergic receptors, should also have significant interactions with morphine. Interestingly, recent evidence supports a neuromodulatroy interaction between agmatine and opioids in which agmatine potentiates some opioid-induced effects. The beneficial potentiating effects of agmatine on morphine analgesia (Kolesnikov *et al*, 1996; Yesilyurt and Uzbay, 2001; Ruiz-Durantez *et al*, 2003) are clearly documented. Agmatine has been shown to reduce the development of dependence to morphine (Aricioglu *et al*, 2004) and attenuates the contractile response to naloxone in morphine-dependent guinea-pig ileum (Aricioglu *et al*, 2003). This latter effect is partly abolished by pretreatment with yohimbine and is almost completely abolished by idazoxan. Furthermore, agmatine attenuates withdrawal syndrome in morphine-dependent rats (Aricioglu *et al*, 2003; Aricioglu-Kartal and Uzbay, 1997) and decreases fentanyl self-administration (Morgan *et al*, 2002).

Yesilyurt and Uzbay (2001) have reported that agmatine potentiates the antinociceptive action of morphine through α_2-adrenoceptors. The involvement of α_2-adrenoceptors in other central effects of agmatine and in its interaction with opioids has been also reported by others (Aricioglu *et al*, 2003; Roerig, 2003; Zomkowski *et al*, 2002; Ruiz-Durantez *et al*, 2003). We have recently shown that agmatine potentiates the anticonvulsant effect of morphine, by a yohimbine-reversible mechanism (Riazi *et al*, 2005). While the effects of agmatine on morphine-induced analgesia, tolerance, dependence, and seizure threshold alteration have been shown in several studies, the possible effect of agmatine on reward system and its interaction with morphine in this regard has not yet been investigated.

Conditioned place preference (CPP) has been widely used to assess the rewarding effect of different systems including opioids (Tzschentke, 1998). The test is based upon the principle that, when a primary reinforcer is paired with a contextual stimulus, the contextual stimulus can acquire secondary reinforcing properties. These secondary reinforcing properties, which are presumably established due to a Pavlovian contingency, are thought to be capable of eliciting an operant approach response or place preference which results in a significant increase in the time spent in the drug-paired place. Morphine has been shown to produce a significant and dose dependent effect on the magnitude of place preference (Tzschentke, 1998; Langroudi *et al*, 2005). Likewise, the effects of various drugs on acquisition and expression of morphine-induced place preference have also been assessed (Bardo *et al*, 1984; Bardo and Bevins, 2000). Considerable evidence indicates that α_2-adrenergic agonists and antagonists can induce CPP or conditioned place aversion (CPA; see review Tzschentke, 1998). The α_2-agonist clonidine is shown to produce CPP over a range of doses (Asin and Wirtshafter, 1985; Tierney *et al*, 1988), while the α_2 antagonist yohimbine has been found to produce CPA (File, 1986). Also increasing evidence indicates the effect of α_2-adrenergics on opioid-induced CPP. Clonidine blocks the rewarding effects of morphine in rats withdrawn from morphine; however, it has no effect on morphine-induced CPP in drug naïve animals (Nader and Van der Kooy, 1996). Yohimbine is shown to limit the preference induced by morphine in male rats (Morales *et al*, 2001).

In the present study, we have investigated the effect of agmatine on the acquisition of morphine-induced CPP. Moreover, we have assessed the possible involvement of α_2-adrenoceptors using selective α_2-adrenoceptor agonist, UK 14 304 and selective α_2-antagonists, yohimbine and RX821002.

MATERIALS AND METHODS

Animals

Male NMRI mice (Pasteur Institute of Iran), weighing 20–30 g were used. The animals were housed six per cage in a temperature-controlled ($22 \pm 3^{\circ}$C) colony room. They were maintained in a 12 h on and 12 h off light/dark schedule with *ad libitum* food and water except during experimental procedures. All trials were carried out in the light phase. Subjects were experimentally naïve. Animals were allowed 7 days to acclimatize to the laboratory environment before testing began. Attention was paid to the ethical guidelines for investigations of experimental pain in conscious animals. The protocol had been approved by the committee of ethics of the faculty of Sciences of Tehran University (357; 8 November 2000).

Drugs

The drugs used in the present study were morphine sulfate (Temad Pharmaceutical, Tehran, Iran), agmatine sulfate, RX821002, UK 14 304 (Sigma, Germany), and yohimbine (Sigma, UK). The UK 14 304 was initially dissolved in dimethylsulfoxide (DMSO) and further diluted in saline until the adequate mixture (0.5% DMSO-saline, v/v) was reached. All other drugs were dissolved in sterile physiological saline solution to such concentrations that requisite doses were administered in a volume of 10 ml/kg. Morphine was injected subcutaneously (s.c.), while others were administered intraperitoneally (i.p.) in all experiments. Appropriate vehicle controls (saline or 0.5% DMSO-saline) were performed for each experiment.

Place Preference Apparatus

Two-compartment place preference apparatus were used. Place conditioning was conducted using a biased procedure. The apparatus were made of wood and consisted of two square-based compartments ($15 \times 15 \times 30H$ cm each). In order to distinguish the two compartments, visual and sensory texture cues were used; one compartment was painted in black and the other compartment was painted white. A black texture covered the floor of the black compartment. During the conditioning phases, the two compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. In such apparatus, mice preferred the black compartment significantly.

Experimental Procedure

Measurement of CPP. CPP consisted of three phases: familiarization and preconditioning, conditioning, and postconditioning.

Familiarization and preconditioning. On the first day of the trials (ie, familiarization) and the second day (ie, preconditioning), each mouse was placed separately into the apparatus for 10 min, while they could freely access both compartments. The time spent in each compartment was recorded on the preconditioning day. Placement in each compartment was considered as placement of the front paws and the head. The preconditioning score was measured as the subtraction of the preferred compartment staying time from the nonpreferred compartment staying time. After the test, the animals were grouped randomly.

Conditioning. This phase consisted of six conditioning sessions held in six consecutive days. The duration of each session was 40 min and the mice were confined to the considered compartment, by isolating the compartment using a removable partition. Animals received morphine, agmatine, UK 14 304, and all drug combinations in the nonpreferred compartment (defined in the preconditioning phase, which was the white compartment in our experiments), while yohimbine and RX821002 alone were administered in the preferred compartment on days 1, 3, and 5 of the conditioning phase. Vehicles were injected in the opposite compartment on days 2, 4, and 6 of the conditioning phase.

Postconditioning. This phase was carried out in the ninth day of the trials (24 h after the last conditioning session, with no preceding injections) in a drug-free state. As in the preconditioning phase, the partition was raised and the animals were placed in the apparatus for 10 min, with free access to both compartments and the time spent in each compartment was recorded in real time. Experimenters were blind to groups and treatments. The postconditioning score was measured in the same way as the preconditioning score. The difference between post- and preconditioning scores was considered as change in preference score (CIP).

Measurement of locomotor activity. Locomotion was measured, based on a method used previously by Tzschentke and Schmidt (1997), during the test sessions (Belzung and Barreau, 2000) in the drug-paired compartment with slight modifications. To measure the locomotor activity, the ground areas of the two compartments were divided into two equal segments by a transverse line and locomotion for each animal was measured as the number of crossings from one-half to the other over 10 min testing in the postconditioning day by a separate experimenter blind to groups and treatments. None of the drugs and treatments that were used in these experiments altered locomotor activity.

CPP Experimental Design

Dose–response effects of place conditioning produced by morphine. In this experiment, we established a dose–response function for morphine place conditioning. Five different doses of morphine sulfate (0.5, 1, 2.5, 5, and 7.5 mg/kg) were tested for producing place preference. Animals received morphine immediately before confinement to the conditioning apparatus. A control group that received saline (10 ml/kg, s.c.) in all sessions was included in order to confirm that the injection and conditioning schedule did not affect the time spent in the compartments.

Effects of agmatine on the acquisition of place preference conditioning per se *and in the presence of morphine*. To assess the effect of agmatine on the acquisition of place preference, five doses of agmatine (1, 5, 10, 20, and 40 mg/kg) were injected 30 min prior to placement in the nonpreferred compartment, under the schedule described above. One additional group received saline (10 ml/kg, i.p.) 30 min prior to placement in the apparatus and served as a control.

In order to investigate the interaction of agmatine and morphine on the acquisition of CPP, 16 groups of animals received different doses of agmatine (1, 5, and 10 mg/kg) or saline (10 ml/kg, i.p.) 30 min before the administration of the various noneffective doses of morphine (0.01, 0.05, 0.1, and 0.5 mg/kg), obtained from morphine dose–response experiment, under the schedule.

Effect of UK 14 304, and its combination with agmatine on morphine-induced place preference. This part consisted of two experiments. In the first experiment, nine groups of animals received two doses of selective α_2-agonist, UK 14 304 (0.1 and 0.5 mg/kg) and its vehicle (10 ml/kg, i.p.) 35 min before the administration of morphine (0.05 and 0.5 mg/kg) or saline (10 mg/kg, s.c.) under the schedule. In the second experiment, in order to study the effect of coadministration of *per se* noneffective doses of UK 14 304 and agmatine on the acquisition of place preference induced by morphine, six groups of animals received UK 14 304 (0.1 and 0.5 mg/kg) or its vehicle (10 ml/kg, i.p.) 5 min before the administration of agmatine (1 mg/kg). Animals received morphine sulfate (0.05 mg/kg) or saline (10 ml/kg, s.c.) 30 min after administration of agmatine and were placed in the apparatus immediately, under the schedule.

Effect of yohimbine or RX821002 on the acquisition of place preference conditioning in the presence of morphine or/and agmatine. In this part of experiment, effects of coadministration of yohimbine or RX821002 plus morphine on the acquisition of place preference were studied. A total of 18 groups of mice (6–8 per group) received yohimbine (0.001 and 0.005 mg/kg), RX821002 (0.05, 0.1, and 0.5 mg/kg) or saline (10 ml/kg, i.p.) 35 min before the administration of morphine (0.05, 0.5, and 5 mg/kg) under the schedule. Mice were placed in the apparatus immediately after morphine injection.

Effects of coadministration of yohimbine or RX821002 and agmatine on the acquisition of place preference were also examined. In this experiment, mice (5–8 per group) received yohimbine (0.001, 0.005 mg/kg) or saline (10 ml/kg, i.p.) 5 min before the administration of agmatine (5 mg/kg). In another set of animals, RX821002 (0.05, 0.1 and 0.5 mg/kg) or saline (10 ml/kg, i.p.) were injected 5 min prior to agmatine (5 mg/kg) injection. Animals were then placed in the apparatus 30 min after injection of agmatine under the schedule.

Two other experiments were designed to observe the possible involvement of α_2-adrenoceptors in agmatine influence on morphine-induced place preference. Yohimbine (0.001 and 0.005 mg/kg) or saline (10 ml/kg, i.p.) plus agmatine (5 mg/kg) were injected 35 and 30 min prior to subcutaneous administration of morphine (0.05 mg/kg),

respectively. A group receiving two intraperitoneal saline injections 35 and 30 min prior to morphine (0.05 mg/kg) administration was also included. Animals were immediately placed in the apparatus following the schedule.

In the next experiment, more selective α_2-antagonist with very low affinity for imidazoline receptors, RX821002 (0.05, 0.1 and 0.5 mg/kg) or saline (10 ml/kg) was injected 5 and 35 min before the administration of agmatine (5 mg/kg) and morphine (0.05 mg/kg) under the schedule, respectively.

To assess the effects of yohimbine and RX821002 alone on the acquisition of place preference, eight groups were studied. Yohimbine (0.001, 0.005. 0.01, and 0.05 mg/kg), RX821002 (0.05, 0.1 and 0.5 mg/kg) or saline (10 ml/kg, i.p.) were injected 35 min prior to placement in the preferred compartment of apparatus under the schedule. The ability of yohimbine and RX821002 to induce CPP or aversion on the postconditioning day was evaluated.

Elevated Plus Maze

In the biased design of place conditioning if a drug has a strong anxiolytic effect that could overcome the initial aversion for the nonpreferred compartment, it may increase the preference scores for that particular compartment (Tzschentke, 1998). As place preference conditioning experiments were conducted as a biased procedure in this study, the following experiments were designed to observe whether agmatine alone or in combination with morphine was able to induce anxiolytic effects or not. Anxiety levels were assessed using the elevated plus maze (EPM), which exploits the conflict between the animal's innate tendency to explore novel areas with their aversion for heights and open spaces.

The method was basically the same as those used in previous experiments (Lister, 1987; Cao and Rodgers, 1997). The apparatus was a wooden, cross-shaped maze, consisted of four arms arranged in the shape of a plus sign. Two of the arms had no side or end walls (open arms; $30 \times 5 \times 0.25\,cm^3$). The other two arms had side walls and end walls, but were open on the top (closed arms; $30 \times 5 \times 15\,cm^3$). Where the four arms intersect, there was a square platform of $5 \times 5\,cm^2$. The maze was elevated to a height of 50 cm. The animals were carried to the laboratory and left there undisturbed for 1 h before the experiment. A relatively dark box was used to hold the mice in before exposure to the maze in order to increase their exploratory behavior (Pellow and File, 1986). The tests were performed in a silent environment under dim light. Each mouse was individually placed on the central platform facing toward an open arm and was observed for 5 min by two observers sitting in the same room. The number of entries into the open arms and closed arms and the total time spent in the open arms and closed arms were measured. Entry was defined as placement of all four paws in the arms. The percentage of open arm entries (%OAE) and open arm qtime (%OAT) as the standard anxiety indices (Sanders *et al*, 2003) were calculated as follows: (a) %OAE = the ratio of entries into open arms to total entries $\times$ 100; (b) %OAT = the ratio of times spent in the open arms to total times spent in any arms $\times$ 100. Total arm entries were considered as the index of locomotor activity (Pellow and File, 1986). Animals received agmatine (1, 5, and 10 mg/kg) 30 min prior to saline (10 ml/kg, s.c.) or morphine (0.05 and 0.5 mg/kg) and were placed in the plus maze under the procedure explained above.

Statistical Analysis

All results are presented as mean $\pm$ SEM Data were assessed by two-way analysis of variance (ANOVA) or, when appropriate, one-way ANOVA. If a significant F-value was obtained, *post hoc* analyses (Tukey–Kramer's multiple comparison tests) were performed to determine the effects of various treatments on induction of place preference, changes in locomotion, %OAT and %OAE compared to appropriate vehicle groups. *P*-values less than 0.05 were considered as significant. Calculations were performed using the SPSS statistical package (version 11.5). ED_{50} values were determined using Graphpad Prism software (version 4.03).

RESULTS

Dose–Response Curve for Place Preference Conditioning Produced by Morphine in Opioid-Naïve Mice

Figure 1 shows the dose–response curve for place conditioning induced by morphine in mice. In preconditioning session, mean staying time in the white compartment was 187.02 $\pm$ 9.27 s (mean $\pm$ SEM) out of 600 s, thus the white compartment was chosen as the drug-paired compartment. Statistical analysis indicated that morphine induces place preference (one-way ANOVA; $F(5,40) = 4.832$, $P = 0.002$). Tukey–Kramer multiple comparison tests revealed that doses of 1–7.5 mg/kg of morphine induced place preference in comparison to control group; however, saline (10 ml/kg, s.c.) or morphine 0.5 mg/kg failed to produce significant conditioning in animals and no preference for either compartment was seen. The maximum response was

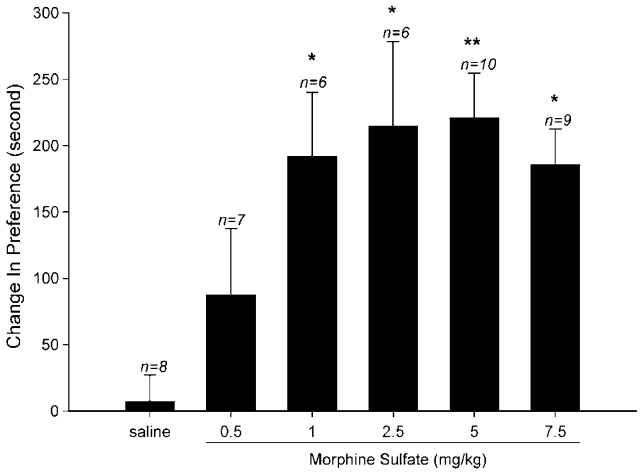

Figure 1 Effects of morphine on CPP induction in opioid-naïve mice. In a 6-day schedule, animals received saline (10 ml/kg, s.c.) or morphine (0.5–7.5 mg/kg, s.c.) in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. The data are shown as means of change in preference $\pm$ SEM *$P < 0.05$, **$P < 0.005$ different from the group treated with saline (Tukey–Kramer's multiple comparison tests).

observed with 5 mg/kg of morphine and ED_{50} for morphine in producing CPP was 0.898 mg/kg.

Effects of Agmatine on the Acquisition of Place Preference Conditioning *Per Se* and in the Presence of Morphine

Figure 2 shows the effect of different doses of agmatine on place preference. Statistical analyses did not show any significant effect for agmatine on place preference (one-way ANOVA; $F(5,40) = 0.764$, $P = 0.581$). Although agmatine 5, 10, 20, and 40 mg/kg increased the staying time by 28.5, 43.7, 92, and 106.3 s, respectively, they were not statistically significant.

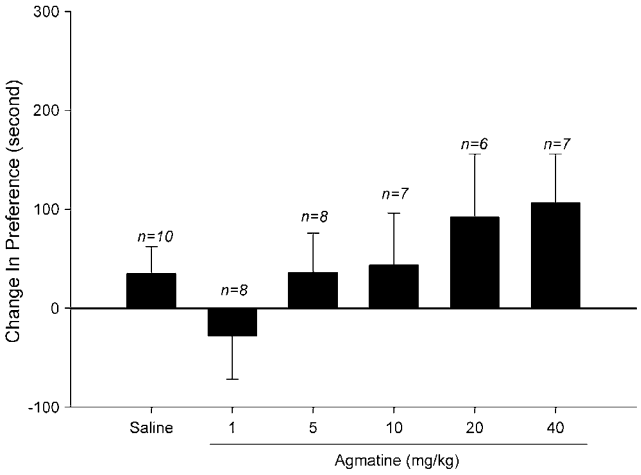

Figure 2 Effects of agmatine on CPP in mice. In a 6-day schedule, animals received saline (10 ml/kg, i.p.) or agmatine (1–40 mg/kg, i.p.) in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. The data are shown as means of change in preference ± SEM. Analysis revealed that no group showed a statistical significant difference.

Figure 3 shows the effect of different doses of agmatine in combination with various noneffective doses of morphine on producing CPP. Two-way ANOVA indicated a significant interaction between morphine and agmatine (factor morphine, $F(3,88) = 23.628$, $P < 0.001$; factor agmatine, $F(3,88) = 54.579$, $P < 0.001$; factor morphine × agmatine, $F(9,88) = 3.268$, $P = 0.002$). *Post hoc* analyses showed that agmatine had enhanced the effect of morphine on producing CPP. Coadministration of agmatine (1 mg/kg, i.p.) with morphine (0.01 and 0.05 mg/kg) failed to produce CPP; while agmatine 1 mg/kg coadministered with morphine (0.1 and 0.5 mg/kg) induced a significant CPP. Agmatine 5 mg/kg in combination with morphine (0.05, 0.1, and 0.5 mg/kg, s.c) induced significant CPP. Agmatine (10 mg/kg) produced CPP while coadministered with *per se* noneffective doses of morphine (0.01, 0.05, 0.1, and 0.5 mg/kg, s.c.). According to the first experiment, the morphine dose–effect function saturates at 5 mg/kg of morphine; therefore, the magnitude of place preference produced at this dose was considered as a maximum effect and ED_{50} values for morphine alone and morphine in combination with various doses of agmatine were calculated relative to this maximum effect. ED_{50} for morphine to induce place preference in the presence of agmatine 1, 5, and 10 mg/kg were 0.081, 0.016, and < 0.01 mg/kg, respectively, showing that agmatine 1, 5, and 10 mg/kg were able to enhance the rewarding properties of morphine in producing CPP for 11, 56.13, and > 89.8 times, respectively.

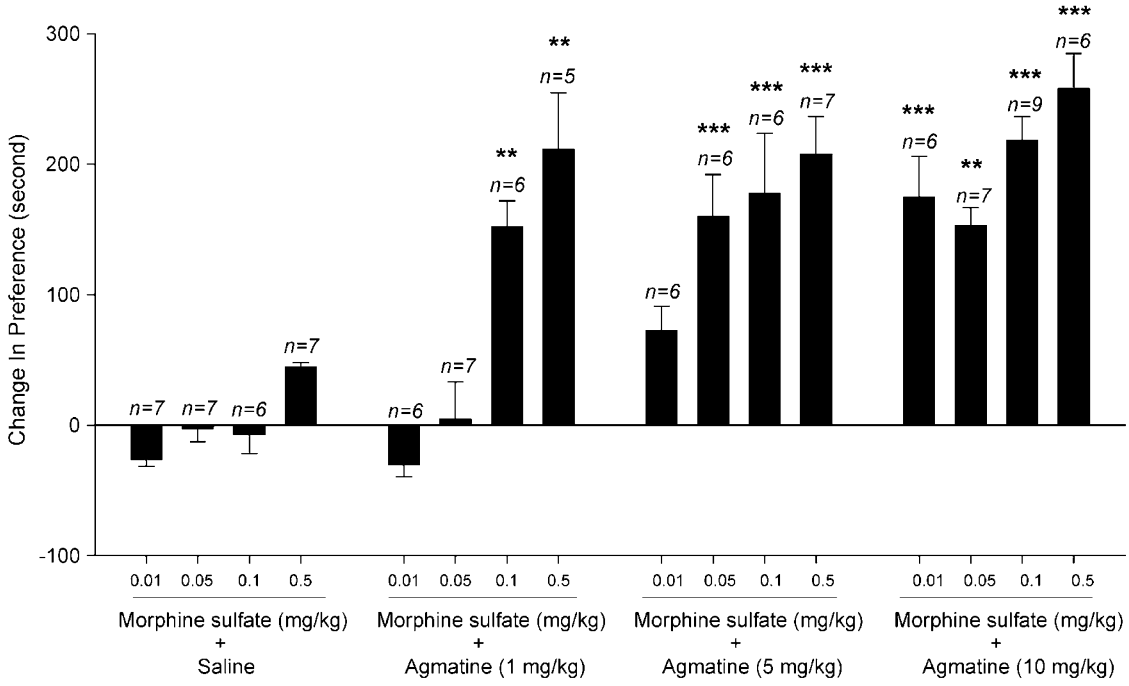

Figure 3 Effects of agmatine on acquisition of morphine-induced CPP in mice. In a 6-day schedule, animals received saline (10 ml/kg, i.p.), or agmatine (1, 5, 10 mg/kg, i.p.) 30 min before injection of morphine (0.01, 0.05, 0.1, 0.5 mg/kg, s.c.) and were placed in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. The data are shown as means of change in preference ± SEM. $^{**}P < 0.005$, $^{***}P < 0.001$ different from the groups pretreated with saline (Tukey–Kramer's multiple comparison tests).

Effect of Agmatine Alone or with Morphine on Anxiety

Two-way ANOVA analysis indicated that agmatine or its combination with morphine had no significant effect on %OAT (factor agmatine, $F(2,45) = 0.602$, $P = 0.552$; factor morphine, $F(2,45) = 0.218$, $P = 0.805$; factor agmatine × morphine, $F(4,45) = 0.840$, $P = 0.507$), %OAE (factor agmatine, $F(2,45) = 0.970$, $P = 0.387$; factor morphine, $F(2,45) = 0.282$, $P = 0.755$; factor agmatine × morphine, $F(4,45) = 0.973$, $P = 0.432$), and locomotor activity (factor agmatine, $F(2,45) = 2.357$, $P = 0.106$; factor morphine, $F(2,45) = 1.195$, $P = 0.312$; factor agmatine × morphine, $F(4,45) = 0.166$, $P = 0.955$). The %OAT (mean ± SEM) for agmatine 1, 5, and 10 mg/kg plus saline were 29.41 ± 2.97, 26.23 ± 3.47, and 29.15 ± 2.95, respectively; the %OAE (mean ± SEM) for animals receiving agmatine (1, 5, and 10 mg/kg) prior to saline injection were 41.2 ± 2.73, 37.7 ± 1.48, and 32.35 ± 3.05, respectively. The above result suggests that agmatine in the absence or presence of morphine, with the doses used in this experiment, does not seem to have any effect on anxiety-related behaviours.

Effect of UK 14 304, and its Combination with Agmatine on Morphine-Induced Place Preference

Figure 4 shows the effect of UK 14 304 on morphine CPP. Statistical analysis indicated that UK 14 304 was able to induce a significant place preference in combination with lower and *per se* noneffective doses of morphine (two-way ANOVA; factor morphine, $F(2,48) = 29.796$, $P < 0.001$; factor UK 14 304, $F(2,48) = 17.891$, $P < 0.001$; factor morphine × UK 14 304, $F(4,48) = 6.468$, $P < 0.001$). UK 14 304 (0.1 and 0.5 mg/kg) in combination with saline or morphine (0.05 mg/kg), also UK 14 304 (0.1 mg/kg) with morphine (0.5 mg/kg) did not produce significant place preference; however, a significant effect was seen in mice receiving UK 14 304 (0.5 mg/kg) 35 min prior to morphine (0.5 mg/kg) administration ($P < 0.001$ in comparison with saline/saline, saline/morphine 0.5 mg/kg and UK 14 304 0.5 mg/kg/saline control groups).

The effect of UK 14 304 in combination with agmatine on morphine place preference illustrated a significant interaction (Figure 5) (one-way ANOVA; $F(5,31) = 5.748$, $P = 0.001$). *Post hoc* analyses showed that UK 14 304 enhanced the effect of agmatine on morphine CPP. UK 14 304 (0.5 mg/kg) in combination with agmatine (1 mg/kg) and morphine (0.05 mg/kg) produced a significant place preference ($P = 001$, $P = 0.032$, and $P = 0.002$ in comparison with vehicle/agmatine 1 mg/kg/saline, UK 14 304 0.5 mg/kg/agmatine 1 mg/kg/saline, and vehicle/agmatine 1 mg/kg/morphine 0.05 mg/kg control groups, respectively). Although UK 14 304 (0.1 mg/kg) in combination with agmatine (1 mg/kg) and morphine (0.05 mg/kg) increased the staying time in the drug-paired compartment for 83.66 s, it did not produce a statistically significant effect.

Effect of Yohimbine or RX821002 on the Acquisition of Place Preference Conditioning in the Absence and Presence of Morphine and/or Agmatine

In animals receiving yohimbine (0.001 and 0.005 mg/kg) prior to morphine (0.05, 0.5, and 5 mg/kg), two-way ANOVA indicated that no interaction between yohimbine and morphine existed on producing place preference or aversion (factor yohimbine, $F(2,52) = 0.059$, $P = 0.943$; factor morphine, $F(2,52) = 34.987$, $P < 0.001$; factor yohimbine × morphine, $F(4,52) = 0.457$, $P = 0.767$) (Figure 6). In addition, no significant interaction between RX821002 and morphine was seen in the acquisition of place preference (two-way ANOVA; factor RX821002, $F(3,67) = 0.456$, $P = 0.714$; factor morphine, $F(2,67) = 47.573$, $P < 0.001$; factor RX821002 × morphine, $F(6,67) = 0.472$, $P = 0.826$) (Figure 7).

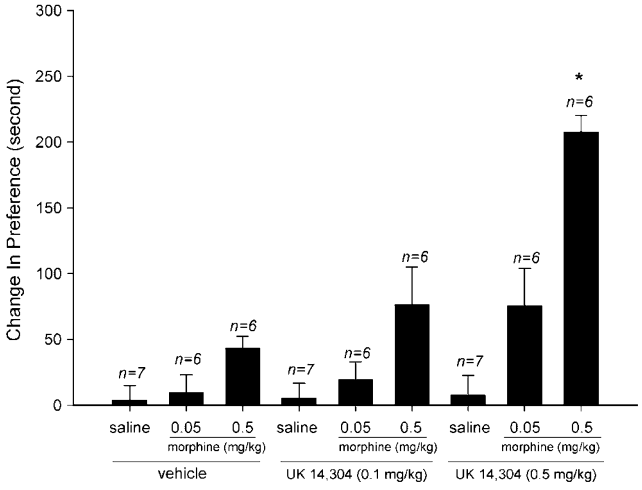

Figure 4 Effect of UK 14 304 on CPP induced by morphine in mice. Animals received vehicle (0.5% DMSO-saline; 10 ml/kg, i.p.), or UK 14 304 (0.1 and 0.5 mg/kg, i.p.) 35 min prior to administration of morphine (0.05 and 0.5 mg/kg, s.c.) and were placed in the nonpreferred compartment in the 1st, 3rd, and 5th days of the conditioning phase. The data are shown as means of change in preference ± SEM. *$P < 0.001$ different from the groups pretreated with 0.5% DMSO-saline (Tukey–Kramer's multiple comparison tests).

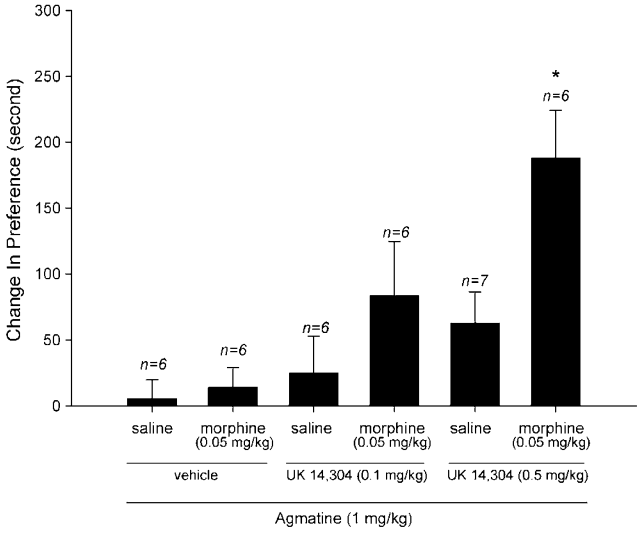

Figure 5 Effect of coadministration of UK 14 304 and agmatine on place preference induced by morphine. UK 14 304 (0.1 and 0.5 mg/kg, i.p.) or its vehicle (10 ml/kg, i.p.) was administered 5 min before agmatine (1 mg/kg, i.p.) and 35 min before morphine (0.05 mg/kg, s.c.) or saline (10 ml/kg, s.c.) in the 1st, 3rd, and 5th day of conditioning. The data are shown as means of change in preference ± SEM. *$P < 0.005$ different from vehicle/agmatine/morphine group (Tukey–Kramer's multiple comparison tests).

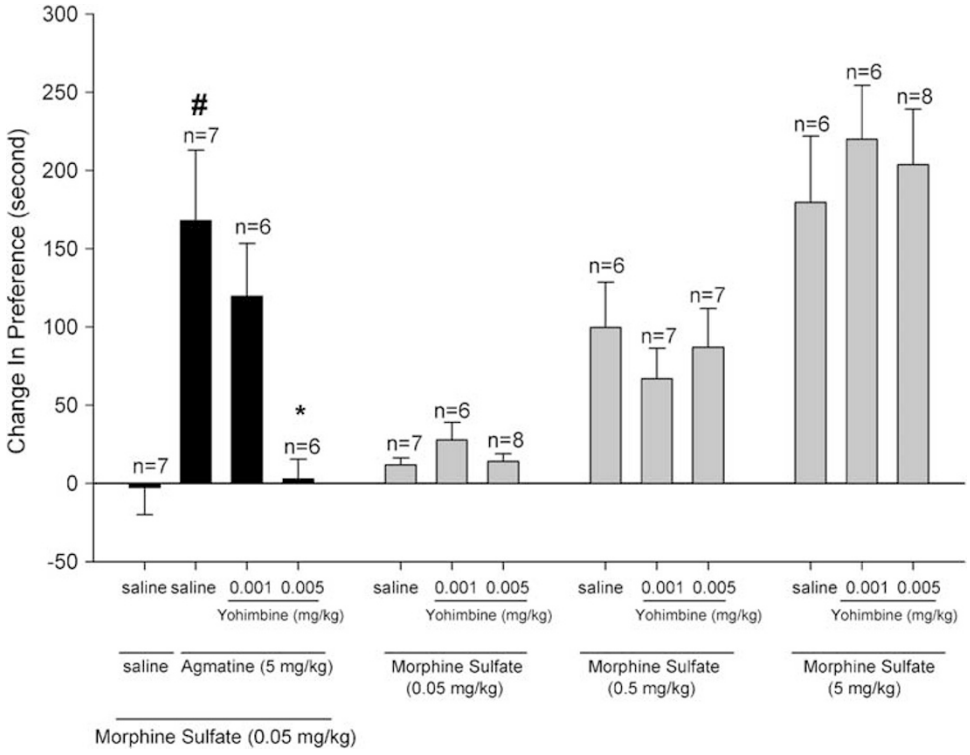

Figure 6 Effects of coadministration of yohimbine and yohimbine plus agmatine on morphine-induced CPP in mice. Black bars: in a 6-day schedule, animals received saline (10 ml/kg, i.p.) or yohimbine (0.001 and 0.005 mg/kg, i.p.) 5 min before injection of agmatine (5 mg/kg, i.p.) or saline (10 ml/kg, i.p.) and 35 min before morphine (0.05 mg/kg, s.c.) and were placed in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. Gray bars: animals received yohimbine (0.001 and 0.005 mg/kg, i.p.) or saline (10 ml/kg, i.p.) 35 min before the administration of morphine (0.05, 0.5, and 5 mg/kg, s.c.) and were placed in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. The data are shown as means of change in preference ± SEM $^{*}P<0.01$ different from the group receiving saline/agmatine (5 mg/kg)/morphine (0.05 mg/kg), $^{\#}P<0.005$ different from the group receiving saline/saline/morphine (0.05 mg/kg) (Tukey–Kramer's multiple comparison tests).

Coadministration of yohimbine or RX821002 plus agmatine showed that no significant interaction existed between yohimbine (0.001 and 0.005 mg/kg) and agmatine (5 mg/kg) (one-way ANOVA; $F(2,15)=0.399$, $P=0.678$) or RX821002 (0.05, 0.1 and 0.5 mg/kg) and agmatine (5 mg/kg) (one-way ANOVA; $F(3,21)=0.166$, $P=0.918$) on the acquisition of place preference (data not illustrated).

Figure 6 shows the effect of yohimbine on the enhancement of morphine-induced place preference produced by agmatine. Statistical analysis revealed that a significant interaction exists between yohimbine and the effect of agmatine on morphine CPP (one-way ANOVA, $F(3,22)=7.832$, $P=0.001$). Further analysis revealed that although yohimbine (0.001 mg/kg) had decreased the enhancing effect of agmatine on morphine-induced place preference, this effect was not statistically significant; however, yohimbine (0.005 mg/kg) had completely blocked the enhancing effect of agmatine on the acquisition of morphine-induced CPP.

A significant interaction between RX821002 and the enhancing effect of agmatine on morphine CPP exists, as shown in Figure 7 (one-way ANOVA, $F(3,26)=6.645$, $P=0.002$). RX821002 (0.1 and 0.5 mg/kg) significantly reversed the enhancing effect of agmatine on the acquisition of morphine CPP; while RX821002 (0.05 mg/kg) did not show a significant effect in this regard.

To see whether yohimbine or RX821002 alone could affect the acquisition of place preference in mice, animals received saline (10 ml/kg, i.p.), yohimbine (0.001, 0.005, 0.01, and 0.05 mg/kg) or RX821002 (0.05, 0.1, and 0.5 mg/kg) prior to placement in the preferred side of the apparatus. Animals exhibited no place preference or aversion for either of the compartments (one-way ANOVA; $F(7,44)=0.110$, $P=0.997$) (data not shown).

DISCUSSION

Recent evidence suggests that agmatine, which is an intermediate in polyamine biosynthesis, might be a neurotransmitter in mammals. Agmatine is synthesized in the brain, stored in synaptic vesicles in regionally selective neurons, accumulated by uptake, released by depolarization, and inactivated by agmatinase (Reis and Regunathan, 2000). The central effects of agmatine are diverse and have been linked to its ability to bind α_2-adrenoceptors and imidazoline binding sites, as well as its function on various isoforms of nitric oxide synthase, blockade of NMDA-receptor-mediated effects, and its metabolite turnover into putrescine and spermine (Raasch *et al*, 2001).

The current study concerns the effects of intraperitoneal administration of agmatine on the acquisition of morphine-induced place preference. CPP paradigm used in this study represents an animal model to assess the rewarding effect of

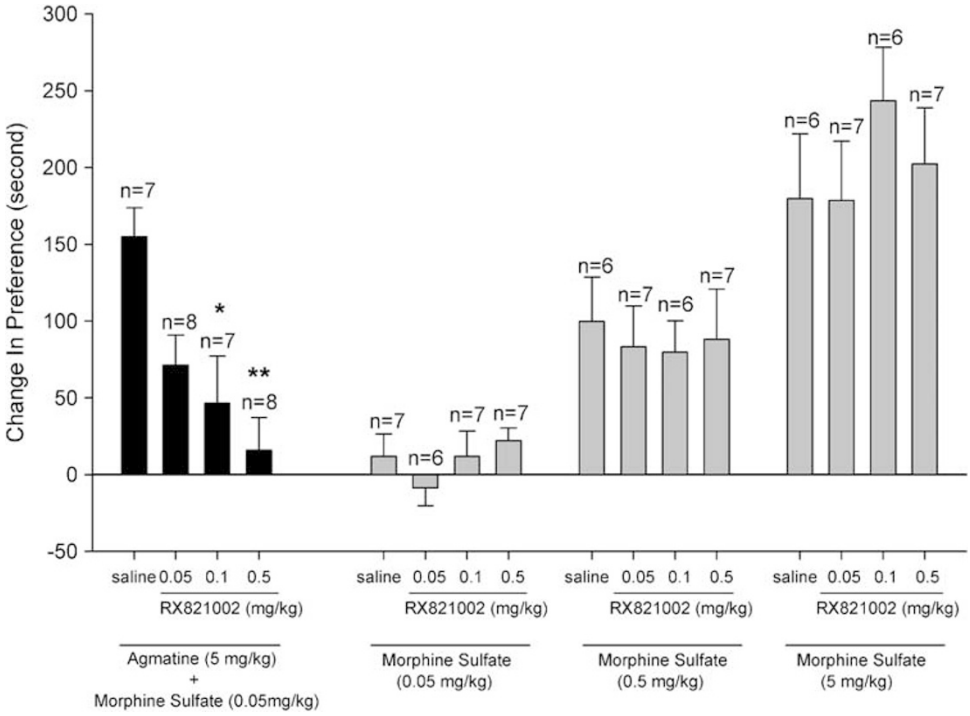

Figure 7 Effects of coadministration of RX821002 and RX821002 plus agmatine on morphine-induced CPP in mice. Black bars: in a 6-day schedule, animals received saline (10 ml/kg, i.p.) or RX821002 (0.05, 0.1, and 0.5 mg/kg, i.p.) 5 min before injection of agmatine (5 mg/kg, i.p.) and 35 min before morphine (0.05 mg/kg, s.c.) and were placed in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. Gray bars: animals received RX821002 (0.05, 0.1 and 0.5 mg/kg, i.p.) or saline (10 ml/kg, i.p.) 35 min before the administration of morphine (5 mg/kg, s.c.) and were placed in the nonpreferred compartment in the 1st, 3rd, and 5th days of conditioning. The data are shown as means of change in preference ± SEM. $^{*}P<0.05$ and $^{**}P<0.005$ different from the group receiving saline/agmatine (5 mg/kg)/morphine (0.05 mg/kg) (Tukey–Kramer's multiple comparison tests).

different systems including opioids (Bardo *et al*, 1984). Our data indicate that morphine induces a significant CPP in a dose-dependent manner in mice, which is consistent with our previous findings and results of others in this respect (Suzuki *et al*, 1995; Tzschentke, 1998; Zarrindast *et al*, 2003; Langroudi *et al*, 2005). The present work shows for the first time that agmatine alone does not show any effect on place conditioning; however, concomitant administration of agmatine with lower and *per se* noneffective doses of morphine (as low as 0.01 mg/kg) induce a synergistic rewarding effect using place preference conditioning paradigm. It has been suggested that agmatine, in doses of 20 and 40 mg/kg, causes a mild anxiolytic-like behavior (Lavinsky *et al*, 2003). The biased design of place conditioning used in this study, has often been criticized because it is susceptible to yielding false-positive results in place conditioning experiments. For example, if a drug has a strong anxiolytic component that could overcome the initial aversion for the nonpreferred compartment, it may increase the preference scores for that particular compartment (Tzschentke, 1998). We have investigated the anxiolytic effects of agmatine and its combination with morphine using elevated plus-maze, in order to exclude the involvement of these effects in inducing the place conditioning in biased paradigm. Neither agmatine, nor its combination with morphine with the mentioned doses shows a significant anxiolytic effect, in plus-maze test. Therefore, the enhancing effect of agmatine on morphine-induced CPP could not be attributed to their anxiolytic effects.

This interaction of agmatine and morphine in reward system is to some extent similar to previous reports of potentiating effect of agmatine on analgesic and anticonvulsant properties of opioids and suggests possible existence of common molecular mediators of these interactions (Sanchez-Blazquez *et al*, 2000; Roerig, 2003; Yesilyurt and Uzbay, 2001; Ruiz-Durantez *et al*, 2003; Kekesi *et al*, 2004; Riazi *et al*, 2005). Agmatine enhances morphine-induced analgesia in a dose-dependent manner (Kolesnikov *et al*, 1996; Yesilyurt and Uzbay, 2001; Ruiz-Durantez *et al*, 2003). Acute administration of low dose of agmatine prevents tolerance following chronic morphine treatment for 10 days (Kolesnikov *et al*, 1996). Agmatine prevents naloxone-precipitated abstinence syndrome in morphine-dependent rats and attenuates all of the signs of morphine withdrawal syndrome dose-dependently (Aricioglu-Kartal and Uzbay, 1997; Uzbay *et al*, 2000; Li *et al*, 2002; Aricioglu *et al*, 2004). Exposure of rats to morphine for 3 days is shown to decrease the levels of agmatine in the liver, kidney, brain, aorta, and intestine; also, precipitation of withdrawal syndrome by injecting naloxone further decreases agmatine levels in these tissues, providing evidence that endogenous agmatine may play an important role in regulating morphine tolerance, dependence, and withdrawal symptoms (Aricioglu-Kartal and Regunathan, 2002). Moreover, substances involved in the metabolism of endogenous agmatine, such as aminoguanidine and α-difluoromethylornithine, could modulate the pain threshold, morphine analgesia, and tolerance (Lu *et al*, 2003).

Nader and Van der Kooy (1996) have shown that clonidine blocks the rewarding effects of morphine in rats withdrawn from morphine, while it has no effect on morphine-induced CPP in drug naïve animals. Nevertheless, an illicit use of α_2-agonist clonidine among opiate addicts has been shown that does not seem to be exclusively directed to alleviate withdrawal (Anderson *et al*, 1997; Beuger *et al*, 1998). This effect could be related to a possible reinforcing effect of α_2-agonists such as clonidine, as shown in animal models of drug addiction (Asin and Wirtshafter, 1985). Growing body of evidence supports the involvement of α_2-adrenoceptors in mediating the actions of agmatine. Like clonidine, agmatine has been shown to bind to α_2-adrenoceptors (Pinthong *et al*, 1995). Agmatine alters the firing rate of locus ceruleus neurons *in vivo* (Pineda *et al*, 1996; Ruiz-Durantez *et al*, 2002) and induces α_2 adrenoceptors-dependent antinociception (Onal and Soykan, 2001). Moreover, agmatine has agonistic activity at prejunctional α_2-adrenoceptors in the rat tail artery (Gonzalez *et al*, 1996). It is demonstrated that agmatine elicits a significant antidepressant-like effect that is completely prevented by pretreatment of animals with yohimbine (Zomkowski *et al*, 2002). Agmatine is shown to increase ethanol-induced gastric mucosal injury and this effect is partly abolished by pretreatment with yohimbine (Utkan *et al*, 2000). It has been shown that the potentiating effect of agmatine on morphine-induced analgesia is mediated by α_2-adrenoceptors (Yesilyurt and Uzbay, 2001; Roerig, 2003). Attenuation of contractile responses to naloxone in morphine-dependent guinea-pig ileum is partly abolished by pretreatment with yohimbine and is almost completely reversed by idazoxan, showing an α_2-adrenergic and imidazoline-receptor-mediated mechanism for such actions of agmatine (Aricioglu *et al*, 2003). These findings are in agreement with our previous reports of an α_2-adrenoceptor-mediated anticonvulsant effect for the higher doses of agmatine (5 mg/kg or more) (Demehri *et al*, 2003). In addition, we have recently reported that agmatine and morphine exert synergistic anticonvulsant effect, which is mediated via α_2-adrenergic pathway (Riazi *et al*, 2005). Both opioid receptors and α_2-adrenoceptors belong to a superfamily of G protein-coupled receptors and their interaction may involve reciprocal feedback regulation through shared signaling mechanisms (Saunders and Limbird, 1999; Liang *et al*, 1998).

In light of mentioned findings we have studied the possible involvement of α_2-adrenoceptors, known to mediate some physiologic effects of agmatine (Li *et al*, 1994; Roerig, 2003; Zomkowski *et al*, 2002; Ruiz-Durantez *et al*, 2003). UK 14 304, which is a highly selective α_2-adrenoceptor agonist (Cambridge, 1981) with low affinity for I_1 receptors (I_1/α_2 affinity ratio = 0.01; Bricca *et al*, 1993; Ernsberger *et al*, 1992), was used to support our hypothesis that agmatine enhances the effects of morphine on producing CPP via a mechanism involving α_2-adrenoceptors. UK 14 304 (0.5 mg/kg) in combination with a subeffective dose of morphine produces significant CPP. Also, the combination of noneffective doses of UK 14 304 and agmatine enhances morphine place preference, implying the additive effect between these two drugs. Moreover, we have assessed the effects of α_2 antagonists in this regard. Yohimbine, a selective α_2 adrenoceptor antagonist (Goldberg and Robertsson, 1983) with very low affinity for I_1 binding sites (I_1/α_2 affinity ratio = 0.01; Ernsberger *et al*, 1987, 1992; Hamilton *et al*, 1988; Senard *et al*, 1990), with a dose of 0.005 mg/kg is incapable of affecting place preference or altering morphine CPP; however, it completely blocks the potentiating effect of agmatine on morphine-induced place preference. Yohimbine with higher doses is shown to induce CPA (File, 1986) and it tends to limit the place preference induced by morphine (Morales *et al*, 2001). This discrepancy would be explained by administration of lower doses of yohimbine in the present work. RX821002 is a potent α_2-adrenoceptor antagonist with very low affinity for I_1 and I_2 binding sites (Clarke and Harris, 2002; Galitzky *et al*, 1990; Miralles *et al*, 1993). The greater selectivity of RX821002 renders it much superior to yohimbine as a tool for probing physiological actions at α_2-receptors (Clarke and Harris, 2002). RX821002 (0.5 mg/kg) completely abolishes the agmatine potentiation of morphine CPP, implying the involvement of α_2-adrenoceptors due to its very low affinity for imidazoline receptors. Meanwhile, the interaction between agmatine and morphine in modulation of rewarding properties of morphine may also involve the imidazoline receptors, a point that warrants further investigation.

In conclusion, the present study is the first report of the potentiating effect of agmatine on morphine-induced CPP. This finding implies a potential novel approach to utilize the rewarding effects of opioids in some clinical situations with such low doses that may be devoid of their adverse effects, while the issue should be carefully investigated. Moreover, this effect is enhanced by UK 14 304 and abolished by yohimbine and RX821002, indicating the involvement of α_2-adrenoceptors in this regard.

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