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Inhibition by agmatine on morphine-induced conditioned place preference in rats

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Abstract

Our previous studies demonstrated the ability of exogenous agmatine to inhibit tolerance to and physical dependence on morphine in mice, rats and monkeys. The present study further evaluated the effect of agmatine on the psychological dependence induced by morphine in conditioned place preference assay. Agmatine (0.75–20 mg/kg, s.c.) co-administered with morphine during the conditioning sessions completely abolished the acquisition of morphine-induced conditioned place preference in rats, which was associated with activation of imidazoline receptors. Agmatine (0.75–10 mg/kg, s.c.) administered on the test day inhibited the expression of the place preference. After 30 days of extinction of conditioned place preference, agmatine 2.5 and 40 mg/kg inhibited the priming effect of morphine 0.5 mg/kg on the place preference. Furthermore, agmatine inhibited the increased expression of FosB in the nucleus accumbens caused by chronic morphine. All these results suggest that agmatine could inhibit morphine-induced psychological dependence and relapses by affecting the expression of transcription factor FosB.

Keywords: Agmatine; Morphine; Conditioned place preference; Imidazoline receptor

1. Introduction

Drug addiction is a chronic, relapsing disease of the brain (Leshner, 1997). Continuous administration of drugs including opioids induces adaptive changes in the central nervous system that is responsible for tolerance, physical dependence, sensitization, craving and relapse. Opioid addiction involved not only counteradaptive changes of endogenous opioid peptides and its receptor systems, but adaptive changes of many other non-opioid neuronal transmitter systems, such as noradrenergic, dopaminergic, 5-hydroxytryptaminergic, glutamate and γ-aminobutyric acid systems (Shalev et al., 2002).

Endogenous agmatine may act as a new neurotransmitter or neuromodulator (Reis and Regunathan, 1999), by antagonizing the *N*-methyl-D-aspartate (NMDA) receptor (Gibson et al., 2002), inhibiting nitric oxide synthase (NOS) (Galea et al., 1996), binding to α2-adrenoceptors (Sugawara et al., 2001) and imidazoline binding sites (Reis and Regunathan, 2000). Previous studies showed that exogenous agmatine enhanced morphine analgesic effect (Yesilyurt and Uzbay, 2001; Ruiz-Durantez et al., 2003), inhibited the development of tolerance to morphine analgesia including acute- and chronic-spinal morphine tolerance (Kolesnikov et al., 1996; Li et al., 1998, 1999a; Fairbanks and Wilcox, 1997), inhibited ethanol and morphine-withdrawal syndromes (Uzbay et al., 2000; Aricioglu-Kartal and Uzbay, 1997; Li et al., 1998, 1999b). Such effect of agmatine was related to activation of imidazoline receptors, so the imidazoline receptors system was also postulated to be an important system that regulates opioid dependence (Su et al., 2000, 2003). Moreover, Morgan et al. reported that agmatine attenuated the escalation of fentanyl self-administration when administered before the escalation of intake and may mediate neuroadaptive events related to chronic opioid self-administration (Morgan et al., 2002). So

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it is likely that agmatine could also modulate the reinforcing effect of morphine.

Conditioned place preference (CPP) paradigm has been widely used as a model for studying the reinforcing effect of drugs with dependent tendency. Many drugs such as cocaine, amphetamine, morphine, heroin and ethanol produce reinforcing effect and induce CPP for the drug-paired side after several conditioning sessions (Carr et al., 1988). It seems likely that treatments that decrease these reinforcing actions of drugs in animal models would be effective in diminishing the intake of drugs of abuse in human. In the present study CPP model was used to determine the effect of agmatine on the rewarding of morphine in rats.

Moreover, accumulating evidence suggests that the expression of ΔFosB and the chronic Fos-related antigens are augmented after chronic treatment with many drugs of abuse, such as cocaine and morphine (Chen et al., 1995, 1997; Nye and Nestler, 1996). Thus ΔFosB may represent a molecular mechanism that could initiate and sustain changes in gene expression that persist long after drug exposure (Nestler et al., 2001). ΔFosB is a splice variant of FosB with a truncated C-terminus, so in the present study we assessed FosB expression in nucleus accumbens in rats that developed CPP with an antibody directed against the N-terminus of the FosB molecule.

The purpose of the present study was to examine the effect of agmatine on the acquisition and expression of morphine-induced CPP in rats. We also studied the potential effect of agmatine on the priming effect by low dose of morphine on CPP. Meanwhile, expression of FosB in nucleus accumbens of morphine-induced CPP rats was observed.

2. Materials and methods

2.1. Animals

Male Wistar rats (Beijing Animal Center, China) weighing 180–220g were used in all experiments. The rats were grouped 6 per cage and accustomed to the experimental environment for 5–6 days before the place conditioning sessions with the temperature maintained at 24±1 °C and relative humidity at 50%. Animals were maintained on a 12 h light dark cycle and given free ad libitum access to food and water, in strict compliance with the guidelines set for the use of experimental animals by the European Community.

2.2. Drugs

Morphine hydrochloride was purchased from Qinhai Pharmaceutical Factory, China. Idazoxan hydrochloride, yohimbine hydrochloride, MK-801 and agmatine sulfate were obtained from Sigma (St. Louis, MO, USA). Rabbit polyclonal antibody for FosB was obtained from Santa Cruz (H-75, Santa Cruz Biotechnology, Santa Cruz, CA, USA). All drugs were dissolved in 0.9% saline to final concentrations and injected in a volume of 1.0 ml/kg. Morphine and agmatine were given subcutaneously (s.c.), the other drugs were given intraperitoneally (i.p.).

2.3. Place conditioning procedure

The CPP apparatus consisted of two equal-sized Plexiglas compartments (18 × 22 × 22 cm), one with a white box and the other with a black box jointed by a wall with a sliding door (Med Associates, USA). For testing, the sliding door was raised 12 cm above the floor to allow the rat free access to both sides of the box.

Conditioning sessions were conducted twice daily which involved alternate injection of the conditioning drug or saline and each session was separated by a period of 6 h. Conditioning training persists for 9 continuous days. Each conditioning session was 45 min in duration. At 8:00 to 12:00 on the first training day, rats in all the groups were injected saline and immediately confined to the black compartment (not drug-paired side) of the shuttle box; at 14:00 to 18:00 the animals were confined to the white compartment (drug-paired side) after saline was administered to the vehicle group and drug to the experiment groups. On the second day, the procedure was performed in reverse order, that is, at 8:00 to 12:00 the animals were confined to the white compartment after saline was administered to the vehicle group and drug to the experiment groups; at 14:00 to 18:00 rats in all groups were injected saline and confined to the black compartment. Test sessions were carried out 1 day after the last training session in the drug-free state. On the test day, the sliding door was opened to allow rats free access to both sides of the box for 15 min. The time that the rats spent on the drug-paired side (the white compartment) was recorded.

2.4. Effect of agmatine on morphine induced CPP

To evaluate whether agmatine (0.75, 2.5, 10, 40 mg/kg, s.c.) alone could induce CPP, rats were tested after being treated following the above-mentioned conditioning procedure for 9 days.

In order to study the effect of agmatine on the acquisition of morphine-induced CPP, morphine (3 mg/kg, s.c.) was used during the conditioning sessions and agmatine (0.75–20 mg/kg, s.c.) was injected 30 min before morphine administration once per day. The rats were tested on the test day in a drug-free state. Idazoxan (1–9 mg/kg, i.p.) or yohimbine (1–5 mg/kg, i.p.) was given intraperitoneally 15 min before agmatine (2.5 mg/kg, s.c.) injection to determine whether the effect of agmatine on morphine-induced CPP was related to imidazoline receptors or α2-adrenoceptors.

To observe the effect of agmatine on the expression of morphine-induced CPP, morphine (3 mg/kg, s.c.) was used during the conditioning sessions in all the groups except saline-treated group. On the test day, saline (1 ml/kg, s.c.), MK-801 (0.1 mg/kg, i.p.) or agmatine (0.75–10 mg/kg, s.c.) was injected 30 min before the 15 min test. The duration for which the rats stayed in the drug-paired side was recorded.

Meanwhile, we also evaluated the effect of agmatine (2.5–40 mg/kg, s.c.) on the priming effect of morphine 0.5 mg/kg on CPP induced by morphine. After the 9 days of conditioning sessions with morphine (3 mg/kg, s.c.) in all the groups except the saline group, the rats were maintained in a drug-free state for about 30 days of extinction. During the extinction period, a 15 min test was carried out once a week until the extinction of CPP. On the next day, agmatine (2.5–40 mg/kg, s.c.) was injected 30 min before morphine (0.5 mg/kg, s.c.) administration, priming test was performed for 15 min immediately after injection of morphine 0.5 mg/kg and the duration for which the rats stayed in the drug-paired side was recorded.

2.5. FosB immunohistochemistry

In order to study the effect of agmatine on the expression of FosB during the development of morphine-induced CPP, morphine (3 mg/kg, s.c.) was used during the conditioning sessions and agmatine (2.5–20 mg/kg, s.c.) was injected concomitantly 30 min before morphine administration. After the effect of agmatine on the acquisition of morphine-induced CPP was tested, animals were deeply anesthetized with sodium pentobarbital, and perfused transcardially with 100 ml saline, followed by 50 ml 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains of the rats were then removed, left in fixative state for 2 h and transferred to phosphate-buffered 30% sucrose solution at 4 °C until they sank. Brains were sectioned on a freezing microtome at 20 μm thickness, and the sections were mounted onto glass slides coated with poly-L-lysine, and stored at − 20 °C until use. Prior to immunohistochemistry, slides were allowed to thaw for 10 min at room temperature.

Immunohistochemistry was performed using a non-biotin horseradish peroxidase (HRP) detection system (PV6000, America Zymed). The primary rabbit polyclonal antibody for FosB (H-75) was used. Sections were washed three times in 0.01 M PBS and then pretreated with 0.3% H_2O_2 for 30 min. Sections were washed three times in PBS and then placed in 0.01 M sodium citrate buffer (pH 6.0) and heated at 92 °C to 98 °C for 10 min in a microwave. Slides were left to cool in the same sodium citrate buffer for approximately 20 min and washed three times in PBS. Sections were incubated in primary FosB antibody (diluted 1:750 in 0.01 M PBS containing 2% goat serum and 0.15% Triton X-100) for 48 h at 4 °C. On the next day, sections were washed three times in PBS and incubated in peroxidase-labelled secondary goat anti-rabbit IgG for 30 min at 37 °C. Slides were washed three times in PBS and then reacted with nickel-enhanced diaminobenzidine for 10 min. Slides were dehydrated and coverslipped prior to viewing with a light microscope. Omission of the primary or secondary antiserum from an immunohistochemical run resulted in complete absence of cellular staining (data not shown).

Counts of FosB-like immunoreactivity were made using Image-Pro image analysis software. Sections were viewed at 20 × object lens magnification and the number of FosB positive neurons in nucleus accumbens was quantified. Counts of FosB positive neurons were made by a single treatment-blind observer within standard square measuring grids that were placed over nucleus accumbens.

2.6. Data analysis

Conditioning scores represented the time spent in the drug-paired place (the white compartment). The data were expressed as mean ± S.E.M. Analyses between two groups were conducted using a Student's t test. Statistical analyses among three or more groups were performed using an analysis of variance (ANOVA), and the significance between individual dose conditions and the corresponding control group was determined by Dunnett's t test. A value of $P<0.05$ was considered significant.

3. Result

3.1. Effect of agmatine alone on place conditioning (Fig. 1)

As shown in Fig. 1, rats treated chronically with morphine 3 mg/kg for 9 days demonstrated preference to the drug-paired side ($t=5.5027$, $P<0.01$, $n=15$). Agmatine (0.75, 2.5, 10, 40 mg/kg, s.c.), however, was not able to induce CPP or conditioned place aversion (CPA). The scores of treatment groups were not significantly different from those of the saline control group ($F_{(4,50)}=1.78$, $P>0.05$, $n=10–15$). This result inferred that agmatine itself had no tendency of psychological dependence.

3.2. Effect of agmatine on the acquisition of morphine-induced CPP (Fig. 2)

As shown in Fig. 2A, rats treated with morphine 3 mg/kg showed place preference with the mean conditioning score of 557 s in the drug-paired side. Pairing of agmatine (0.75–20 mg/kg, s.c.) with each injection of morphine (3 mg/kg, s.c.) during the conditioning sessions completely abolished the acquisition of morphine-induced CPP and the mean score of rats in the 20 mg/kg groups was 331 s, which was significantly lower than that of the morphine treated group ($F_{(6,70)}=2.67$, $P<0.05$, $n=11$).

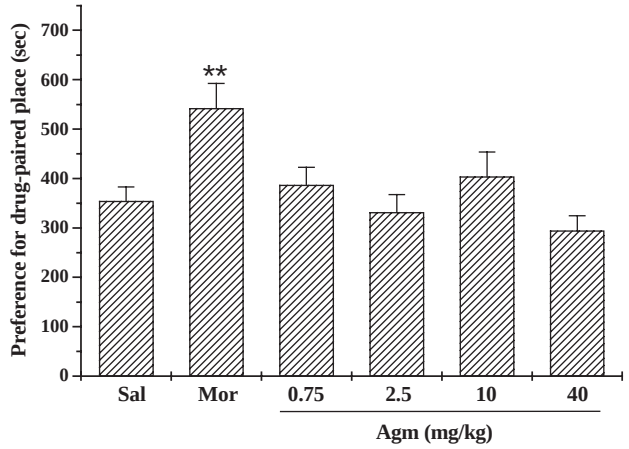

Fig. 1. The effect of agmatine on place conditioning. Conditioning sessions, conducted twice daily for 9 days, involved the alternate injection of saline (Sal, 1 ml/kg, s.c.) and morphine (Mor, 3 mg/kg, s.c.) or agmatine (Agm, 0.75, 2.5, 10, 40 mg/kg, s.c.). Test session was carried out 1 day after the last training session in the drug-free state for 15 min. The time that the rats spent in the drug-paired side (the white compartment) was measured. Each value was expressed as mean±S.E.M. of 10–15 rats in each group. $^{**}P<0.01$, compared with saline group. Student's t test was used between the morphine and saline group. One-way ANOVA was used among several agmatine groups and the saline group.

Idazoxan (9 mg/kg, i.p.) alone affects neither the conditioning preference nor the place preference induced by morphine (data not shown). However, idazoxan (1–9 mg/kg, i.p.) dose-dependently reversed the inhibition by agmatine 2.5 mg/kg on the acquisition of morphine-induced CPP. The mean score of idazoxan 9 mg/kg group was 539 s, which was significantly different from that of agmatine plus morphine group, 373 s ($P<0.05$, $n=11$, Fig. 2B). This result inferred the possible role of imidazoline receptors in this process. Because agmatine was able to bind with α_2-adrenoceptors, the effect of yohimbine on the action of agmatine was observed. Yohimbine 1–5 mg/kg (i.p.) co-administered with agmatine and morphine did not change the effect of agmatine on morphine-induced CPP in rats. The time of which the rats stayed in the white box in yohimbine groups was not significantly different from that

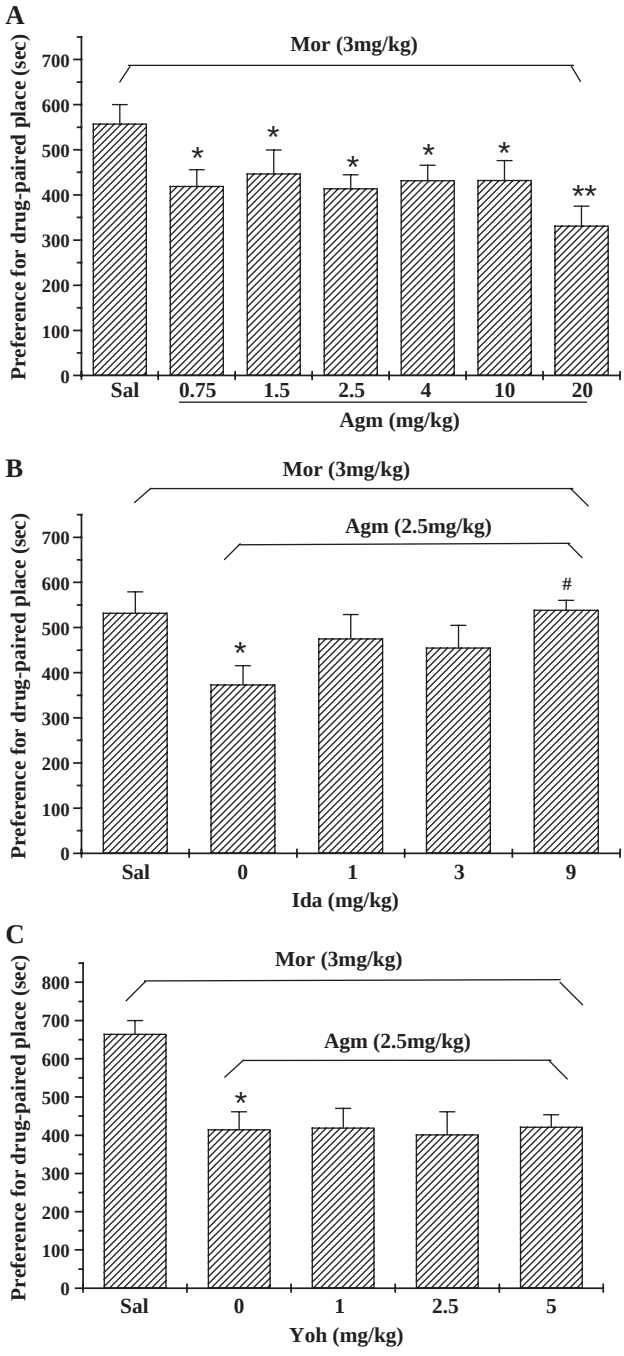

Fig. 2. A. The effect of agmatine on acquisition of morphine-induced CPP. Rats were trained by conditioning sessions twice daily for 9 days and saline (Sal, 1 ml/kg, s.c.) or agmatine (Agm, 0.75–20 mg/kg, s.c.) was co-administered 30 min prior to each injection of morphine (Mor, 3 mg/kg, s.c.) during the conditioning sessions. Test session was carried out 1 day after the last training session in the drug-free state for 15 min. The time that the rats spent in the drug-paired side (the white compartment) was measured. Each value was expressed as mean±S.E.M. of 11 rats in each group. $^{*}P<0.05$, $^{**}P<0.01$, compared with the saline plus morphine group. One-way ANOVA followed by Dunnett's t test. B. The effect of idazoxan on the inhibition by agmatine on CPP induced by morphine. Rats were trained by conditioning sessions twice daily for 9 days. saline (Sal, 1 ml/kg, s.c.) or agmatine (Agm, 2.5 mg/kg, s.c.) was co-administered 30 min prior to each injection of morphine (Mor, 3 mg/kg, s.c.) during the conditioning sessions and idazoxan (Ida, 1–9 mg/kg, i.p.) was given 15 min before agmatine. Test session was carried out 1 day after the last training session in the drug-free state for 15 min. The time that the rats spent in the drug-paired side (the white compartment) was measured. Each value was expressed as mean±S.E.M. of 11 rats in each group. $^{*}P<0.05$, compared with saline plus morphine group by Student's t test. $^{\#}P<0.05$, compared with agmatine plus morphine group by one-way ANOVA followed by Dunnett's t test. C. The effect of yohimbine on the inhibition by agmatine on CPP induced by morphine. Rats were trained by conditioning sessions twice daily for 9 days. Saline (Sal, 1 ml/kg, s.c.) or agmatine (Agm, 2.5 mg/kg, s.c.) was co-administered 30 min prior to each injection of morphine (Mor, 3 mg/kg, s.c.) during the conditioning sessions and yohimbine (Yoh, 1–5 mg/kg, i.p.) was given 15 min before agmatine. Test session was carried out 1 day after the last training session in the drug-free state for 15 min. The time that the rats spent in the drug-paired side (the white compartment) was measured. Each value was expressed as mean±S.E.M. of 11 rats in each group. $^{*}P<0.05$, compared with saline plus morphine group by Student's t test.

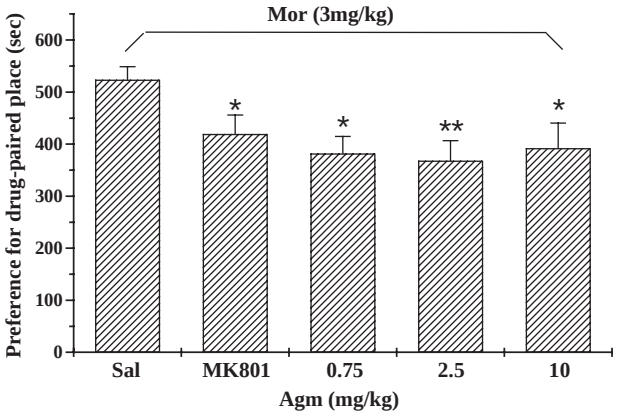

Fig. 3. The effect of agmatine on the expression of CPP induced by morphine. Conditioning sessions were conducted twice daily for 9 days, with the alternate injection of saline (Sal, 1 ml/kg, s.c.) and morphine (Mor, 3 mg/kg, s.c.). Test session was carried out 1 day after the last training session in the drug-free state for 15 min. On the test day, saline (Sal, 1 ml/kg, s.c.), MK801 (0.1 mg/kg, i.p.) or agmatine (Agm, 0.75–10 mg/kg, s.c.) was injected 30 min before the test. The time that the rats spent in drug-paired side (the white compartment) was measured. Each value was expressed as mean ± S.E.M. of 14 rats in each group. *$P<0.05$, **$P<0.01$, compared with the morphine/saline group. One-way ANOVA followed by Dunnett's t test.

of rats in the agmatine plus morphine group ($P>0.05$, $n=11$, Fig. 2C). This result inferred that the effect of agmatine might not be related to the α_2-adrenoceptors.

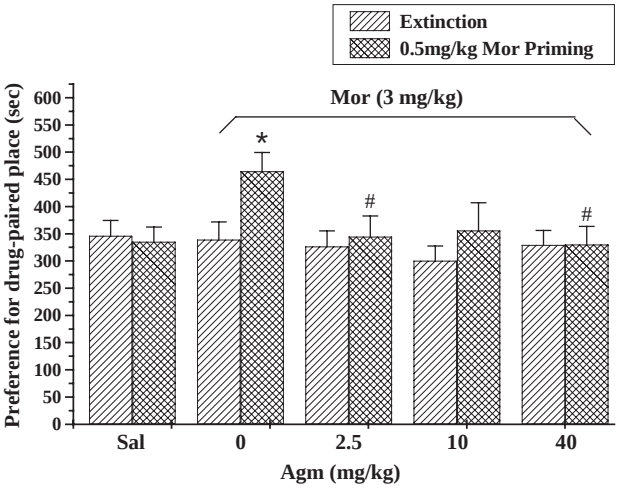

Fig. 4. The effect of agmatine on the priming effect of morphine on CPP. Conditioning sessions were conducted twice daily for 9 days involved alternate injection of saline (Sal, 1 ml/kg, s.c.) or morphine (Mor, 3 mg/kg, s.c.). Rats were trained for conditioned preference to the drug-paired side by morphine before they were maintained in drug-free state for about 30 days and test was carried out once a week until the extinction of CPP. A priming test was performed after complete extinction of CPP in rats. On the priming test day, saline or agmatine (Agm, 2.5–40 mg/kg, s.c.) was injected 30 min prior to morphine injection (0.5 mg/kg, s.c.) and the rats were placed into the boxes immediately after morphine injection, then the priming effect was observed. The time that the rats spent in the drug-paired side (the white compartment) was measured. Each value was expressed as mean ± S.E.M. of 13 rats in each group. *$P<0.05$, compared with previous saline group by morphine priming by Student's t test. $^{\#}P<0.05$, compared with the previous morphine group by morphine priming, one-way ANOVA followed by Dunnett's t test.

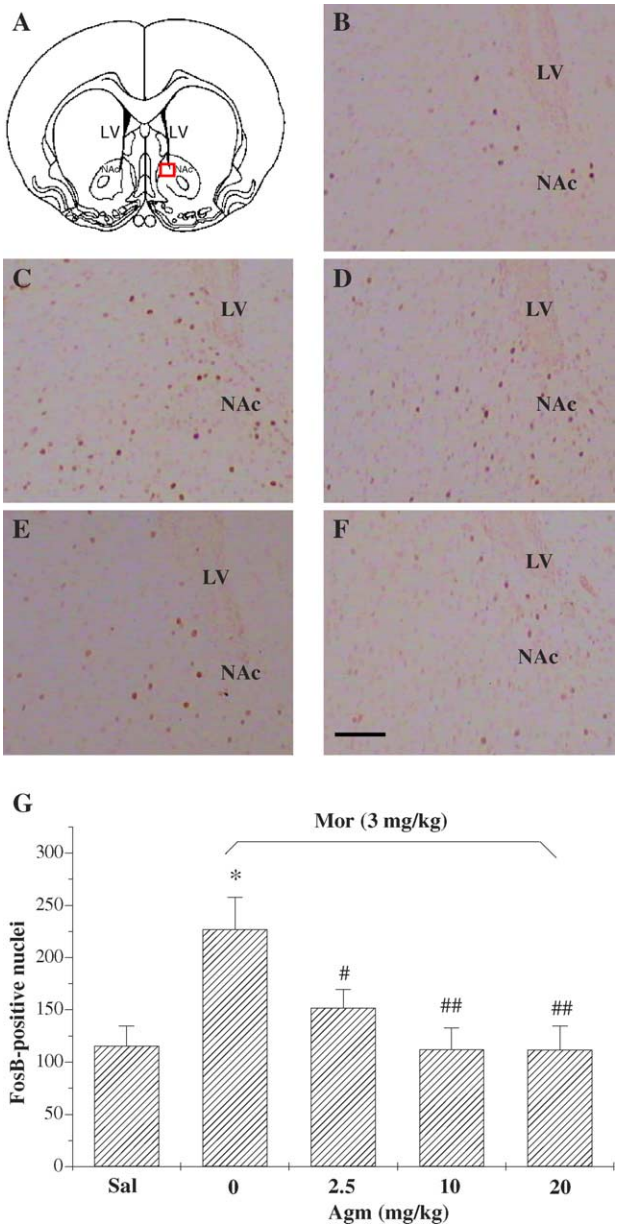

Fig. 5. The effect of agmatine on FosB expression in nucleus accumbens of morphine-induced CPP rats. The outline around the nucleus accumbens was shown in A and the red square marked the area in which the following slides displayed. Photomicrographs representing the sections immunostained with the H-75 anti-FosB were shown in B–F. FosB-positive neurons were predominately situated in nucleus accumbens of brain. A few FosB-positive neurons were observed in saline (Sal, 1 ml/kg, s.c.) control animals (B), while an increasing number of FosB-positive neurons were seen in the morphine-treated (Mor, 3 mg/kg, s.c.) rats (C). The effect of agmatine (Agm, 2.5, 10, 20 mg/kg, s.c.) on FosB expression of morphine-induced CPP rats as shown in D–F. LV, lateral ventricle; NAc, nucleus accumbens. The counts of positive neurons were expressed as mean ± S.E.M. of 6 rats in each group and shown in G. *$P<0.05$, compared with saline group by Student's t test. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the morphine group by one-way ANOVA followed by Dunnett's t test. Scare bar = 50 μm.

3.3. Effect of agmatine on the expression of morphine-induced CPP (Fig. 3)

After the conditioning sessions induced by morphine, the rats were injected with saline (1ml/kg, s.c.) or agmatine (0.75–10 mg/kg, s.c.) on the test day and placed into the shuttle boxes 30 min later. As shown in Fig. 3, a NMDA receptor antagonist MK-801 (0.1 mg/kg, i.p.) significantly inhibited the expression of morphine-induced CPP in rats, and this result was consistent with that of other studies (Suzuki et al., 2000). The scores of three morphine/agmatine groups were lower than those of the morphine/saline group and there was significant statistical difference among these groups ($F_{(3,50)}=3.66$, $P<0.05$, $n=14$). This result suggested that agmatine inhibited the expression of CPP induced by morphine.

3.4. Effect of a single injection of agmatine on priming effect by morphine (Fig. 4)

After 30 days of extinction, CPP induced by morphine disappeared with the scores of all drug treatment groups the same as those of the saline group. On the next day all groups of rats were placed into the boxes immediately after being injected morphine 0.5 mg/kg or agmatine (2.5, 10 or 40 mg/kg) plus morphine 0.5 mg/kg and the time spent in the drug-paired side was recorded. Compared with the previous saline group, morphine 0.5 mg/kg significantly primed CPP of the previous morphine group, the time the rats spent in the white box increased from 339 s to 464 s. Agmatine inhibited the priming effect of morphine 0.5 mg/kg, and the scores of agmatine 2.5 mg/kg and 40 mg/kg group were 345 s and 330 s, respectively, which were significantly different from that of morphine group ($P<0.05$, $n=13$, Fig. 4).

3.5. Effect of agmatine on FosB expression in nucleus accumbens of morphine-induced CPP rats (Fig. 5)

Compared with the saline control group, the number of FosB positive neurons in nucleus accumbens significantly increased during the development of CPP induced by morphine. The number increased from 116 to 227. Agmatine 2.5, 10 and 20 mg/kg inhibited the effect of morphine, with which the number of FosB positive neurons reduced to 152, 112, 111 respectively ($F_{(3.20)}=4.87$, $P<0.05$, $n=6$, Fig. 5A–G). This result inferred that the inhibition by agmatine on morphine-induced CPP might be related to the effect on expression of the transcription factor FosB.

4. Discussion

In the present study, we found that agmatine (0.75, 2.5, 10, 40 mg/kg, s.c.) alone didn't produce place preference or place aversion in the rat model for CPP. However, at the dose of 0.75–20 mg/kg, agmatine inhibited the acquisition and expression of CPP induced by morphine. Idazoxan inhibited the effect of agmatine while yohimbine did not. Furthermore, agmatine inhibited the priming effect of morphine on CPP. The effect of agmatine on the acquisition of CPP induced by morphine was related to its inhibition of the expression of immediate early gene FosB.

With the model of self-administration in rats, Morgan et al. demonstrated that agmatine attenuates the escalation of fentanyl self-administration when administered before the escalation of intake occurs (Morgan et al., 2002). This is the first report on the inhibitory effect of agmatine on opioid psychological dependence. Our present study further proved the effect of agmatine on the rewarding effect of the drug on the CPP animal model. CPP paradigm has been widely used to study the reinforcing effect of psychostimulants, such as cocaine, amphetamine and opioids (Bardo et al., 1995). Agmatine inhibited the acquisition of morphine-induced CPP, which suggested that agmatine prevented the development of psychological dependence and inferred the possible application of agmatine to the prevention of positive reinforcement for opioids. Agmatine itself did not induce CPP or CPA, which suggested that the effect of agmatine on morphine-induced CPP is not based on its own effect on spontaneous behavior, but related to the interaction between the morphine-opioid receptor system and agmatine and its action system. All these results are similar to the effect of agmatine on morphine analgesia, tolerance to and physical dependence on morphine (Li et al., 1998, 1999a,b).

Agmatine binds with the α_2-adrenoceptors (Sugawara et al., 2001), activates imidazoline receptors (Reis and Regunathan, 2000), antagonizes NMDA receptor (Gibson et al., 2002), blocks calcium channel (Weng et al., 2003) and inhibits NOS activity (Li et al., 1999c), and all these five targets are closely related to morphine dependence. In the present study, idazoxan, as an imidazoline receptors antagonist, reversed the inhibition of agmatine on morphine-induced CPP in a dose-dependent manner. However, yohimbine did not antagonize the inhibition by agmatine. Thus, despite the action of agmatine on different targets, the effect of agmatine on the acquisition of CPP induced by morphine is at least partly related to activation of imidazoline receptors rather than α_2-adrenoceptors. These results are consistent with our previous work, which showed the effect of agmatine on morphine analgesia, tolerance to and physical dependence on morphine were also related to the imidazoline receptors (Su et al., 2003).

Expression of morphine induced CPP exhibits drug-induced pathological memory process. In this study, besides its effect on acquisition of morphine-induced CPP, agmatine also inhibited the expression of morphine-induced CPP, which inferred that agmatine modulates the reminiscence of the reinforcing effect of drugs. This result pointed to the possible role of agmatine in the therapy of psychological dependence. As reported by Suzuki, MK-801 inhibited the expression of CPP induced by morphine (Suzuki et al., 2000), the only difference between our study and Suzuki's

result is the inhibitory rate for 20% and 70%, which might be attributed to different animal species (rats and mice) and different conditioning sessions in our laboratory and Suzuki's. Morphine-induced CPP is persistent over time and can be reinstated by morphine after extinction (Mueller et al., 2002), which simulates the relapsing process of drugs and often reflects the long-term memory of animals on the rewarding effect of these kinds of drugs. Agmatine inhibited the priming effect of morphine 0.5 mg/kg after 30 days of CPP extinction, which showed the inhibition by agmatine of the recalling of long-term rewarding memory on opioids. This effect is important for the application of agmatine to the prevention of drug relapse and was further convinced us of the inhibition by agmatine of psychological dependence on morphine.

Recently, ΔFosB was supposed to be an important transcription factor that reflects the development of psychological dependence. The expression of ΔFosB will increase after chronic morphine treatment (Chen et al., 1995, 1997; Nye and Nestler, 1996), and the induction of ΔFosB may contribute to sensitized responses to drug exposure (Nestler et al., 2001). In the current study, during the development of CPP, morphine significantly increased the number of cells that expressed FosB, indicating that this immediate early gene is an indicator for the rewarding effect of morphine. Agmatine co-administration with morphine inhibited this increase and this effect is parallel to its inhibition of morphine-induced CPP. This result inferred the possible mechanisms for the effect of agmatine on morphine induced CPP and provided further evidence that FosB plays an important role in the development of psychological dependence on morphine.

In conclusion, agmatine inhibited the acquisition and expression of morphine-induced CPP, and blocked the priming effect of morphine on CPP. All these effects may be produced through activation of the imidazoline receptors and related to inhibition of FosB expression. These results inferred the possible use of agmatine in the prevention and treatment of psychological dependence on opioids. The exact mechanisms of agmatine on opioid receptors system need to be further studied.

Acknowledgements

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