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# Agmatine blocks acquisition and re-acquisition of intravenous morphine self-administration in rats

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ABSTRACT

Our previous studies showed that agmatine inhibits morphine-induced conditioned place preference, locomotor sensitization and drug discrimination in rats. In the present study, we investigated the effects of agmatine on intravenous morphine self-administration in rats. At a dose of 80 mg/kg/infusion, agmatine did not substitute for intravenous morphine (0.5 mg/kg/infusion) self-administration, suggesting that agmatine itself has no reinforcing effect. However, pretreatment with agmatine (40 or 80 mg/kg, i.g.) significantly inhibited the acquisition of intravenous morphine self-administration as assessed by the nose-poke response and morphine intake. The mean number of days required to meet the acquisition criteria for intravenous morphine self-administration was significantly prolonged. After acquisition of intravenous morphine self-administration, chronic administration of agmatine (40 or 80 mg/kg × 30 days, bid, i.g.) during the extinction period significantly prevented the re-acquisition of intravenous morphine self-administration. The ability of agmatine to inhibit the acquisition and re-acquisition of intravenous morphine self-administration suggests a possible use of agmatine in the treatment of opioid dependence.



## 1. Introduction

Drug addiction is a complicated behavioral disturbance characterized by physical dependence, psychological dependence and relapse. Psychological dependence, which is characterized by euphoria, drug craving, compulsive drug seeking and drug taking (Mendelson and Mello, 1996; O'Brien, 1997), is the major reason for drug relapse. Opioids enhance the activity of specific neurobiological circuits commonly described as "brain reward systems". The rewarding or reinforcing effects of drugs are related mainly to the nucleus accumbens (NAc) and the ventral tegmental area (VTA), while multiple brain sites are involved in compulsive drug-seeking and drug-taking behavior of addictive drugs.

Craving is the strong motivation to reinstate both drug-seeking and drug-taking behavior. Relapse can be triggered by direct exposure to drugs (de Wit, 1996), by stimuli previously associated with drug taking (Carter and Tiffany, 1999), or by exposure to stressors (Kreek and Koob, 1998). Experiences of the rewarding effect lead from controlled drug use to uncontrollable drug consumption, which is characterized by the escalation of drug self-administration. Even after a long period of extinction, escalation of drug self-administration can still be triggered rapidly by re-exposure to drugs under the drive of strong motivation and the pathological memory of the reinforcement of the drugs.

For a long time, researchers have believed that the neurobiological basis of this disease is the adaptation of the opioid receptor system at molecular, cellular and neural network levels after long-term exposure to opioids (De Vries and Shippenberg, 2002; Nestler, 2004). Based on this hypothesis, many new drugs that target opioid receptors, such as methadone and naltrexone, have been developed for the therapy of psychological dependence and prevention of opioid relapse. In recent years, many non-opioid receptors, such as dopamine, NMDA, 5-HT, GABA, have come to be seen as being involved in drug dependence and relapse (Heidbreder and Hagan, 2005). Ligands binding to these receptors modulate the pharmacological effects of opioids including enhancement of opioid analgesia, and inhibition of tolerance to and dependence on opioids. These substances were defined as "opioid function modulators" (Su et al., 2003). These results provided a new basis for developing new drugs that attenuate opioid dependence and relapse. Many compounds have been found that target non-opioid receptors that have anti-relapse effects. For example, in preliminary clinical studies, memantine hydrochloride, a non-competitive NMDA receptor antagonist, inhibited morphine intake behavior in addicts (Heidbreder and Hagan, 2005) and blocked reinstatement of morphine-conditioned reward (Popik et al., 2006). These studies provided direct experimental evidence that it would be possible to find other effective anti-opioid compounds that target non-opioid receptors.

Agmatine, a product of L-arginine decarboxylatation, is an endogenous ligand of the imidazoline receptor. It was isolated in the mammalian

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central nervous system (CNS) in 1994 (Li et al., 1994). Besides binding to the imidazoline receptor, agmatine also antagonizes the NMDA receptor (Gibson et al., 2002), blocks calcium channels (Weng et al., 2003) and inhibits nitric oxide synthase (NOS) activity (Li et al., 1999b). Systemic administration of agmatine was found to enhance the analgesic effects of morphine (Yeşilyurt and Uzbay, 2001; Ruiz-Durantez et al., 2003), inhibit tolerance to morphine analgesia (Kolesnikov et al., 1996; Li et al., 1998, 1999a; Fairbanks and Wilcox, 1997), and attenuate morphine and alcohol withdrawal syndromes (Uzbay et al., 2000; Aricioglu-Kartal and Uzbay, 1997; Li et al., 1998, 1999a). In addition, it produces neuroprotective effects in persistent pain and neuronal injury models (Fairbanks et al., 2000). Although agmatine itself did not induce conditioned place preference, locomotion sensitization and drug discrimination behavior, it significantly inhibited morphine-induced conditioned place preference, locomotion sensitization and drug discrimination (Wei et al., 2005, 2007; Su et al., 2008b), and inhibited intravenous fentanyl self-administration (Morgan et al., 2002). These findings suggest that agmatine may have pharmacotherapeutic potential in the treatment of opioid dependence, including inhibition of craving and relapse to drug-taking and drug-seeking behaviors.

The intravenous drug self-administration model is a commonly used animal model to study the drug-seeking and drug-taking behavior of addictive drugs (Ahmed and Koob, 1997, 1998). In 2002, agmatine was found to inhibit intravenous fentanyl self-administration (Morgan et al., 2002). Agmatine (30 mg/kg; i.v.) inhibited escalation of intravenous fentanyl self-administration in rats when it was administered before the escalation occurred, which suggested a possible preventive role of agmatine in the reinforcement of opioid dependence. However, the potential of agmatine to induce escalation of intravenous self-administration and the possible effect of agmatine on the acquisition and re-acquisition of intravenous morphine self-administration after extinction period are not clear.

In the present study, we used this animal model to observe the effects of intragastric administration of agmatine on the acquisition and re-acquisition of intravenous morphine self-administration in rat models. We hoped that this would provide evidence supporting its possible use in the treatment of opioid dependence and relapse.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats initially weighing 200–220 g were used. Rats were grouped 6 per cage, acclimated to the housing conditions, and handled for 3–4 days before experiments to minimize handling stress during testing. Animals were maintained on a 12 h light/dark cycle (lights on between 7:00 A.M. and 7:00 P.M.) and given *ad libitum* access to food and water. After surgery, all rats were individually housed in operant chambers and allowed to recover for 3 days. Subsequently, rats received free access to water and a daily ration of 20 g food, which maintained their body weight and ensured an adequate responding range to test the effects of pharmacotherapy. All experimental procedures were conducted in accordance with the guidelines for the use of experimental animals approved by the local ethical committee and the Institutional Review Committee on Animal Care and Use.

### 2.2. Apparatus

The drug self-administration system included a data collecting system, a computer-controlled system, operant chambers, a food pump, a constant speed infusion pump, and an intravenous transfusion channel (aero-filter, valve for counter flow prevention, rolling axis) for rats. Rats were trained under programmed procedures and the data were recorded automatically by a computer. There were two symmetric nose pokes installed on the same wall of the chamber. The rats received an infusion of morphine after a nose poke on the right nose-poke hole under the fixed ratio (FR) schedule, with no reinforcement for the wrong nose poke.

### 2.3. Drugs

Morphine hydrochloride was purchased from Qinhai Pharmaceutical Factory, China. Agmatine sulfate was synthesized by the Beijing Institute of Pharmacology and Toxicology, China. Sulbactam was provided by the Institute of Clinical Pharmacology of Peking University. All drugs were dissolved in normal saline (NS) to their final concentrations and injected in a volume of 1.0 ml/kg. Drug solutions were added daily to the intravenous reservoir at room temperature. Agmatine sulphate was given intragastrically (i.g.).

### 2.4. Procedure for drug self-administration test

#### 2.4.1. Substitution of agmatine for intravenous morphine self-administration

Rats were anesthetized with pentobarbital (40 mg/kg, i.p.) during surgery. Silastic venous catheters were embedded through the femoral vein with the tip terminating at the opening of the right atrium. Rats were put into the chamber after the surgery. A venous catheter was connected with a constant speed infusion pump through a cannula connector. Rats could freely move in the cage. After 3 days of routine anti-infection with sulbactam (25 mg/kg, i.m.), the training period was begun (on day 4). Rats were fasted during the training session and free access to water and food was added after the experiment to allow the rats to maintain stable body weights.

To evaluate the substitutability of agmatine for intravenous morphine self-administration, six rats were initially trained with morphine (0.5 mg/kg/infusion) for intravenous self-administration under a FR1 (fixed rate 1, 1 nose poke for one infusion) schedule on day 4 after surgery. Each session lasted 4 h (8:30–12:30). The catheter was washed? before each session with 0.5 ml NS. After the required? number of infusions, rats were kept stable for 5 consecutive days. It was then determined whether agmatine (80 mg/kg/infusion) could substitute for morphine and maintain the reinforcing effects associated with morphine. This test lasted 4 days (days 6 to 9). On day 10, morphine (0.5 mg/kg/infusion) was again used and the number of infusions was recorded for 4 days (days 10 to 13). In the laboratory, room temperature was maintained at $22 \pm 2$ °C and the relative humidity at 70%.

#### 2.4.2. Effect of agmatine on the escalation of intravenous morphine self-administration

Rats were divided into 3 groups and treated with agmatine (0, 40 or 80 mg/kg, i.g.) before each morphine training session on day 4 after surgery, respectively. Each group consisted of 8 rats.

Rats were trained with morphine (1.0 mg/kg/infusion) for intravenous self-administration for 40 consecutive days under FR 1 to FR 5 schedules. On each experimental day, NS or agmatine (40 or 80 mg/kg; i.g.) was administrated 30 min before the training session with morphine. The nose-poke response and the morphine intake (mg/kg) of rats were recorded. Acquisition of self-administration behavior was defined as 10 episodes of drug infusion during a single session. The mean number of days to meet the acquisition criteria was defined as the time (in days) for the acquisition of intravenous morphine self-administration.

#### 2.4.3. Effect of agmatine treatment during the extinction period on the re-acquisition of intravenous morphine self-administration

Rats were initially trained with morphine (1.0 mg/kg/infusion) for intravenous self-administration for 40 consecutive days under FR 1 to FR 5 schedules. After the acquisition of intravenous morphine self-administration, all rats were taken out of the operant chamber and placed in ordinary cages for extinction of self-administration behavior.

Twenty four rats were divided into 3 groups of 8 per group. During the extinction period, the rats in the 3 groups were treated with NS or agmatine – 40 and 80 mg/kg, bid, i.g. – respectively for 30 days. After 30 days of treatment, all rats were operated on again and trained with morphine (1.0 mg/kg/infusion, FR5) for re-acquisition of intravenous morphine self-administration without agmatine treatment. The nose-poke response, morphine intake (mg/kg) and mean number of days to meet criteria for the re-acquisition of intravenous morphine self-administration were recorded.

### 2.5. Data analysis

Data are expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's *t* test was used to analyze between group differences in the mean number of days required to meet acquisition criteria. For substitution experiments (agmatine replacing intravenous morphine self-administration), one-way ANOVA followed by Bonferroni-corrected *t* tests were used for statistical analysis. Two way ANOVA followed by Bonferroni-corrected Student's *t* tests were used to analyze differences in nose-poke response and morphine intake (mg/kg) among groups at different times after agmatine treatment. A *P* value of less than 0.05 was chosen as the critical criterion for statistical significance.

## 3. Results

### 3.1. Substitution of agmatine for intravenous morphine in self-administration

After being trained with morphine (0.5 mg/kg/infusion) for intravenous drug self-administration under an FR1 schedule, all rats acquired self-administration behavior. The number of infusions averaged 25.9. After 5 days of stable intravenous morphine self-administration, agmatine (80 mg/kg/infusion) replaced morphine. The infusion number during the 4 days of agmatine substitution (days 6 to 9) was 4.5, which was significantly lower than the infusion number (25.9) before agmatine substitution ($t=7.59$, $P<0.001$). This finding implied that morphine-associated drug-taking behavior could not be generalized to agmatine, and that agmatine does not have the same characteristics as morphine in this model. After restoration of intravenous morphine self-administration from days 10 to 13, the infusion number increased to 13.5, which was significantly different from that for days 6 to 9 ($t=3.03$, $P<0.05$). However, the average infusion number during days 10 to 13 was still lower than that before substitution ($t=4.39$, $P<0.01$). This result suggested an effect of agmatine on the reinforcement of morphine and the possible effect on morphine relapse in the drug self-administration model. See Fig. 1.

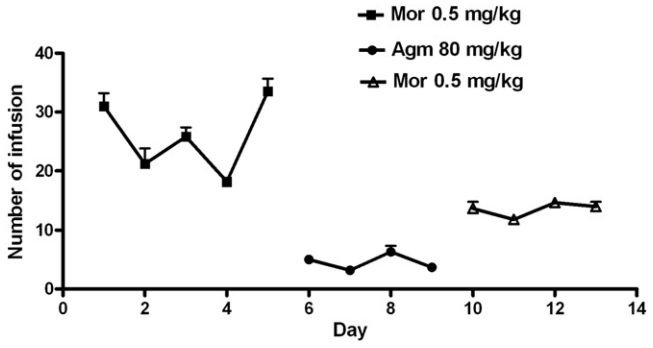


**Fig. 1.** Agmatine (Agm, 80 mg/kg/infusion) substitution for morphine (Mor) did not maintain intravenous morphine self-administration behavior in rats previously treated with morphine (0.5 mg/kg/infusion). $n=6$, means ± S.E.M. One-way ANOVA followed by Bonferroni-corrected Student's *t* tests.

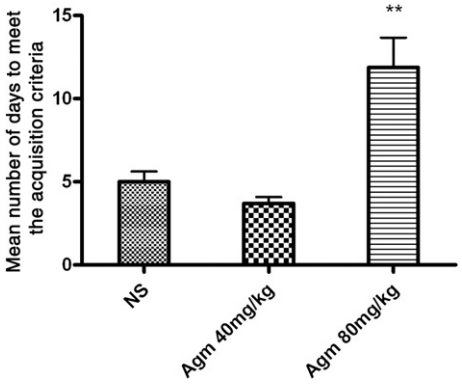


**Fig. 2.** Agmatine (Agm) prolonged the mean number of days required to meet the acquisition criteria for the acquisition of intravenous morphine (Mor) self-administration. $n=8$, means ± S.E.M. $^{**}P<0.01$, compared with the NS group, one-way ANOVA followed by Dunnett's *t* test.

### 3.2. Agmatine inhibits the escalation of intravenous morphine self-administration

After a morphine training period, rats in the NS group acquired stable drug self-administration behavior. In the agmatine treated groups, all rats also acquired intravenous morphine self-administration after the training period, which implied that agmatine at the doses involved (40 and 80 mg/kg) does not completely block the acquisition of intravenous morphine self-administration. However, the mean number of days to meet the acquisition criteria in the group treated with 80 mg/kg agmatine was prolonged to 11.9 ($t=4.37$, $P<0.01$). This indicated that agmatine inhibits the acquisition of intravenous morphine self-administration and might be useful for the prevention of morphine dependence. See Fig. 2.

Fig. 3A shows the mean number of nose pokes per 4-h session during the 40-day training period. The average nose-poke response of the rats in the NS group reached 113.8 times per session. There was a significant difference among the three groups ($F_{2,\ 39}=379.18$, $P<0.0001$). Agmatine at 40 mg/kg (i.g.) had no effect on the nose-poke response (an average of 110.8 times per session) compared with the NS group. Agmatine at 80 mg/kg (significantly decreased the nose-poke response to 45.8 times per session. Bonferroni-corrected Student's *t* tests revealed statistically significant differences between the agmatine 80 mg/kg group and the NS group for single day means for days 3, 8–14, 16–23, 25, 34–35, 37, and 40.

Fig. 3B shows the average morphine intake (mg/kg) per 4-h session during the 40-day training period. The average morphine intake (mg/kg) in the NS group was 6.76 mg per session. There was a significant difference among the three groups ($F_{2,\ 39}=922.66$, $P<0.0001$). Agmatine treatment significantly decreased morphine intake (mg/kg) during the training period. The morphine intakes (mg/kg) in the 40 and 80 mg/kg agmatine treated groups were 3.28 and 1.78 per session, respectively. Bonferroni-corrected Student's *t* tests revealed between group differences on single day means for days 1, 4–24, 33, and 40 for the 40 mg/kg agmatine group and for days 1, 3–34, 40 for the 80 mg/kg agmatine group, compared with the NS group.

### 3.3. Agmatine treatment during the extinction period inhibits the re-acquisition of intravenous morphine self-administration

After 30 days of extinction, the nose-poke response decreased to 29.25 times per session in morphine pre-treated rats, which implied the extinction of intravenous morphine self-administration behavior. In the NS group, the rats re-acquired intravenous morphine self-administration after re-exposure to morphine, and the mean number of days to meet the acquisition criteria was 2.13. After the rats were treated with 40 or 80 mg/kg agmatine (i.g.) for 30 days during

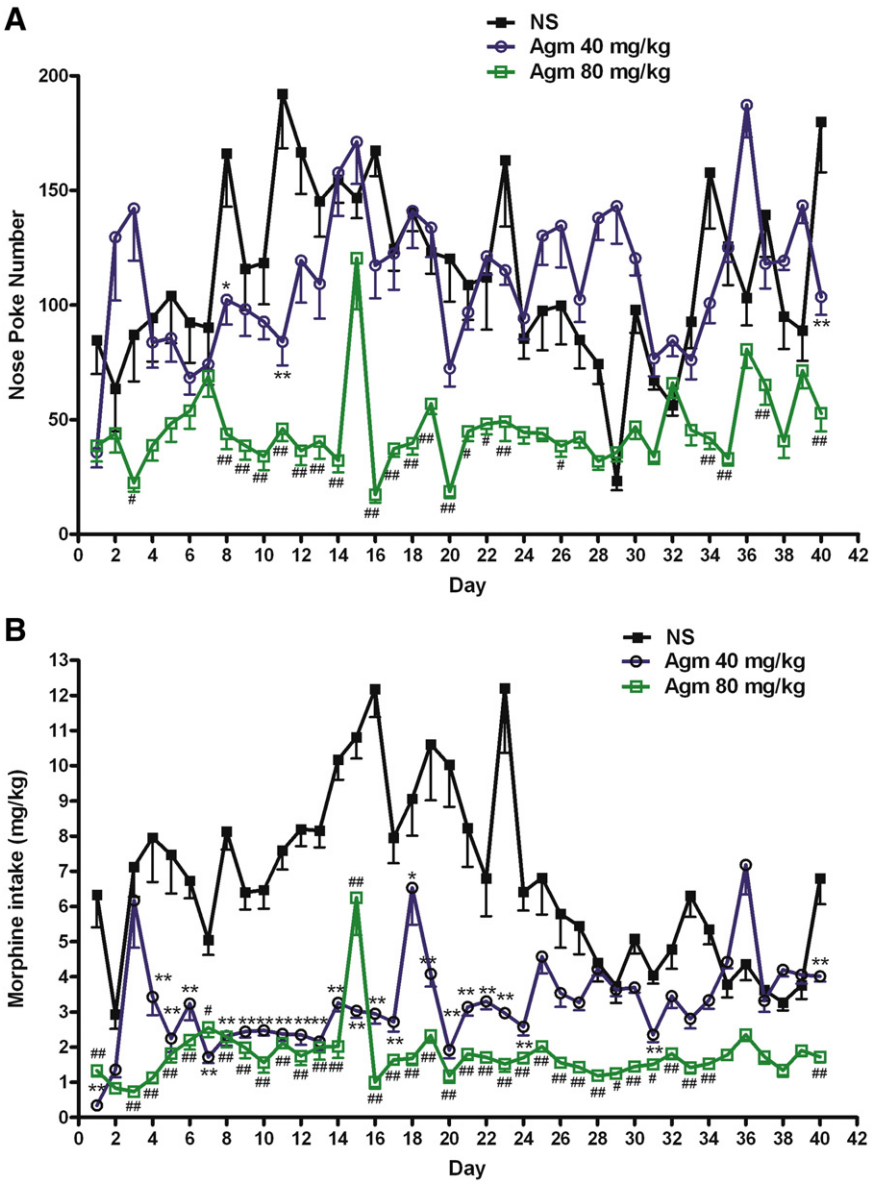


**Fig. 3.** Agmatine (Agm) inhibited the escalation of intravenous morphine (Mor) self-administration in respect to the nose-poke response (Panel A) and morphine intake (mg/kg) (Panel B). $n=8$, means ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, showed differences between NS and Agm 40 mg/kg groups; $^{\#}P<0.05$, $^{\#\#}P<0.01$ showed differences between NS and Agm 80 mg/kg groups; two way ANOVA followed by Bonferroni-corrected Student's *t* tests.

extinction, the mean numbers of days to meet the acquisition criteria for the re-acquisition of intravenous morphine self-administration were prolonged to 6.71 ($t=4.65$, $P<0.01$) and 11.86 ($t=9.88$, $P<0.01$) days, respectively, which were significantly different from that of the NS group. This result implies that agmatine inhibits the re-acquisition of morphine self-administration and might be useful for the prevention of opioid relapse. See Fig. 4.

After 30 days of extinction, the nose-poke response decreased to 29.25 times per session in morphine pre-treated rats while in the 40 and 80 mg/kg agmatine treated groups the nose-poke response decreased to 10.29 and 3.14 times per session on the first day after re-exposure to morphine. Fig. 5A shows the mean nose-poke number per 4-h session during the 25 days (days 35 to 59) of the morphine re-training period. The average nose-poke response of the rats in the NS group reached 103.0 times per session, which indicated the re-acquisition of intravenous morphine self-administration. After the rats were treated with 40 or 80 mg/kg agmatine (i.g.) for 30 days during the extinction period, the average nose-poke responses of the rats

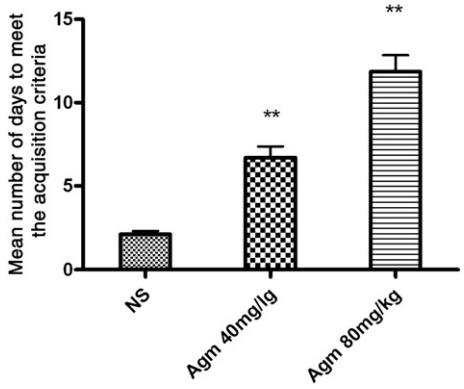


**Fig. 4.** Agmatine (Agm) prolonged the mean number of days to meet criteria for the re-acquisition of intravenous morphine (Mor) self-administration. $n=8$, means ± S.E.M. $^{**}P<0.01$, compared with the NS group, one-way ANOVA followed by Dunnett's *t* test.

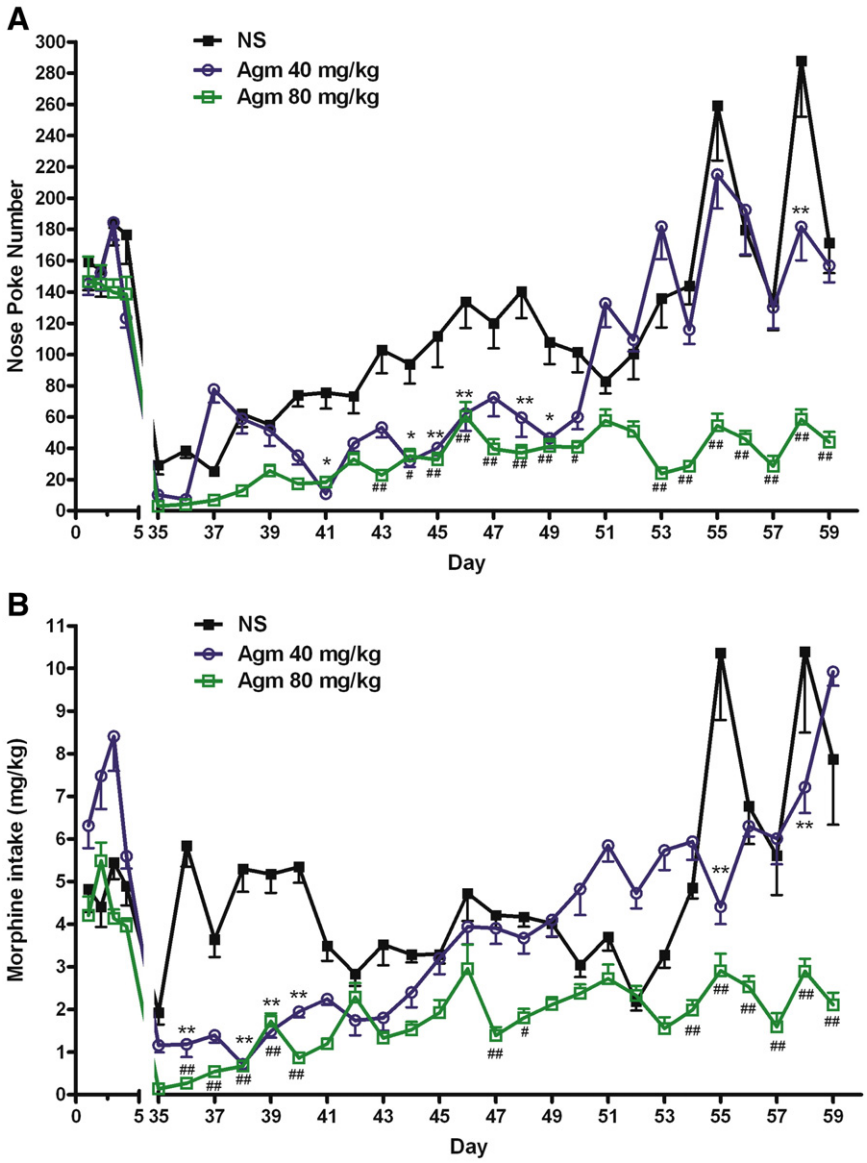


**Fig. 5.** Agmatine (Agm) inhibited the re-acquisition of intravenous morphine (Mor) self-administration in respect to the nose-poke response (Panel A) and morphine intake (mg/kg) (Panel B). $n = 6$, means ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, showed differences between NS and Agm 40 mg/kg groups; $^{\#}P<0.05$, $^{\#\#}P<0.01$ showed differences between NS and Agm 80 mg/kg groups; two way ANOVA followed by Bonferroni-corrected Student's *t* tests.

decreased to 60.0 times and 33.0 times per session, respectively. There was a significant difference between the three groups ($F_{2, 24} = 262.19$, $P<0.0001$). Bonferroni-corrected Student's *t* tests revealed between groups differences on single day means for days 41, 44–46, 48–49, 58 in the 40 mg/kg agmatine group and for days 41, 43–50, 53–59 for the 80 mg/kg agmatine group, compared with the NS group.

Fig. 5B shows the average morphine intake (mg/kg) per 4-h session during the 25 days of the morphine re-training period. The average morphine intake (mg/kg) of the rats in the NS group during the 25 days of the morphine re-training period was 4.76 mg per session. There was a significant difference between the three groups ($F_{2, 24} = 241.42$, $P<0.0001$). After the rats were treated with 40 or 80 mg/kg agmatine (i.g.) for 30 days during the extinction period, the average morphine intake (mg/kg) of the rats decreased to 3.83 mg and 1.75 mg per session, respectively. Bonferroni-corrected Student's *t* tests revealed between groups differences on single day means for days 36, 38–40, 55, 58 in the 40 mg/kg agmatine group and for days 36–40, 47–48, 54–59 for the 80 mg/kg agmatine group, compared with the NS group. See Fig. 5A and B.

## 4. Discussion

In the present study, 80 mg/kg/infusion of agmatine did not substitute for intravenous morphine self-administration. Instead it inhibited the escalation of intravenous morphine self-administration with respect to the nose-poke response and morphine intake (mg/kg). Agmatine (40, 80 mg/kg, i.g.) treatment during the extinction period significantly inhibited the re-acquisition of intravenous morphine self-administration displayed by prolongation of the mean number of days to meet the acquisition criteria and by a decrease in the nose-poke response and in morphine intake (mg/kg). These findings suggest that agmatine may have therapeutic effects on morphine-induced escalation of intravenous self-administration and relapse.

In recent years, opioid dependence and relapse have been postulated to relate not only to the opioid receptor system, but to many non-opioid receptor systems as well. Thus, in addition to the inhibition of naloxone on the acquisition of drug self-administration and drug discrimination by directly antagonizing the opioid receptor (Walker et al., 2004; Greenwald and Roehrs, 2005), many non-opioid ligands were also

reported to influence psychological dependence and relapse induced by opioids (Heidbreder and Hagan, 2005). In our previous study, agmatine was found to inhibit morphine-induced conditioned place preference (Wei et al., 2005), behavioral sensitization (Wei et al., 2007) and drug discrimination (Su et al., 2008b) through non-opioid imidazoline receptor mechanisms. In 2002, Morgan found that agmatine inhibited the escalation of intravenous fentanyl self-administration in rats (Morgan et al., 2002). These results imply a possible preventive and therapeutic role for agmatine against opioid induced escalation of intravenous self-administration.

To evaluate the effect of agmatine on opioid dependence and relapse, the dependence potential of agmatine should be determined first. In our previous study, agmatine exhibited no dependence potential in our physical dependence model (Su et al., 2008a), our conditioned place preference animal model (Wei et al., 2005) and our drug discrimination animal model (Su et al., 2008b). In drug self-administration tests, a psychoactive stimulus of the same kind of drugs can be generalized as was exhibited by substitution. Thus, through substitution of drug self-administration, we can sub-classify different pharmacological types of drugs. In the present study, agmatine (80 mg/kg/infusion) did not substitute for morphine (0.5 mg/kg/infusion) induced reinforcement. Whether agmatine induces aversive and punishing self-administration behaviors that are maintained by the opioid is very important. In our previous work, agmatine did not induce conditioned place aversion (Wei et al., 2005) and exhibited very low toxicity in rats and mice (data not reported). These results, which were consistent with our previous study which found that agmatine did not bind to opioid receptors (Su et al., 2003), indicated that agmatine is not a member of the opioid class. To sum up, these effects indicate that agmatine does not have a euphoric effect like morphine does; it has different pharmacological actions not shared by morphine or opioids.

In the present study, agmatine was administrated before opioids to evaluate whether agmatine influences the reinforcing effects of opioids. Agmatine co-administrated with morphine significantly prevented the escalation of intravenous morphine self-administration, like its effect on the escalation of fentanyl intake (Morgan et al., 2002). However, although agmatine prolonged the mean number of days to meet the acquisition criteria for the acquisition of intravenous morphine self-administration, it did not block the acquisition of drug self-administration completely, further implying that agmatine exerts its effect through non-opioid mechanisms of opioid dependence. Based on these results, we conclude that agmatine intervenes in neuro-adaptation during chronic morphine treatment, which suggests a possible clinical application of agmatine in the prevention of positive reinforcement for opioids.

Morphine-induced psychological dependence is persistent over time and can be reinstated by morphine after long-term extinction, which simulates the relapsing process of drugs and to some extent reflects the long-term memory of animals of the rewarding effect of these kinds of drugs. Agmatine inhibited the priming effect of 0.5 mg/kg morphine after 30 days' extinction of conditioned place preference (Wei et al., 2005), which shows an inhibition by agmatine of the recalling of long-term rewarding memory for opioids. Morgan reported that agmatine at a relatively high dose, 30 mg/kg (i.v.), attenuates the escalation of intravenous fentanyl self-administration when administered before the escalation of intake and may mediate neuro-adaptive events related to chronic opioid self-administration (Morgan et al., 2002). Based on his work, we further investigated the possible role of agmatine in the relapse into opioid self-administration. In the present study, agmatine (40, 80 mg/kg, bid, i.g.) treatment for 30 days during the extinction period significantly inhibited the escalation of intravenous morphine self-administration. This effect of agmatine is important for its possible application in the prevention of drug relapse, and further demonstrates inhibition by agmatine of the reinforcing effect and possible relapse of opioid abuse. As is known, the neuronal events mediating drug-seeking behavior are to some degree dissociable from those mediating drug-taking behaviors (Shalev et al., 2002). Drug priming, cues, and stressors can also trigger drug reinstatement (Shaham et al., 1996). Through the use of these kinds of animal models, we plan to further investigate the effects of agmatine on the motivation underlying drug craving behavior.

Agmatine binds to the $\alpha_2$-adrenoceptor (Sugawara et al., 2001), activates imidazoline receptors (Reis and Regunathan, 2000), antagonizes the NMDA receptor (Gibson et al., 2002), blocks the calcium channel (Weng et al., 2003) and inhibits NOS activity (Li et al., 1999b). All five of these targets are closely related to morphine dependence. It has been reported that agmatine enhances morphine-induced analgesia dose-dependently in mice via the $\alpha_2$-adrenoceptor (Yeşilyurt and Uzbay, 2001). Agmatine inhibits ethanol and morphine withdrawal syndromes (Uzbay et al., 1997, 1998, 2000; Adams et al., 1995; Aricioglu-Kartal and Uzbay, 1997), which might be related with inhibition on NOS (Li et al., 1999b). In addition, an interaction between NO and NMDA receptors has been reported (Garthwaite, 1991; Uzbay and Oglesby, 2001) and this interaction may also be important in the inhibitory effects of agmatine on morphine intake in rats. Morgan attributed the effect of agmatine to the block of NMDA receptors and inhibition of NOS activity (Morgan et al., 2002). In our previous work, agmatine was shown to inhibit morphine-induced conditioned place preference and locomotion sensitization through activation of imidazoline receptors (Wei et al., 2005, 2007). Based on the present data, we are not sure about the exact mechanisms for the effects of agmatine. However, the effects of agmatine on intravenous morphine self-administration might relate to all these receptors, enzymes and their interactions. Therefore, the exact receptor mechanisms underlying the effects of agmatine require further studies.

In conclusion, agmatine did not substitute for intravenous morphine self-administration, which implies that agmatine does not have a euphoric effect like morphine. Agmatine attenuated but did not completely inhibit the acquisition of intravenous morphine self-administration. Agmatine significantly inhibited drug intake behavior indicated by decreases in nose-poke responses and in morphine intake (mg/kg), which suggests a possible use of agmatine in the prevention of morphine addiction. Chronic treatment with agmatine during the extinction period inhibits the re-acquisition of intravenous morphine self-administration after re-exposure to morphine, which suggests a possible role of agmatine in the prevention of opioid relapse. Agmatine appears to have little if any potential for creating a reinforcing effect. It had a modulatory effect on morphine-induced psychological dependence and could be useful for preventing relapse in opioid dependent individuals.

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