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Modulation of opioid analgesia by agmatine

Yuri Kolesnikov [a,b], Subash Jain [b], Gavril W. Pasternak [a,c,*]

[a] *The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA*
[b] *Department of Anesthesiology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA*
[c] *Departments of Neurology and Neuroscience and Pharmacology, Cornell U. Medical College, New York, NY 10021, USA*

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Abstract

Administered alone, agmatine at doses of 0.1 or 10 mg/kg is without effect in the mouse tailflick assay. However, agmatine enhances morphine analgesia in a dose-dependent manner, shifting morphine's ED_{50} over 5-fold. A far greater effect is observed when morphine is given intrathecally (9-fold shift) than after intracerebroventricular administration (2-fold). In contrast to the potentiation of morphine analgesia, agmatine (10 mg/kg) has no effect on morphine's inhibition of gastrointestinal transit. δ-Opioid receptor-mediated analgesia also is potentiated by agmatine, but κ_1-receptor-mediated (U50,488H; *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] benzeneacetemide) and κ_3-opioid receptor-mediated (naloxone benzoylhydrazone) analgesia is not significantly enhanced by any dose of agmatine tested in this acute model. In chronic studies, agmatine at a low dose (0.1 mg/kg) which does not affect morphine analgesia acutely prevents tolerance following chronic morphine dosing for 10 days. A higher agmatine dose (10 mg/kg) has a similar effect. Agmatine also blocks tolerance to the δ-opioid receptor ligand [D-Pen^2,D-Pen^5]enkephalin given intrathecally, but not to the κ_3-opioid receptor agonist naloxone benzoylhydrazone. Despite its inactivity on κ_1-opioid analgesia in the acute model, agmatine prevents κ_1-opioid receptor-mediated tolerance. These studies demonstrate the dramatic interactions between agmatine and opioid analgesia and tolerance.

Keywords: Agmatine; Morphine; Analgesia; Tolerance

1. Introduction

Clonidine, an imidazoline and a centrally active hypotensive agent, interacts with both the adrenergic α_2-adrenoceptor receptors and the non-adrenergic imidazoline receptors (Bousquet et al., 1984; Bricca et al., 1989; Coupry et al., 1987; Ernsberger et al., 1987, 1990a, b; Coupry et al., 1990; Michel et al., 1990; Michel and Ernsberger, 1992; Michel and Insel, 1989). Imidazoline receptors have been defined by their dependence on an imidazoline ring for binding. Several subclasses, imidazoline I_1 and imidazoline I_2 receptors, have been proposed based upon ligand selectivity and regional distributions (Michel and Insel, 1989; Michel and Ernsberger, 1992). Imidazoline receptors are widely distributed in the brain, where they are expressed in both neurons and glia (Regunathan et al., 1993; Regunathan et al., 1995). Imidazoline receptors appear to mediate many of the hypotensive actions of clonidine (Ernsberger et al., 1987, 1988; Reis et al., 1995).

Recently, an endogenous imidazoline receptor ligand was isolated from cow and rat brain and found to be agmatine (Li et al., 1994). Agmatine, an amine generated by the decarboxylation of L-arginine by the enzyme arginine decarboxylase (Tabor and Tabor, 1984), had been identified in bacteria and other lower life forms, but it had not previously been found in mammals. Agmatine binds to both imidazoline receptors and α_2-adrenoceptors (Li et al., 1994) and is widely distributed in the brain, viscera and serum of rats (Raasch et al., 1995), including astrocytes (Regunathan et al., 1995). In view of the major influence on opioid analgesia by clonidine (Yaksh and Noueihed, 1985), we now have examined the effects of agmatine on opioid analgesia.

* Corresponding author. Department of Neurology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA. Tel.: 212 639-7046; fax: 212 794-4332; e-mail: pasterng@mskmail.mskcc.org.

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2. Methods and materials

Male CD-1 mice (25–35 g, Charles River Breeding Laboratory, Bloomington, MA, USA) were used in all studies. Animals were maintained on a 12 h light/dark cycle and given ad libitum access to food and water. Morphine sulfate, [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and U50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetemide) were obtained from the Research Technology Branch of NIDA (Rockville, MD, USA). Naloxone benzoylhydrazone was synthesized as previously described (Luke et al., 1988). Idazoxane, yohimbine and agmatine were purchased from Sigma Chemicals (St. Louis, MO, USA). All drugs were administered subcutaneously, except for idazoxane which was administered intraperitoneally. Agmatine was given 15 min prior to morphine and yohimbine and idazoxane 5 min prior to agmatine. Intrathecal and intracerebroventricular injections were performed under general halothane anesthesia, as previously reported (Paul et al., 1990).

Analgesia was assessed quantally using the mouse tailflick assay 30 min after administration of the opioid (Paul et al., 1990). Analgesia was defined as a doubling or greater of the baseline latency for each mouse, which typically ranged from 2 to 3 s. All studies employed groups of at least 10 mice. Dose-response curves comprised at least three doses of drug and were evaluated using the Bliss program (Umans and Inturrisi, 1981). Comparisons of single doses of drugs were evaluated for significance using the Fisher exact test. Gastrointestinal transit was determined by measuring the motility of a charcoal meal in untreated and treated groups of animals as previously described (Paul and Pasternak, 1988). Significance in the gastrointestinal transit studies was determined by Student's *t*-test.

3. Results

3.1. Analgesia

Agmatine enhances morphine analgesia in naive mice in a dose-dependent manner with the highest dose giving a 5-fold shift of morphine's ED_{50} (Table 1). Systemic agmatine also increases the analgesic actions of centrally administered morphine (Table 1). Although agmatine appears to shift the dose-response curve for i.c.v. morphine over 2-fold, this shift does not achieve statistical significance. However, its actions are more prominent against intrathecal morphine ($P < 0.05$), whose potency is increased 9-fold.

The increased analgesic response elicited by the combination of morphine and agmatine may be mediated through imidazole receptors. Idazoxan reverses the enhanced analgesia seen with the combination of morphine with agmatine (Fig. 1). Idazoxan given alone has no effect upon tailflick latencies and lacks any analgesic activity (data not shown). When administered only with morphine, idazoxan also is inactive, in contrast to yohimbine (5 mg/kg s.c.), which completely blocks morphine analgesia (data not shown).

The enhanced analgesia seen with opioid/agmatine combinations is not restricted to morphine. δ-Opioid receptor systems also are affected. Agmatine potentiates DPDPE analgesia in a dose-dependent manner, although only the higher agmatine dose achieves statistical significance. In contrast, agmatine has little effect

Table 1
Effects of agmatine on opioid analgesia

Treatment		ED_{50}	95% confidence limits	ED_{50} ratio
Morphine (s.c.)		4.4 mg/kg	(3.6, 5.3)	
+ agmatine	0.1 mg/kg	3.2 mg/kg	(1.7, 4.9)	1.4
	1 mg/kg	2.6 mg/kg	(1.6, 4.2)	1.7
	10 mg/kg	0.84 mg/kg	(0.39, 1.7)	5.2
Morphine (i.c.v.)		474 ng	(293, 717)	
+ agmatine	10 mg/kg	211 ng	(125, 313)	2.2
Morphine (i.t.)		742 ng	(413, 1142)	
+ agmatine	10 mg/kg	82 ng	(42, 158)	9
DPDPE (i.t.)		312 ng	(213, 489)	
+ agmatine	0.1 mg/kg	169 ng	(71, 292)	1.8
	10 mg/kg	67 ng	(38, 130)	4.7
U50,488H (s.c.)		4.7 mg/kg	(3.1, 6.7)	
+ agmatine	0.1 mg/kg	3.9 mg/kg	(2.4, 5.8)	1.2
	10 mg/kg	3.3 mg/kg	(1.8, 4.8)	1.4
Naloxone benzoyl-hydrazone (s.c.)		50 mg/kg	(30, 89)	
+ agmatine	10 mg/kg	46 mg/kg	(25, 83)	1.1

ED_{50} values with 95% confidence limits were determined from at least three doses of agonist alone or with the indicated dose of agmatine.

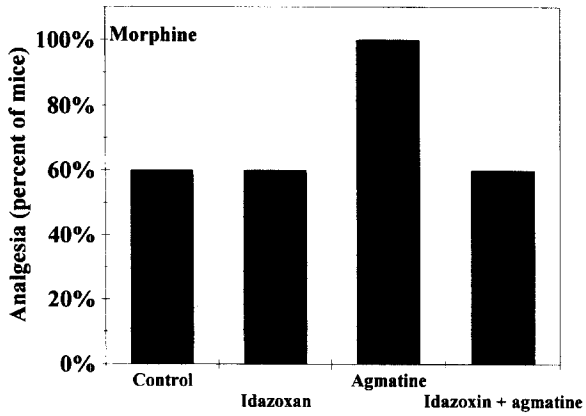

Fig. 1. Effect of idazoxan and agmatine on morphine analgesia. Groups of mice ($n = 10$) received morphine (5 mg/kg) alone (control), with idazoxan (2 mg/kg) 15 min prior to morphine, agmatine (10 mg/kg) 15 min before morphine or both idazoxan and agmatine together 15 min prior to the morphine. Agmatine significantly increases morphine analgesia ($P < 0.05$). Addition of idazoxan reverses this effect ($P < 0.05$).

upon the analgesic actions of either the κ_1-opioid receptor drug U50,488H or the κ_3-opioid agent naloxone benzoylhydrazone (Table 1). The slight trend against U50,488H is not significant.

3.2. Tolerance

Agmatine prevents the development of tolerance to morphine (Fig. 2). Given alone, morphine's initial analgesic response is lost by 5 days. Coadministration of agmatine at either 0.1 mg/kg or 10 mg/kg prevents this loss of analgesic responsiveness. It is interesting that the lower dose of agmatine, which does not potentiate morphine in naive mice, fully protects against tolerance for 10 days. In addition to preventing tolerance to morphine, single doses of agmatine restore the analgesic sensitivity towards morphine in tolerant mice (Table 2). Even the lowest agmatine dose, which has

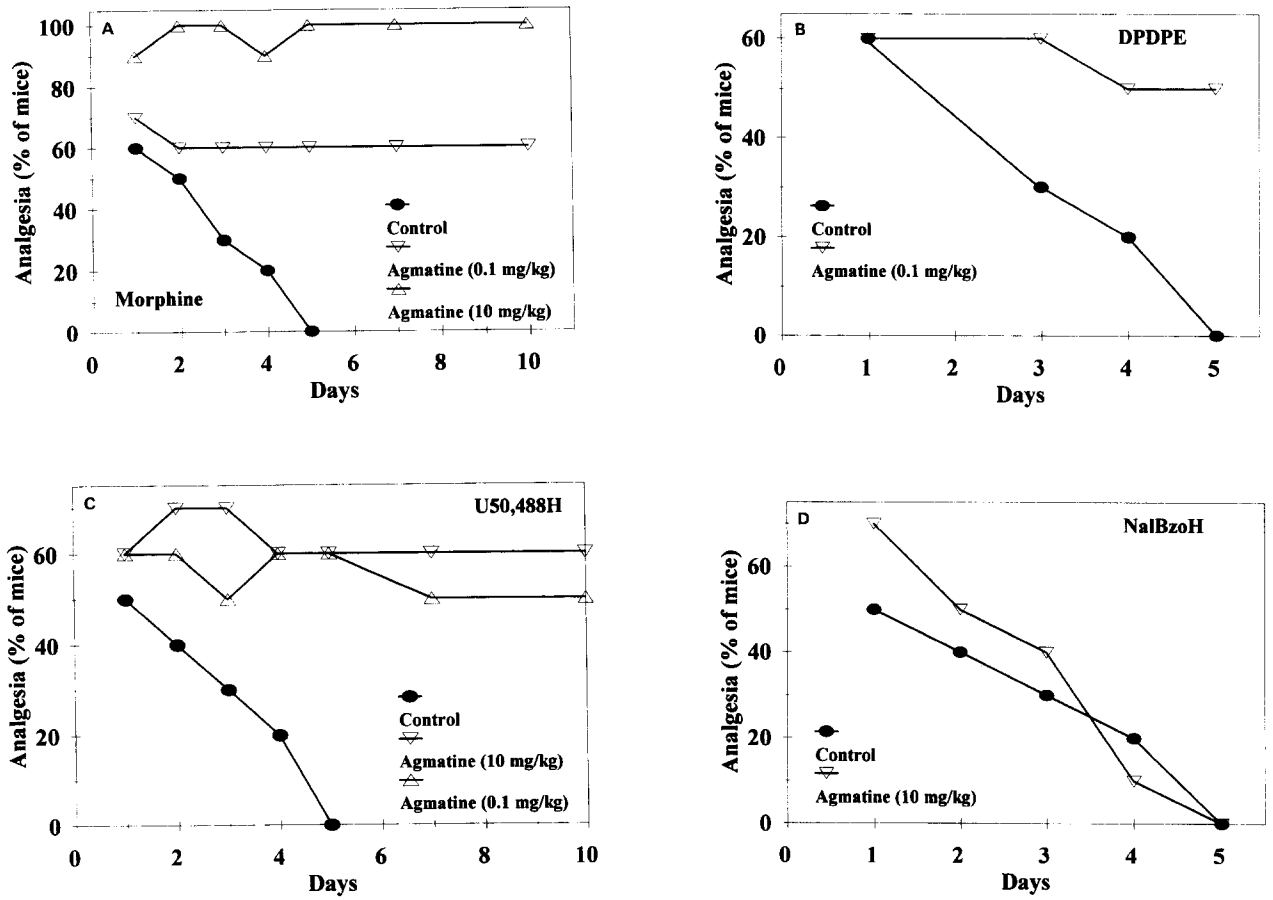

Fig. 2. Effect of agmatine on opioid tolerance. (A) Groups of mice ($n \geq 10$) received daily doses of morphine (5 mg/kg s.c.) alone (Control) or with agmatine at 10 or 0.1 mg/kg s.c. At 5 days, the control group is significantly less than either the 0.1 mg/kg ($P < 0.005$) or the 10 mg/kg ($P < 0.001$). (B) Groups of mice ($n \geq 10$) received daily doses of DPDPE (300 ng i.t.) alone (Control) or with agmatine at 0.1 mg/kg s.c. At 5 days, the control group is significantly different from the agmatine group ($P < 0.02$). (C) Groups of mice ($n \geq 10$) received daily doses of U50,488H (5 mg/kg s.c.) alone (Control) or with agmatine at 10 or 0.1 mg/kg s.c. At 5 days, the control group is significantly less than either agmatine group ($P < 0.005$). (D) Groups of mice ($n \geq 10$) received daily doses of naloxone benzoylhydrazone (50 mg/kg s.c.) alone (Control) or with agmatine at 10 mg/kg s.c. The groups are not significantly different.

Table 2
Effect of single doses of agmatine on the analgesic sensitivity of opioid-tolerant mice

Treatments		Day 1	Day 5
Morphine (5 mg/kg)		60%	0%
+agmatine	0.1 mg/kg	70%	60%
	10 mg/kg	90%	80%
U50,488H (5 mg/kg)		50%	0%
+agmatine	0.1 mg/kg	60%	10%
	10 mg/kg	60%	60%
Naloxone benzoylhydrazone (50 mg/kg)		70%	0%
+agmatine	10 mg/kg	50%	0%

Groups of mice ($n = 10$) received the stated doses of morphine, U50,488H or naloxone benzoylhydrazone daily and the analgesic response was determined on the first and fifth days. Additional groups received the stated doses of agonists and on the first or fifth day were tested with the agonist along with the stated dose of agmatine. All comparisons were made on day 5 between no agmatine and agmatine groups. In the morphine-treated groups, both agmatine doses significantly increased the response ($P < 0.005$). Only the higher agmatine dose (10 mg/kg) significantly enhances U50,488H analgesia ($P < 0.005$). No significant effect is seen with the agmatine/naloxone benzoylhydrazone combination.

only minimal effects on morphine analgesia when given acutely, returns analgesic sensitivity to naive levels.

The low agmatine dose also prevents tolerance to the δ-opioid receptor drug DPDPE (Fig. 2). When given alone, repeated administration of DPDPE results in a loss of analgesic response by the fifth day. Coadministration of agmatine at only 0.1 mg/kg prevents this decreased response. In a similar manner, coadministration of agmatine maintains the analgesic sensitivity of the κ_1-opioid receptor analgesic U50,488H with repeated dosing (Fig. 2). Like morphine, U50,488H-tolerant mice regain their analgesic sensitivity following single injections of agmatine. However, only the higher agmatine dose is effective. In contrast to the μ-, δ- and κ_1-opioid receptor systems, agmatine neither prevents the development of κ_3-opioid receptor-mediated tolerance (Fig. 2) nor does it reverse preexisting κ_3-opioid receptor-mediated tolerance (Table 2).

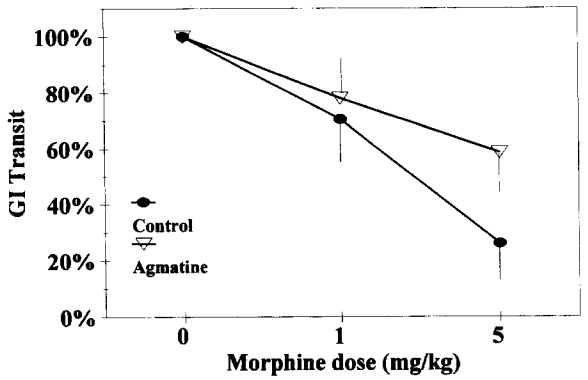

Fig. 3. Effect of agmatine on the inhibition of gastrointestinal transit by morphine. Groups of mice ($n = 10$) received either nothing or morphine at 1 or 5 mg/kg s.c. plus either nothing or agmatine (10 mg/kg s.c.). Gastrointestinal transit was determined as described in Methods and materials. Results are given as the percent of control for mice who received no morphine. All values receiving morphine are different from controls ($P < 0.02$) but the control and agmatine groups are not different from each other.

3.3. Gastrointestinal transit

In an effort to determine whether agmatine has similar effects upon all of morphine's actions, we also investigated gastrointestinal transit. Morphine alone inhibits the distance traveled by the charcoal meal in a dose-dependent manner (Fig. 3). Addition of agmatine does not increase morphine's effects.

4. Discussion

The current study demonstrates important interactions between agmatine and opioids, both in opioid naive and tolerant mice. Acutely, agmatine potentiates morphine and DPDPE analgesia. The agmatine effects against morphine are dose-dependent and sensitive to the idazoxan. Idazoxan does not influence tailflick latencies when administered alone and does not influence morphine analgesia. The lack of effect on morphine analgesia differs dramatically from yohimbine, which we and others (Iglesias et al., 1992) find actively blocks morphine analgesia. This distinction between idazoxan and yohimbine suggests that the actions of agmatine may be mediated through imidazoline receptors.

DPDPE analgesia also is enhanced by agmatine but there are only minimal interactions between agmatine and either the κ_1-opioid receptor agonist U50,488H or the κ_3-opioid receptor-mediated analgesic naloxone benzoylhydrazone. The slight trend with U50,488H is not very impressive and does not achieve statistical significance while naloxone benzoylhydrazone analgesia is totally indifferent to the inclusion of agmatine. The manner in which agmatine influences opioid analgesia is unclear. While it may reflect direct cellular interactions, it seems more likely to involve interacting neuronal circuits as has been demonstrated for μ/δ-opioid receptor synergy (Rossi et al., 1994) and the interaction between sigma drugs and opioids (Chien and Pasternak, 1993). Clearly, this is an area which will require additional investigation.

The ability of agmantine to selectively potentiate morphine analgesia without affecting the inhibition of gastrointestinal transit argues against a simple pharmacokinetic explanation for agmatine's actions. If agmatine were simply increasing morphine levels through an indirect effect on metabolism and/or elimination, simi-

lar actions would be expected for both gastrointestinal transit and analgesia. This distinction between these two morphine actions might prove clinically useful since its suggests that drugs such as agmatine might increase morphine's therapeutic index. The utility of coadministering morphine and agmatine might be further enhanced by administering the agmatine epidurally and/or intrathecally in view of the profound actions at the spinal level.

Agmatine prevents tolerance to μ- (morphine), δ- (DPDPE) and κ_1-opioid receptor-mediated (U50,488H) analgesia, but its mechanism(s) of action remain unclear. Agmatine might be acting directly upon the mechanisms leading to tolerance or it might simply potentiate opioid actions in tolerant animals much as it does to morphine and DPDPE in naive mice, particularly in view of its ability to restore analgesic sensitivity following a single dose. However, the blockade of tolerance requires very low agmatine doses which have little potentiating effect in the acute model. This is particularly evident with U50,488H which is not significantly affected in the acute model by any agmatine dose. This rapid reversal of morphine 'tolerance' by agmatine also may prove useful clinically.

Agmatine joins a number of agents which act on the NMDA receptor/nitric oxide synthase cascade in the modulation of opioid tolerance (Trujillo and Akil, 1991, 1994; Babey et al., 1994; Bhargava, 1994; Elliott et al., 1994; Gutstein and Trujillo, 1993; Inturrisi, 1994; Kolesnikov et al., 1992, 1993a, b, 1994; London et al., 1994; Thorat et al., 1993; Adams et al., 1993; Ben-Eliyahu et al., 1992; Tiseo and Inturrisi, 1993). Like NMDA receptor antagonists and nitric oxide synthase inhibitors, agmatine blocks μ- and δ-, but not κ_3-opioid receptor-mediated tolerance in mice (Kolesnikov et al., 1993a, b; Babey et al., 1994; Elliott et al., 1994). Although other groups have suggested that κ_1-opioid receptor-mediated tolerance can be modulated (Bhargava, 1995; Bhargava and Thorat, 1994), we find that most NMDA antagonists and nitric oxide synthase inhibitors have little effect upon κ_1-opioid receptor-mediated tolerance, with the exception of the competitive NMDA receptor antagonist NPC17792 (Kolesnikov et al., 1993a, b). The opioid receptor profile of agmatine's actions against tolerance is much like NPC17742, with both agents preventing κ_1-, as well as μ- and δ-opioid, tolerance.

In conclusion, agmatine is a newly recognized neuropharmacological active agent. Our studies indicate that agmatine can greatly influence the analgesic actions of specific opioid receptor agonists and can modulate opioid tolerance.

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