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Influence of idazoxan on analgesia, tolerance, and physical dependence of morphine in mice and rats *in vivo*[1]

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KEY WORDS idazoxan; agmatine; morphine; imidazoline receptors; morphine dependence

ABSTRACT

AIM: To study the influence of idazoxan (Ida), an antagonist of imidazoline receptors (I - R), on analgesia, tolerance, and physical dependence of morphine. **METHODS**: The effects of Ida on pain threshold and morphine analgesia were observed in mouse acetic acid writhing test and 55 ℃ hot plate test. The effect of Ida on morphine tolerance and physical dependence were observed in mouse tolerant model and in mice and rat models. **RESULTS**: Ida (3 - 9 mg/kg) significantly decreased the pain threshold by 120 % in acetic acid writhing test and by 39 % in 55 ℃ hot plate test of mice, respectively. It inhibited the analgesic effect of morphine in a dose-dependent manner. Ida promoted the development of tolerance to morphine in mice and induced the abstinence syndrome in morphine-dependent mice and rats similar to naloxone. **CONCLUSION**: I-R and its endogenous ligand agmatine might participate in the pain threshold and influence morphine analgesia as well as negatively regulate tolerance to and physical dependence on morphine.

INTRODUCTION

Agmatine has been long known to be a constituent of bacteria, plants, and a range of invertebrates, and believed to be a metabolic intermediate in the formation of polyamines in these organisms. Until 1994, it was proved to be existing in mammalian tissues. Later, it was postulated to be an endogenous ligand for imidazoline receptors (I - R)[1]. In 1996, Kolesnikov first reported the pharmacological effects of agmatine on opioids. They found that agmatine enhanced morphine analgesia and inhibited the tolerance to morphine[2]. Research works in our laboratory further shows that agmatine itself has a weak analgesic effect and inhibits tolerance to and substance dependence on morphine in mice and rats *in vivo* and in guinea pig ileum *in vitro*[3-5]. All these effects of agmatine are based on the activation of I - R and can be antagonized by I - R selective antagonist idazoxan (Ida)[3-5]. Agmatine does not directly interact with opioid receptors, it has no influence on the down-regulation or the lowering of ligand binding capability of opioid receptor which takes place in the pathophysiologic process of tolerance and dependence. Agmatine inhibits the NOS activity by activation of I - R and by substrate competition, which is correlated with its effects on inhibition of naloxone-precipitated withdrawal jumps in morphine dependent mice[6].

Agmatine is an endogenous ligand of imidazoline receptors. Since exogenous agmatine has so much obvious influence on morphine actions, it is possible that endogenous agmatine and I-R may have effects on pain threshold, opioid analgesia, tolerance to, and dependence on opioid. In the present research, we use Ida to observe the possible influence of endogenous agmatine on the effects of morphine.

MATERIALS AND METHODS

Male and female (1:1) mice (20 g ± *s* 2 g, Grade II, Certificate № 01-3023) and male Wistar rats (200 g ± *s* 20 g, Grade II, Certificate № 01-3039) were used. Ida was purchased from Research Biochemicals International (Natick MA, USA). Morphine hydrochloride and acetic acid were produced by Qinghai Pharmaceutical Factory and Beijing Chemical Plant, respectively. Naloxone hydrochloride was from Sigma (St Louis, MO, USA).

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At least 10 mice or rats were used in each group. Ida and naloxone were given intraperitoneally (ip); morphine was injected subcutaneously (sc).

Pain threshold test Ida was ip 30 min prior to determination of pain threshold[7]. Mice in acetic acid writhing test were injected 0.1 % acetic acid (20 mL/kg, ip), which is the minimal concentration able to induce positive responses. The number of writhing, characterized by a wave of contraction of the belly followed by extension of the hind limbs, was counted for 15 min after ip acetic acid. In 55 ℃ hot plate test[8], pain threshold (the period from the contact of the limbs of mice with the hot plate to the kicking or licking of the hind limbs) was determined before drug administration and 30 min after Ida injection.

Morphine analgesia test Ida was injected 30 min prior to sc morphine, then 30 min later, the pain threshold was determined. Mice in acetic acid writhing test were ip 0.6 % acetic acid (20 mL/kg) 30 min after sc morphine. The number of writhings was counted for 15 min thereafter. In 55 ℃ hot plate test, pain threshold of mice were determined for each mouse before drug administration and 30 min after morphine injection. Analgesia was expressed as possible maximal analgesic percentage (PMAP) = [(latency after medication − baseline latency)/(60 s − baseline latency) × 100 %].

Tolerance test In order to set up a tolerance model, mice were pretreated with morphine (30 mg/kg, tid for 3 d, sc). Different doses of Ida or normal saline were given 30 min prior to morphine. Baseline latency was determined at d 1 for each mouse before any drug administration and reassessed at the end of chronic treatment program (d 4). In 55 ℃ hot plate test, analgesia was evaluated by PMAP as mentioned above. In mouse heat radiation tail-flick assay[9], the tail-flick latency (TFL) was defined as the time from the onset of radiant heat to tail withdrawal, the analgesia was also evaluated by PMAP [PMAP = (latency after medication − baseline latency)/(16 s − baseline latency) × 100 %].

Physical dependence test[10] The animals were pretreated with normal saline or increasing doses of morphine (90 − 210 mg·kg^{-1}·d^{-1}) for 5 d. Normal saline, naloxone or different doses of Ida were injected ip, 6 h after the last injection of morphine, the number of jumps in the first 30 min and loss of body weight in the first 1 h were observed. *In rat physical dependence test*[11], male rats were pretreated with normal saline or increasing doses of morphine (30 − 150 mg·kg^{-1}·d^{-1}) for 5 d. Normal saline, naloxone or different doses of Ida were injected by ip route at 6 h after the last injection of morphine, the abstinence syndrome in the first 15 min and loss of body weight in the first 1 h were recorded. The withdrawal signs were recorded as follows: jumping, writhing, wet dog shakes, and teeth chattering were counted and scored as 1 − 3 scales; ptosis, diarrhea, and hostility on handling were scored on a different scale[11].

Statistic analysis *t*-Test was used to analyze all the data.

RESULTS

Decrease in the pain threshold of mice By observing dose-responses to different concentrations of acetic acid to induce pain, we determined that 0.1 % acetic acid (20 mL/kg, ip) was the minimal dosage capable of inducing writhing responses of 100 % mice tested (data not shown). The writhing number was about 4 for each mouse within the period of 15 min after injecting the acid. Ida did not induce the writhing responses in mice directly even at the highest doses (data not shown) but it could significantly increase the writhing number induced by 0.1 % acetic acid by 120 % *vs* control (n = 16, $P < 0.05$). In 55 ℃ hot plate test, Ida significantly shortened the time for mice to stay on the hot plate in a dose-dependent manner ($n = 20$, $P < 0.05$). The pain threshold was 61 % as much as that of control. These results indicated that Ida was able to lower the pain threshold in mice (Tab 1).

Tab 1. Effect of Ida (ip) on basic pain threshold in mice acetic acid writhing test ($n = 16$) and in 55 ℃ hot plate test ($n = 20$). $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* NS.

Group	Dose (mg/kg)	Frequency of writhing	Pain Threshold/s
NS	−	4.4 ± 5.0	12.4 ± 5.0
Ida	3.0	5.8 ± 3.6	12.2 ± 5.3
	5.2	8.3 ± 6.0^b	9.5 ± 3.8
	9.0	9.7 ± 6.8^c	7.6 ± 4.9^b

NS: normal saline; Ida: idazoxan

Inhibition on analgesic effect of morphine by Ida In mouse acetic acid writhing test, morphine 2.5 mg/kg almost completely inhibited writhing responses of mice to 0.6 % acetic acid compared with that of control. The inhibitory effect of morphine, however, was markedly decreased by pretreatment with Ida. The writhing number was increased from 0.3 to 19.8 for each mouse

(Tab 2). In 55 ℃ hot plate test, morphine (5 mg/kg) significantly prolonged the period for mice to stay on the hot plate compared with normal saline group ($n = 18$, $P < 0.01$). Similar to the results obtained from acetic acid test, Ida also decreased the PMAP of morphine in a dose-dependent manner (Tab 3). These results showed that Ida inhibited the analgesic effect of morphine.

Tab 2. Effect of Ida (ip) on morphine (M) analgesia in mouse acetic acid writhing test. $n = 18$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **M.**

Group	Dose (mg/kg)	Frequency of writhing
NS	–	26 ± 13
M	2.5	0.10 ± 0.20
Ida + M	3.0 + 2.5	0.30 ± 0.70
	5.2 + 2.5	3.0 ± 4.3
	9.0 + 2.5	5.9 ± 5.4[c]
Ida	9.0	20 ± 19

NS: normal saline; Ida: idazoxan.

Tab 3. Effect of Ida (ip) on morphine (M) analgesia in 55 ℃ mouse hot plate test. $n = 20$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* **M.**

Group	Dose (mg/kg)	PMAP
M	5.0	43 ± 22
Ida + M	3.0 + 5.0	34 ± 17
	5.2 + 5.0	31 ± 16
	9.0 + 5.0	27 ± 22[b]

PMAP: possible maximal analgesic percentage = [(latency after medication − baseline latency)/(60 s − baseline latency) × 100 %]; Ida: idazoxan.

Promotion of Ida on morphine tolerance

When mice were pretreated with morphine alone for 3 d, the analgesic effect of morphine 10 mg/kg was decreased significantly, suggesting the occurrence of tolerance. Pretreatment of the animals with Ida alone for 3 d, however, did not influence the analgesic effect of morphine significantly. Co-administration of different dose of Ida with morphine further decreased the analgesic effect of morphine compared with that of morphine group ($n = 10$, $P < 0.05$) in mouse heat radiant tail-flick assay. Similar results were obtained from 55 ℃ hot plate test (Tab 4). These results suggested that Ida promoted the development of tolerance in mice.

Tab 4. Influence of Ida (ip) on morphine analgesia in morphine tolerant mice in the mouse heat radiant tail-flick assay (morphine 10 mg/kg, PMAP1) and in 55 ℃ hot plate test (morphine 20 mg/kg, PMAP2). $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **M.**

Group	Dose (mg/kg)	PMAP1	PMAP2
NS	–	100.0 ± 0.0	89 ± 20[b]
M	30	75 ± 33	61 ± 35
Ida + M	3.0 + 30	76 ± 25	36 ± 21[b]
	5.2 + 30	67 ± 37	43 ± 33
	9.0 + 30	45 ± 19[b]	27 ± 17[c]
Ida	9.0	94 ± 18	82 ± 25

NS: normal saline; Ida: idazoxan; M: morphine. PMAP1 = [(latency after medication-baseline latency)/(16 s-baseline latency) × 100 %]; PMAP2 = [(latency after medication-baseline latency)/(60 s-baseline latency) × 100 %].

Abstinence syndrome induced by Ida Both naloxone and Ida did not induce jumping of the mice which were pretreated with normal saline. Naloxone induced jumping responses in the animals pretreated with morphine. The jumping number of the morphine-dependent mice induced by naloxone was increased significantly compared to that of normal saline. Different doses of Ida also induced jumping response and increased the jumps of the morphine-dependent mice, especially in the 9.0 mg/kg group ($n = 10$, $P < 0.05$) similar to naloxone. In the present test, loss in body weight in the morphine-dependent mice was not significant after either naloxone or Ida administration compared with those of normal saline group (Tab 5).

Tab 5. Effect of Ida (ip) on number of jumps and loss in body weight in morphine-dependent mice. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **NS.**

Group	Dose (mg/kg)	Number of jumps	Loss of body weight/g
NS	–	2.8 ± 6.5	0.50 ± 0.20
Nal	5.0	54 ± 52[c]	0.60 ± 0.20
Ida	3.0	7.9 ± 9.7	0.40 ± 0.20
	5.2	10 ± 11	0.30 ± 0.10
	9.0	31 ± 54[b]	0.40 ± 0.30

NS: normal saline; Ida: idazoxan; Nal: naloxone.

Typical abstinence signs such as jumping, writhing, wet dog shakes, teeth chattering, ptosis, diarrhea, and hostility on handling were seen in the morphine-dependent rats after naloxone administration. Like naloxone, Ida

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also significantly induced abstinence syndrome in the morphine-dependent rats in a dose-dependent manner compared with those of normal saline group, though the abstinent signs were not as severe as those in the naloxone group ($n = 10$, $P < 0.05$, $P < 0.01$) (Tab 6). The loss in body weight of the morphine-dependent rats was significantly different from normal saline group after either naloxone ($n = 10$, $P < 0.05$, $P < 0.01$) or Ida administration ($n = 10$, $P < 0.05$, $P < 0.01$).

Tab 6. Effect of Ida (ip) on abstinence syndrome and loss in body weight in morphine-dependent rats. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* NS.

Group	Dose (mg/kg)	Abstinence score	Body weight lost/g
NS	–	0.7±0.8	2.5±1.1
Nal	5.0	8.7±2.4^c	13.1±4.1^c
Ida	3.0	1.6±1.5	4.1±2.3
	5.2	2.5±1.5^b	4.3±2.7^b
	9.0	3.7±1.7^c	4.9±2.0^c

NS: normal saline; Ida: idazoxan; Nal: naloxone.

DISCUSSION

In 1984, Atlas *et al* partially purified a substance from calf brain that could competitively and completely displace the binding of [^{3}H]clonidine; this material was named clonidine displacing substance (CDS)[12]. In 1994, Li *et al* isolated a molecule of CDS from bovine brain and proved this molecule to be agmatine by mass spectroscopy[1]. Further study found that agmatine could bind to all imidazoline receptors (I_1 and I_2) and was bioactive, so agmatine was assumed to be an endogenous ligand for I – R. Based on this theory, Kolesnikov postulated agmatine might have the same effect as clonidine that influencing opioid analgesia. Studies in our laboratory showed that agmatine had a weak analgesic effect itself and enhanced the analgesic effect of morphine in a dose-dependent manner[3], which could be blocked by Ida, a selective antagonist of I – R, suggesting that I – R might be involved in opioid analgesia. In the present experiment, the results showed that blockade of the effect of endogenous agmatine decreased the basic pain threshold and inhibited morphine analgesia. These observations can be used to explain why exogenous agmatine has a weak analgesic effect and enhances morphine analgesia, indicating that I-R and its endogenous ligand agmatine might be related to the pain threshold and regulation of the opioid analgesia.

Many scholars thought that the mechanism of tolerance to and dependence on opioid was through adaptation and that the adaptation took place on opioid receptors and their signal transduction system at different levels after long term interaction between opioid receptors and high concentrations of opioid. However, recent studies further showed that many non-opioid receptors were relevant to the development of tolerance to and dependence on opioids. For example, NMDA receptor antagonists and NOS inhibitors could prevent the opioid tolerance and substance dependence completely[13,14]; mice without dopamine D_2 receptors could not develop psychological dependence[15]. Furthermore, many endogenous substances were proved to be important during the development of opioid tolerance and dependence, such as dynorphin, CCK, neuropeptide FF and so on[16]. All these phenomena indicate that tolerance to and dependence on opioid are not only connected with the adaptation of opioid receptors and their signal transduction system but also modified by many other substances and receptors. Since agmatine is an endogenous ligand of I – R, and exogenous agmatine can prevent tolerance to and dependence on morphine[4-6,17], it can be reasonably inferred that I – R and endogenous agmatine may be another system to modulate the opioid effects. The present study demonstrates that Ida promotes the development of tolerance to morphine. In the mouse and rat morphine-dependent test, Ida induced the abstinence syndrome of morphine-dependent animals similar to naloxone. These results support the hypothesis that I – R and endogenous agmatine may be another system to modulate the opioid effects and explain why exogenous agmatine could inhibit tolerance to and substance dependence on opioids.

In conclusion, the present results demonstrate that Ida lowers the basic pain threshold, inhibits the morphine analgesia, promotes the tolerance to morphine and induces the abstinence signs of morphine-dependent animals, indicating that I-R and its endogenous ligand agmatine play a very important role in the regulation of opioid actions and the formation of pain threshold as well as proving the existence of cross talk between opioid receptor and imidazoline receptors.

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咪唑克生对吗啡镇痛、耐受和身体依赖的影响[1]

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关键词 咪唑克生；胍丁胺；吗啡；咪唑啉受体；吗啡依赖

目的：观察咪唑克生对吗啡镇痛及吗啡所致耐受和躯体依赖的影响。**方法**：采用小鼠醋酸扭体实验和55 ℃热板实验观察咪唑克生对基础痛阈及吗啡镇痛作用的影响；采用小鼠热辐射甩尾实验和小鼠55 ℃热板实验观察咪唑克生对吗啡耐受形成过程的影响；采用大鼠、小鼠身体依赖模型观察咪唑克生对吗啡所致身体依赖的影响。**结果**：咪唑克生(3 – 9 mg/kg)能显著降低小鼠基础痛阈，抑制吗啡镇痛；加重吗啡所致耐受；诱发大、小鼠发生戒断综合征。**结论**：咪唑啉受体参与痛阈形成；咪唑克生能抑制吗啡镇痛，加重吗啡所致耐受；并诱发吗啡依赖性动物发生戒断综合征。

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