ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1): 81 - 85
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Analgesic effect of agmatine and its enhancement on morphine analgesia in mice and rats

LI Jin[1], LI Xin, PEI Gang[2], QIN Bo-Yi (*Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China*)

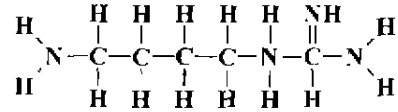
KEY WORDS agmatine; idazoxan; yohimbine; clonidine; analgesia; morphine

ABSTRACT

AIM: To study the effect of agmatine on pain and morphine analgesia. **METHODS:** The effect of agmatine on pain was observed in mouse heat radiant tail-flick test, mouse acetic acid writhing test, and rat 4 % saline test. Its enhancing effect on analgesia of morphine and clonidine was assessed in rat and mouse heat radiant tail-flick tests. **RESULTS:** Agmatine did not significantly prolong tail-flick latency of mice, but reduced the number of acetic acid-induced writhing of mice and inhibited writhing responses to saline completely. It potentiated the analgesic effects of morphine and clonidine in dose-dependent manner and decreased the analgesic ED_{50} of morphine and clonidine by more than 75 % in mouse heat radiant tail-flick test. These effects of agmatine were antagonized by idazoxan. **CONCLUSION:** Agmatine has weak analgesic effects and potentiates morphine and clonidine analgesia by activativation of imidazoline receptors.

INTRODUCTION

Agmatine is an endogenous ligand of imidazoline receptors (I-R), and is biologically active in nervous system and many other tissues in mammals[1,2]. It stimulates the release of catecholamines from adrenal chromaffin cells, increases in arterial blood pressure when injected intracisternally in rats, stimulates the release of insulin from β-cells of pancreatic islet, and increases the release of gonadorelin from hypothalamus[3]. Agmatine potentiates opioid analgesia and prevents the tolerance induced by opioids[4]. The aim of this study was to observe the effects of agmatine and clonidine on pain and morphine analgesia, and to analyze the receptor mechanism(s) of agmatine and clonidine.



Agmatine [4-(aminobutyl) guanidine]

MATERIALS AND METHODS

Male and female (1:1) mice (20 ± *s* 2 g, Certificate № 01-3023) and Wistar rats (220 ± *s* 11 g, Certificate № 01-3039) were used. Agmatine and yohimbine were purchased from Sigma Co; idazoxan and clonidine were products of Research Biochemicals International and Changzhou Pharmaceutical Factory, respectively; morphine and acetic acid were produced by Qinghai Pharmaceutical Factory and Beijing Chemical Plant, respectively. All drugs were given subcutaneously (sc) except idazoxan was injected intraperitoneally (ip). In analgesic test, agmatine, clonidine, or morphine was injected 30 min prior to determination of pain threshold. In the test to observate the effect of agmatine on morphine or clonidine analgesia, it

[1] Correspondence to Assoc Prof LI Jin. Phn 86-10-6693-1621. Fax 86-10-6821-3044. E-mail qinby@nic.bmi.ac.cn
[2] Now in *Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai 200031, China.*
Received 1997-07-02 Accepted 1998-07-16

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1)
E-mail aps@server.shcnc.ac.cn Phn Fax 86-21-6474-2629

was injected 15 min prior to administration of morphine or clonidine. Idazoxan or yohimbine was injected 10 min prior to agmatine or clonidine. At least 10 animals were employed in each group. At least 3 doses of each drugs were injected and ED_{50} were evaluated by Bliss program. Fisher exact test was used to determine the significance of difference between the analgesic effects of single dose of drugs which were expressed as analgesic % and t-test was used to determine the significance of difference between analgesic effects of single dose of drugs which were expressed as possible maximal analgesic % (PMAP).

In analgesia test, the effect of agmatine was determined in mouse heat radiant tail-flick test[5], mouse acetic acid writhing test[6], or rat 4 % saline writhing test. In the tail-flick test, the latency to withdraw tail from a focused light stimulus was measured by a radiant apparatus (type 7360; made in Ugo Bosile Co, Italy). Analgesia was defined as prolongation of latency to twice as long as baseline $\bar{x}$ in the group or even longer. Animals in acetic acid writhing test were injected 0.6 % acetic acid (20 $mL \cdot kg^{-1}$, ip), 30 min after sc of agmatine. The number of writhing, characterized by a wave of contractions of the belly followed by extension of the hind limbs, was counted in 15 min after ip acetic acid. Analgesia was defined as decrease in number of writhing to half of the $\bar{x}$ obtained from all mice in normal saline group or even less. In rat saline test, ip of 4 % saline (2 $mL \cdot kg^{-1}$) induced writhing response of rats. The post-treatment response to stimulation of 4 % saline was observed 30 min after sc agmatine. The analgesia was determined by the disappearance of the writhing response after 4 % saline. All results were expressed as analgesic %.

The effect of agmatine on morphine analgesia was observed in mouse and rat heat radiant tail-flick tests. The results were expressed as PMAP. PMAP = (latency after medication of drug − basline latency)/(10 − basline latency). The enhancing effect of agmatine on the analgesic effects of morphine was determined by the comparison of PMAP between normal saline and agmatine groups. The influence of agmatine on analgesic ED_{50} of morphine or clonidine was observed in mouse heat radiant tail-flick test, in which agmatine was injected by sc, intrathecal (IT), or intracerebroventricular (ICV) injections[7,8].

The influence of agmatine on analgesic time of morphine 10 $mg \cdot kg^{-1}$ (ED_{95}) was examined in mouse heat radiant tail-flick test. The mice were divided into normal saline and agmatine groups. Each group was further divided into 30, 60, 120, and 240 min groups. Saline or agmatine were respectively given to animals 15 min prior to morphine. The pain thresholds were determined at 30, 60, 120, and 240 min after sc morphine. The results were expressed as PMAP.

RESULTS

In the mouse tail-flick test, agmatine (0.1 − 62.5 $mg \cdot kg^{-1}$) did not significantly prolong the latency of pain as compared with baseline. Clonidine prolonged the latency and its ED_{50} was 0.13 (0.10 − 0.17) $mg \cdot kg^{-1}$. Agmatine reduced the number of writhing of mice induced by ip 0.6 % acetic acid and caused disapperance of writhing response of rats induced by ip 4 % saline in a dose-dependent manner. The analgesic ED_{50} of agmatine obtained in mice and rats were 10.1 (6.8 − 15.4) and 14.1 (8.4 − 23.8) $mg \cdot kg^{-1}$, respectively. In mouse acetic acid writhing test, idazoxan inhibited the analgesic effect of agmatine in a dose-dependent manner, but yohimbine had no effect on the action of agmatine. Yohimbine blocked the analgesic effect of clonidine completely (Tab 1).

Agmatine enhanced analgesia of morphine

Tab 1. Influence of drugs on writhing in 15 min in mouse HAc writhing test. $n = 10$ mice in each group. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline.

Drugs mg·kg^{-1}	Frequency of writhing	Analgesia /%
Normal saline	49 ± 13	0
Morphine 2.5	11 ± 10[c]	90
Agmatine 10	20 ± 11[c]	80
Clonidine 0.13	1 ± 2	100
Indazoxan 1.5 + agmatine 10	45 ± 12	0
Indazoxan 3.0 + agmatine 10	34 ± 6[b]	10
Yohimbine 2.5 + agmatine 10	24 ± 10[c]	50
Yohimbine 2.5 + clonidine 0.13	39 ± 11[c]	10

and clonidine in a dose-dependent manner. In mouse and rat tail-flick test, with increasing doses of agmatine sc 15 min prior to morphine, PMAP of morphine at an unchanged dose was increased as compared with those of normal saline group (Tab 2).

Tab 2. Enhancement effects of drugs on morphine analgesia in mouse and rat heat radiant tail-flick test. $n = 10$ animals. $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* morphine.

Drugs/mg·kg^{-1}	Possible maximal analgesia/%	
	Mouse	Rat
Morphine 5	36 ± 17	39 ± 10
Agmatine 0.1 + morphine 5	47 ± 24	–
Agmatine 0.5 + morphine 5	55 ± 26	49 ± 12
Agmatine 2.5 + morphine 5	65 ± 20[b]	53 ± 13[b]
Agmatine 12.5 + morphine 5	92 ± 17[c]	71 ± 22[c]
Clonidine 0.04 + morphine 5	77 ± 24[c]	–

Agmatine potentiated analgesic effects of morphine and clonidine in a dose-dependent manner. In the presence of agmatine, the analgesic ED_{50} of morphine and clonidine were respectively decreased by over 75 % as compared with those obtained in normal saline group (Tab 3).

ICV or IT coadministration of agmatine (12.5 μg for each animal) with morphine potentiated analgesic effect of morphine, but the

Tab 3. Influence of drugs on analgesic ED_{50} of morphine or clonidine in mouse heat radiant tail-flick test. $^cP < 0.01$ *vs* morphine or clonidine, respectively, Bliss Program.

Drugs/mg·kg^{-1}	ED_{50}/ mg·kg^{-1}	95 % confidence limits	ED_{50} ratio
Morphine	7.2	5.6 – 9.2	–
Agmatine 0.5 + morphine	5.3	4.4 – 6.4	1.36
Agmatine 2.5 + morphine	2.4[c]	1.8 – 3.2	3.03
Agmatine 12.5 + morphine	1.6[c]	1.2 – 2.0	4.64
Clonidine	0.2	0.15 – 0.41	–
Agmatine 1.0 + clonidine	0.1[c]	0.03 – 0.11	4.28

potencies of the effects between ICV and IT agmatine were rather different. IT agmatine decreased analgesic ED_{50} of morphine by over 94 % (from 680.7 to 46.0 ng), while ICV agmatine only decreased ED_{50} of morphine by 75 % (from 200.7 to 50.6 ng). Clonidine also potentiated the analgesic effects of morphine in a dose-dependent manner in mouse tail-flick test (Tab 4).

Tab 4. Enhancement effect of clonidine on morphine analgesia in mouse heat radiant tail-flick test. $n = 10$ mice. $\bar{x} \pm s$.
$^bP < 0.05$ *vs* Morphine.

Drugs/mg·kg^{-1}	Possible maximal analgesia/%
Morphine 5	39 ± 12
Clonidine 0.02	5 ± 13
Clonidine 0.04	7 ± 5
Clonidine 0.08	8 ± 10
Clonidine 0.02 + morphine 5	55 ± 19
Clonidine 0.04 + morphine 5	75 ± 21[b]
Clonidine 0.08 + morphine 5	77 ± 18[b]

In mouse tail-flick test, idazoxan sc alone did not influence morphine or clonidine analgesia and lacked any analgesic activity, but yohimbine (2.5 mg·kg^{-1}, sc) completely blocked the analgesic effect of morphine (data not shown). Both idazoxan and yohimbine inhibited the

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

enhancing effects of agmatine and clonidine on morphine analgesia in a dose-dependent manner (Tab 5).

Tab 5. Influence of drugs in the enhancement effects of agmatine on morphine analgesia in mouse heat radiant tail-flick test. $n = 10$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* morphine. $^fP < 0.01$ *vs* agmatine + morphine. $^iP < 0.01$ *vs* clonidine + morphine.

Drugs/mg·kg^{-1}	Possible maximal analgesia/%
Morphine 5	47 ± 27
Agmatine 2.5 + morphine 5	85 ± 24^c
Clonidine 0.05 + morphine 5	75 ± 26^c
Yohimbine 2.5 + morphine 5	10 ± 9^c
Idazoxan 1.5 + morphine 5	49 ± 28
Idazoxan 1.5 + agmatine 2.5 + morphine 5	44 ± 16^f
Yohimbine 2.5 + agmatine 2.5 + morphine 5	27 ± 4^i
Idazoxan 1.5 + clonidine 0.05 + morphine 5	48 ± 15^f
Yohimbine 2.5 + clonidine 0.05 + morphine 5	41 ± 23^f

Although agmatine 10 mg · kg^{-1} enhanced morphine analgesia, it did not prolong the analgesic time in mouse heat radiant tail-flick test. At 240 min after sc of normal saline + morphine or agmatine + morphine, PMAP were < 20 % and showed no significant difference between them (Fig 1).

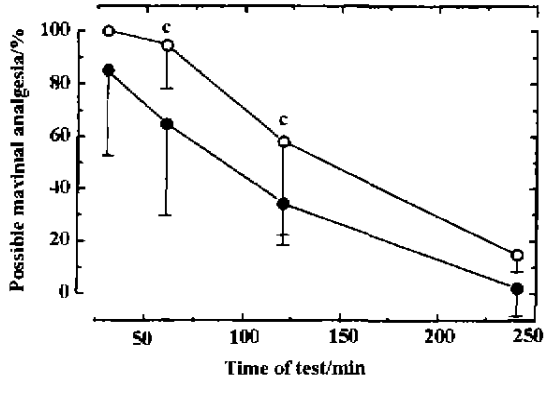


Fig 1. Influence of agmatine 10 mg·kg^{-1} (○) and normal saline (●) on analgesic time of morphine 10 mg·kg^{-1} in mouse heat tail-flick test. $n = 10$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* normal saline.

DISCUSSION

Endogenous ligand of I-R agmatine shows analgesic effects in mouse acetic acid writhing test and rat 4 % saline test, but has no analgesic effect in much stronger nociceptive model, mouse heat radiant tail-flick test. However, clonidine has strong analgesic effect not only in the acetic acid and saline tests, but also in heat radiant test. The effects of agmatine can be blocked by selective I-R antagonist idazoxan, but can not be antagonized by α_2-adrenoceptor antagonist yohimbine. In contrast to agmatine, the clonidine analgesia is antagonized by yohimbine, but not by idazoxan. These results suggest that agmatine has a weak analgesic effect, the mechanism of the effect is different from that of clonidine and might be related to activation of I-R. The analgesic effect of clonidine is much stronger than that of agmatine and the mechanism of the effect might be related to activation of α_2-adrenoceptors. These results seem to be contradiction to the view-point which considers both agmatine and clonidine as agonists of either I-R or α_2-adrenoceptors. In fact, recently accumulated results show that despite recognition at α_2-adrenaceptor binding sites, agmatine failed to produce functional α_2-adrenoceptor activities not only in the isolated guinea pig ileum but also in other functional models of α_2-adrenoceptors[9].

Clonidine is an antihypertensive drug, the effect of which is related to activation of I-R and α_2-adrenoceptors[10]. The current study demonstrates that both agmatine and clonidine enhance the analgesic effect of morphine, which is sensitive to both idazoxan and yohimbine. Idazoxan does not influence tail-flick latency when administered alone and does not inhibit the analgesic effect of morphine. The property of lacking effect on morphine analgesia differs dramatically from yohimbine, which actively blocks morphine analgesia. This distinction between yohimbine and idazoxan suggests that the

enhancing effect of agmatine and clonidine might be mediated through I-R. Agmatine not only potentiates morphine analgesia, but also enhances analgesic effect of clonidine in mouse heat radiant tail-flick test. The results suggest that the mechanism of the action of agmatine might be not related to specialized receptor(s), such as opium receptors or α_2-adrenoceptors. The ability of agmatine given by IT to potentiate morphine analgesia is much greater than that given by ICV, indicating that the main action site of agmatine in enhancing morphine analgesia is at spinal cord. Although agmatine potentiates morphine analgesia, it does not prolong the analgesic time of morphine. The data demonstrate that the action of agmatine might be performed by pharmacodynamics.

In conclusion, agmatine has weak analgesic effect, and potentiates the analgesic effect of morphine and clonidine by activation of I-R, but can not prolong the analgesic time of morphine. The main site of the latter action of agmatine is at spinal cord.

REFERENCES

1 Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ. Agmatine: an endogenous 'clonidine-displacing' substance in the brain. Science 1994; 263: 966 - 9.

2 Raasch W, Regunathan S, Li G, Reis DJ. Agmatine, the bacterial amine, is widely distributed in mammalian tissues. Life Sci 1995; 56: 2319 - 30.

3 Galea E, Regunathan S, Eliopoulos V, Feinstein DL, Reis DJ. Inhibition ofmammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem J 1996; 316: 247 - 9.

4 Kolesnikov Y, Jain S, Pasternak GW. Modulation of opioid analgesia by agmatine. Eur J Pharmacol 1996; 296: 17 - 22.

5 Paul D, Levison JA, Howard DH, Pick CG, Hahn EF, Pasternak GW. Naloxone benzoylhydrazone (NalBzoH) analgesia. J Pharmacol Exp Ther 1990; 255: 769 - 74.

6 Koster R, Aderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc Fed Am Soc Exp Biol 1959; 18: 412 - 8.

7 Popick FR. Application of a new intraventricular injection technique in rat brain norepinehperine studies. Life Sci 1975; 18: 197 - 203.

8 Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. Eur J Pharmacol 1980; 67: 313 - 6.

9 Pinthong D, Wright IK, Hanmer C, Millns P, Mason R, Kendall DA, *et al*. Agmatine recognizes α_2-adrenoceptor binding sites but neither activates nor inhibits α_2-adrenoceptor. Naunyn-Schmiedeberg Arch Pharmacol 1995; 351: 10 - 6.

10 Feldman J, Tibirica E, Bricca G, Dontenwill M, Belcourt A, Bousquet P. Evidence for the involvement of imidazoline receptors in the central hypotensive effects of rilmenidine. Br J Pharmacol 1990; 100: 600 - 4.

胍丁胺对小鼠和大鼠镇痛及增强吗啡镇痛

李 锦, 李 昕, 裴 刚, 秦伯益 (军事医学科学院药物毒物研究所, 北京 100850, 中国)

关键词 胍丁胺; 咪唑克生; 育亨宾; 可乐定; 镇痛; 吗啡

目的: 观察胍丁胺镇痛和对吗啡镇痛的作用. **方法**: 在小鼠热辐射甩尾、醋酸扭体、大鼠4 %盐水实验中观察胍丁胺的镇痛作用; 在小鼠和大鼠热辐射甩尾实验中观察其对吗啡和可乐定镇痛的作用. **结果**: 胍丁胺不延长小鼠甩尾潜伏期, 使小鼠醋酸扭体次数减少、完全抑制大鼠盐水扭体. 在小鼠甩尾实验中, 胍丁胺剂量依赖性地增强吗啡和可乐定的镇痛, 使吗啡和可乐定的镇痛 ED_{50} 减小了 75 %. 胍丁胺的上述作用可被咪唑克生所对抗. **结论**: 胍丁胺通过激动咪唑啉受体而具有较弱镇痛和加强吗啡及可乐定镇痛作用.

(责任编辑 李 颖)