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Effects of agmatine on tolerance to and substance dependence on morphine in mice

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KEY WORDS agmatine; morphine; opioid-related disorders; idazoxan; yohimbine; clonidine

ABSTRACT

AIM: To study the effects of agmatine on tolerance to and dependence on morphine. **METHODS:** Inhibitory effects of agmatine on tolerance to and substance dependence on morphine were observed in mouse tolerant models and in mouse jumping test, respectively. **RESULTS:** Agmatine 0.125 - 2.5 $mg \cdot kg^{-1}$ prevented the development of tolerant to morphine in a dose-dependent manner. Pretreatment of mice with morphine induced an over 3-fold increase in analgesic ED_{50} (20.1, 14.4 - 28.0 $mg \cdot kg^{-1}$) than those with normal saline (6.3, 5.1 - 7.8 $mg \cdot kg^{-1}$). Pretreatment of mice with both of agmatine and morphine made morphine loss the ability to induce tolerance. Withdrawal jumps and loss in body weight induced by naloxone in morphine-dependent mice were prevented by agmatine (2.5 - 10 $mg \cdot kg^{-1}$) in a dose-dependent manner. ED_{50} of naloxone (21.4, 18.4 - 24 $mg \cdot kg^{-1}$) required to precipitate withdrawal jumps in mice pretreated with both agmatine and morphine was 8 times higher than that with morphine alone (2.5, 2.1 - 2.8 $mg \cdot kg^{-1}$). These effects of agmatine were blocked by idazoxan. **CONCLUSION:** Agmatine prevented tolerance to and substance dependence on morphine in mice by activation of imidazoline receptors.

INTRODUCTION

Opioid tolerance and substance dependence limited their clinical use greatly. There were two methods to overcome them, ie, development of powerful analgesics without tolerance and dependence or of drugs which antagonized opioid tolerance and dependence, but did not attenuate their analgesia. Agmatine was recently isolated from brains of mammals (including man) and was considered as endogenous ligand of imidazoline receptors (I-R)[1], although it was previously believed to be restricted to bacteria, plants and invertebrates[2]. Agmatine, 4-(aminobutyl) guanidine, was produced by decarboxylation of *L*-arginine by the enzyme *L*-arginine decarboxylase and was biologically active in mammals. It stimulated the release of catecholamine from adrenal chromaffin cells, increased arterial blood pressure when injected intracisternally in rats, stimulated the release of insulin, and increased the release of gonadorelin from hypothalamus[3]. Agmatine potentiated opioid analgesic effects and antagonized the tolerance induced by them[4]. Our unpublished results also suggested that agmatine itself had a weak analgesic effect, and in addition to potentiating the analgesic effect of opioids, it could also potentiate the analgesic effect of clonidine. In the present study, we attempted to investigate the inhibitory effects of agmatine on tolerance to and substance dependence on morphine and analyzed the relationship between the effects of agmatine and I-R or α_2-adrenoceptors *in vivo* in order to

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provide a clue for development of a new drug which could inhibit opioid tolerance and substance dependence but did not influence analgesia.

MATERIALS AND METHODS

Equal number of male and female Kunming mice obtained from Experimental Animal Center of Academy of Military Medical Sciences (n = 620, Certificate № 01-3023), weighing 20.1 g ± s 2.2 g were used. Agmatine and yohimbine were purchased from Sigma Co. Morphine and clonidine were obtained from Qinghai and Hangzhou Pharmaceutical Factory, respectively. Naloxone was the product of Sihuan Pharmaceutical Factory. All drugs were injected subcutaneously (sc), except for idazoxan which was injected intraperitoneally (ip). Agmatine or clonidine was injected 30 min prior to morphine, while yohimbine or idazoxan was injected 15 min prior to agmatine or clonidine.

In tolerant experiment, analgesia was assessed quantitatively in mice 55 ℃ warm bath test or mouse heat radiant tail-flick assay 30 min after sc morphine. Analgesia was defined as prolongation of latency to twice as long as baseline $\bar{x}$ of all mice in the group or even longer, or evaluated by possible maximal analgesic %: PMAP = (latency after medication − baseline latency)/(10 − baseline latency). In order to set up tolerant model, mice were pretreated with a single dose of morphine 100 mg·kg^{-1}, morphine 10 mg·kg^{-1}(bid, for 9 d) and morphine 20 mg·kg^{-1}(tid, for 3 d), respectively. Agmatine was given after finishing morphine analgesic assay at the time when pain threshold was determined, because it could enhance the analgesia of morphine. On the other hand, to observe the therapeutic effect of agmatine on tolerance, mice were classified into normal saline and agmatine groups. The mice in both of the two groups were further divided into 0, 6, 24, 48, and 72 h groups (n = 20) and pretreated with a single dose of morphine 100 mg·kg^{-1} to induce tolerance to analgesia of morphine 10 mg·kg^{-1} except for 0 h group. Normal saline or agmatine (5 mg·kg^{-1}) was given every 12 h after administration of the single dose of morphine except for 6 h group and the last dose of agmatine was injected 4 h prior to determination of pain threshold. The pain threshold was determined at 6, 24, 48, and 72 h after pretreatment with single dose of morphine.

In substance dependent experiment, mice were pretreated with normal saline, morphine (30 mg·kg^{-1}, tid, for 3 d) and agmatine + morphine, respectively. The degree of substance dependence was evaluated in quantitative and qualitative tests, respectively. In quantitative test[5], the degree of the dependence on morphine was assessed by estimating the amount of naloxone (sc) required to induce withdrawal jumping. It had been shown that there was an inverse relationship between the degree of the dependence and the amount of naloxone required to precipitate withdrawal jumps in mice[6]. The criterion for positive jumping response was that a mouse was required to jump more than 3 times during the first 15 min period after sc naloxone. In qualitative test, the number of jumps in the first 15 min and loss of body weight in the first 1 h after ip naloxone 20 mg·kg^{-1} were observed. To study therapeutic effect of agmatine on withdrawal syndrom, mice were pretreated with morphine (30 mg·kg^{-1}, tid for 3 d) and divided into normal saline and agmatine groups. The mice in both two groups were further divided into 6, 24, 48, and 72 h groups. Normal saline and agmatine (5 mg·kg^{-1}) were injected every 12 h after sc last dose of morphine except for 6 h groups, respectively. The last dose of normal saline or agmatine was injected 15 min prior to naloxone. Naloxone (20 mg·kg^{-1}, ip) was given at 6, 24, 48, and 72 h after sc last dose of

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morphine in corresponding groups. The withdrawal jumps of morphine-dependent mice were determined in the first 15 min after ip naloxone.

All studies employed groups of at least 10 mice each. Dose-response curves comprised at least 3 doses of drugs and were evaluated using Bliss program. Fisher exact test was used for examining the significance of difference between the analgesic % of single dose of drugs and t-test was used for PMAP and other quantitative data.

RESULTS

In qualitative tolerant experiment, agmatine inhibited the development of tolerance induced by sc morphine for 9 d (Fig 1).

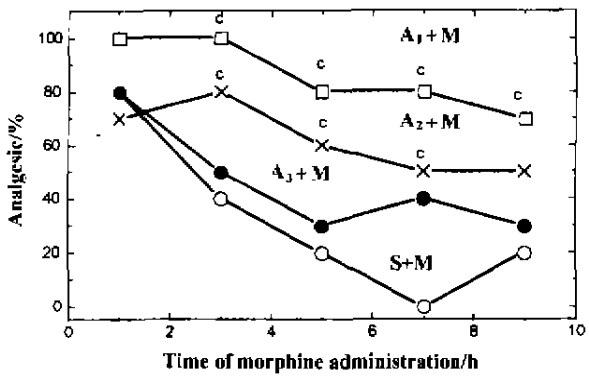

Fig 1. Inhibitory effect of agmatine (A) on tolerance to analgesia of morphine (M) in mouse warm bath test. The mice were treated with normal saline (S), S + M (10 mg·kg^{-1}) or A (A_1 = 2.5, A_2 = 1.25, A_3 = 0.13 mg·kg^{-1}) + M for 9 d. n = 10 mice. $^cP < 0.05$ *vs* S + M group, Fisher exact test.

When mice were pretreated with morphine alone, their responses to the analgesia of morphine 10 mg·kg^{-1} were decreased obviously by d 5. Coadministration of agmatine at 0.13, 1.25, and 2.5 mg·kg^{-1} with morphine prevented the decrease in the analgesic effect of morphine as compared with those pretreated with morphine alone ($P < 0.01$, $n = 10$).

In quantitative tolerant experiment, either repeated (20 mg·kg^{-1}, tid for 3 d) or single large dose (100 mg·kg^{-1}) administrations of morphine caused an increase in its analgesic ED_{50} by about 3-folds as compared with that in naive mice (Tab 1). Agmatine inhibited the increase in a dose-dependent manner. The analgesic ED_{50} of morphine obtained from the mice pretreated with highest dose of agmatine had no significant difference as compared with naive mice.

Tab 1. Effect of agmatine (A) on morphine (M) analgesic ED_{50} of mice pretreated with repeated sc or a single large dose of M in heat radiant tail-flick test. n = 10 mice. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* normal saline (S), Bliss program.

Drug/ mg·kg^{-1}	ED_{50}/ mg·kg^{-1}	95 % Confidence limits	ED_{50} ratio
Repeated dose of M			
S	6.3	5.1 - 7.8	-
M 10	20.1^c	14.4 - 28.0	-
A + M 0.1 + 10	11.2^a	8.5 - 11.9	1.8
A + M 0.5 + 10	8.4^a	6.0 - 11.8	2.4
A + M 2.5 + 10	6.7^a	5.5 - 8.3	3.0
Single dose of M			
S	5.7	4.2 - 7.2	-
M 100	15.4^c	12.3 - 19.1	-
A + M 0.5 + 100	10.0^b	8.1 - 12.3	1.5
A + M 2.5 + 100	5.4^a	4.0 - 7.3	2.9
A + M 12.5 + 100	5.2^a	4.2 - 6.4	3.0

I-R and α_2-adrenoceptor agonist clonidine also prevented the tolerance induced by single large dose of morphine 100 mg·kg^{-1} like agmatine. The effects of the two drugs were not influenced by non-imidazoline α_2-adrenoceptor antagonist yohimbine, but were antagonized by selective I-R blocker idazoxan in a dose-dependent manner (Tab 2).

On the other hand, in addition to preventing the tolerance to morphine, agmatine attenuated the tolerant processes. The tolerance induced by morphine 100 mg·kg^{-1} in mouse tail-flick assay persisted at least over 72 h (Fig 2).

Tab 2. Influence of drugs in the preventive effects of agmatine (A) on morphine (M)-induced tolerance to analgesia of M 10 mg·kg^{-1} in mouse heat radiant tail-flick test. n = 10 mice. $\bar{x} \pm s$. $^cP < 0.01$ vs S, t-test.

Drug/mg·kg^{-1}		Possible maximal analgesia/%
S	–	89 ± 22
S + M	+ 100	31 ± 15^c
A + M	5.0 + 100	82 ± 28
I_1 + A + M	0.3 + 5.0 + 100	40 ± 17^c
I_2 + A + M	1.5 + 5.0 + 100	31 ± 10^c
C + M	0.3 + 100	79 ± 32
I_1 + C + M	0.3 + 0.3 + 100	39 ± 10^c
I_2 + C + M	1.5 + 0.3 + 100	44 ± 24^c
Y + A + M	2.5 + 5.0 + 100	88 ± 25
Y + C + M	2.5 + 0.3 + 100	87 ± 32

S: normal saline; C: clonidine; I: idazoxan; Y: yohimbine.

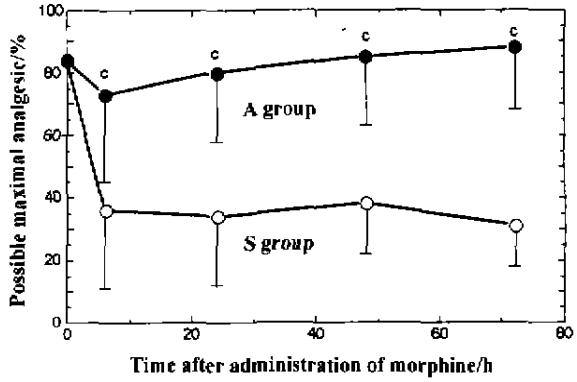

Fig 2. Therapeutic effects of repeated administrations of normal saline (S) or agmatine (A, 10 mg·kg^{-1}) on tolerance to analgesia of morphine (M, 5 mg·kg^{-1}) in mouse tail-flick test. Mice were pretreated with M 100 mg·kg^{-1} to induce tolerance except 0 h group. Pain threshold was determined at 6, 24, 48, and 72 h after administration of large dose of M. n = 20 mice. $\bar{x} \pm s$. $^cP < 0.01$ vs S.

A single dose of agmatine (6-h group) attenuated the tolerant process and restored the sensitivity of mice to morphine analgesia. With increase in the times of administration of agmatine, PMAP recovered gradually to the level in naive mice (Fig 2).

In the substance dependent experiment, naloxone (20 mg·kg^{-1}, ip) induced obvious abstinence syndrome consisting of withdrawal jumps, tremor, shaking, diarrhoea, micturition and loss in body weight of mice pretreated with sc morphine (30 mg·kg^{-1}, tid for 3 d). Administration of agmatine (tid for 3 d) 30 min prior to morphine evoked a significant and dose-dependent decrease in the number of withdrawal jumps and jumping % in first 15 min after administration of naloxone and inhibited the loss in body weight of morphine-dependent mice in the first hour of abstinence syndrome induced by naloxone (Tab 3).

Tab 3. Effect of agmatine (A) on withdrawal jumping % and loss in body weight in morphine (M)-dependent mice. n = 10 mice. $\bar{x} \pm s$ or %. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs normal saline (S), t-test or Fisher exact test.

Drug/mg·kg^{-1}		*Jumping/ %*	*Number of jumps*	*Loss in body weight/g*
S	–	0	0 ± 0	0.12 ± 0.19
M	30 × 3 × 3	100^c	27 ± 55	1.56 ± 0.56^c
A + M	2.5 + 30 × 3 × 3	70^b	14 ± 21	0.23 ± 0.23^a
	5 + 30 × 3 × 3	40^a	19 ± 26	0.10 ± 0.22^a
	10 + 30 × 3 × 3	20^a	10 ± 24	0.16 ± 0.22^a

In quantitative substance dependent experiment, agmatine induced an increase in ED_{50} of naloxone for precipitating withdrawal jumps of morphine-dependent mice in a dose-dependent manner. Coadministration of agmatine (10 mg·kg^{-1}, tid for 3 d) with morphine prevented the development of substance dependence and increased naloxone ED_{50} required for inducing withdrawal jumps by about 8 times as compared with that pretreated with morphine alone (Tab 4).

The above preventive effect of agmatine on the development of substance dependence induced by morphine was not influenced by yohimbine and was antagonized by idazoxan in a

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Tab 4. Effect of agmatine (A) on naloxone ED_{50} for precipitating withdrawal jumps of morphine (M)-dependent mice. S: normal saline.

	Drug/mg·kg^{-1}	ED_{50}/ mg·kg^{-1}	95 % Confidence limits	ED_{50} ratio
S	-	>42.5	-	-
M	30×3×3	2.5	1.9-3.2	-
A+M	2.5+30×3×3	2.5	2.1-2.8	0.96
	5.0+30×3×3	15.3	12.0-18.5	6.00
	10.0+30×3×3	21.4	18.4-24.0	8.41

dose-dependent manner (Tab 5).

Tab 5. Influence of drugs in inhibitory effect of agmatine (A) on mouse withdrawal jumps in the first 15 min after naloxone. $n = 10$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* S, Fisher exact test.

	Drug/mg·kg^{-1}	Jumping/ %	Number of jumps
S	-	10	2 ± 5
S+M	30×3×3	100^c	49 ± 31
A+M	5+30×3×3	20	3 ± 5
C+M	0.3+30×3×3	100^c	94 ± 71
I_1+A+M	0.5+5+30×3×3	90^c	59 ± 59
I_2+A+M	1.5+5+30×3×3	100^c	42 ± 40
I_2+C+M	1.5+0.3+30×3×3	100^c	184 ± 110
Y+A+M	2.5+5+30×3×3	30	4 ± 7
Y+C+M	2.5+0.3+30×3×3	100^c	59 ± 47

S: normal saline; M: morphine; C: clonidine; I: idazoxan; Y: yohimbine.

By contrast with the effect of clonidine on the tolerance, repeated coadministration of clonidine and morphine increased the number of withdrawal jumps of mice induced by naloxone as compared with that pretreated with morphine alone. These effects of clonidine were enhanced by idazoxan and inhibited by yohimbine in some degree (Tab 5).

Agmatine, which prevented the development of substance dependence in mouse experiment, also had a theraputic effect on morphine substance dependence in mice. Multiple doses of agmatine sc in different times prior to naloxone attenuated the process of the dependence on morphine (Fig 3).

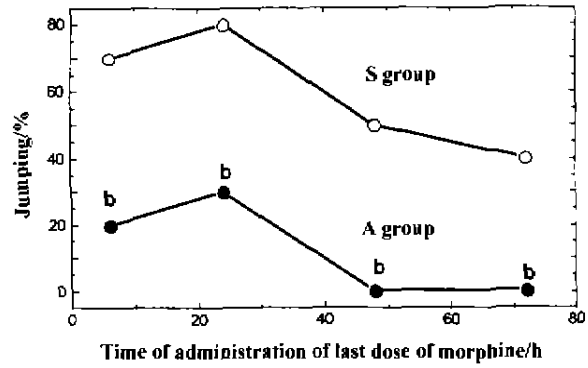

Fig 3. Therapeutic effects of normal saline (S) and agmatine (A, 5 mg · kg^{-1}) on mouse withdrawal jumping % in the first 15 min after administration of naloxone. $n = 10$ mice. $^bP < 0.05$ *vs* S, Fisher exact test.

DISCUSSION

The current research demonstrated that agmatine prevented the development of tolerance to and substance dependence on morphine, and attenuated the processes of them induced by acute or chronic pretreatments with morphine in mice. Our unpublished results and Kolesnikov's work[4] showed that agmatine had a weak analgesic effect and potentiated the analgesic effect of morphine. These results suggested a new kind of chemicals which might be used to prevent and treat opioid tolerance and substance dependence or to potentiate the analgesic effect of opioids.

Although the exact mechanism of preventive and therapeutic actions of agmatine on opioid tolerance and substance dependence was not clear, it might be related to I-R, rather to α_2-adrenoceptors, because these effects of agmatine were not influenced by non-imidazoline structure α_2-adrenoceptor antagonist yohimbine and could be inhibited by selective I-R antagonist idazoxan.

It was reported that antihypertensive drug clonidine was both I-R and α_2-adrenoceptor

agonist[7]. Its effects related to the action of opioids were quite similar to those of agmatine including enhancement of analgesia and inhibition of tolerance[8,9], both of which were sensitive to idazoxan. The differences in effects between agmatine and clonidine were their analgesic potency and influence on withdrawal jumps of mice. Our unpublished results showed that the analgesic effect of clonidine which was antagonized by α_2-adrenoceptor antagonist yohimbine was much stronger than that of agmatine which was sensitive to I-R antagonist idazoxan. The current results demonstrated that clonidine increased the number of withdrawal jumps of morphine-dependent mice, and the effect of clonidine could be enhanced by idazoxan and partly antagonized by yohimbine. Although agmatine could also recognize α_2-adrenoceptor binding sites, it neither activated nor antagonized α_2-adrenoceptor[10]. All these results suggested that the possible reasons for the existence of differences in effects between agmatine and clonidine on opioid actions might be related to the difference in receptors on which they acted. The inhibitory effects of clonidine on tolerance and of agmatine on tolerance and substance dependence induced by pretreatment with morphine in mice were due to activation of I-R. Powerful analgesic effect of and increase in number of withdrawal jumps by clonidine might be related to activation of α_2-adrenoceptors.

In conclusion, agmatine prevented and attenuated morphine tolerance and substance dependent processes. These effects of agmatine might be related to activation of I-R.

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胍丁胺对吗啡所致小鼠耐受和物质依赖的作用

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关键词 胍丁胺；吗啡；阿片类有关的紊乱；咪唑克生；育亨宾；可乐定

目的：观察胍丁胺对吗啡所致耐受和依赖的作用. **方法**：分别在小鼠耐受和跳跃实验中观察胍丁胺抑制吗啡所致耐受和物质依赖的作用. **结果**：胍丁胺 0.125－2.5 mg·kg^{-1}剂量依赖性地阻止小鼠对吗啡耐受. 用吗啡预处理小鼠使吗啡镇痛 ED_{50} (20.1，14.4－28.0 mg·kg^{-1})与盐水组相比(6.3，5.1－7.8 mg·kg^{-1})增加 3 倍以上. 用胍丁胺和吗啡共同预处理小鼠则使吗啡丧失引起耐受的能力. 胍丁胺(2.5－10 mg·kg^{-1})剂量依赖性地抑制由纳洛酮引起的吗啡依赖小鼠之戒断跳跃和体重减轻. 用胍丁胺和吗啡共同处理小鼠使引起小鼠之戒断跳跃所需纳洛酮 ED_{50}(21.4，18.4－24 mg·kg^{-1})较吗啡处理组(2.5，2.1－2.8 mg·kg^{-1})增大 8 倍. 胍丁胺的上述作用均被咪唑克生所阻断. **结论**：胍丁胺通过激活咪唑啉受体而阻止小鼠对吗啡耐受和依赖.

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Partnership between Bioanalytical Systems, Inc (BAS) and Shanghai Institute of Materia Medica (SIMM)

An international partnership has been established between Bioanalytical Systems, Inc (BAS) and Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences. Prof Peter T KISSINGER, the President of BAS and Prof Kai-Xian CHEN, the Director of SIMM recently signed a Master Strategic Agreement.

Both parties will explore mutual opportunities in scientific research in bioanalytical chemistry and the neurosciences. SIMM will help BAS to develop the market in China for BAS instruments and contract bioanalytical services. BAS expects a stronger, faster, better-trained, more innovative workforce, and improved business systems in Shanghai, China. BAS will assist SIMM with alliances with pharmaceutical companies in the USA.

SIMM is one of the most prestigious institutes for the development of new drugs, including traditional medicines, in China. SIMM is located in Shanghai, China. BAS is headquartered in West Lafayette, Indiana with other locations in Kansas, New Jersey, Pennsylvania, and England.