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Effect of intrathecal agmatine on inflammation-induced thermal hyperalgesia in rats

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Abstract

Agmatine, an endogenous ligand, interacts both with the α_2-adrenoceptors and with the imidazoline binding sites. The effect of intrathecally administered agmatine on carrageenan-induced thermal hyperalgesia was investigated by means of a paw-withdrawal test in rats. The effect of agmatine on morphine-induced anti-hyperalgesia was also studied. Intrathecal agmatine in doses larger than 250 μg caused a decrease in the pain threshold, with vocalization and agitation lasting for several hours in all animals. Agmatine alone at 1–100 μg did not give rise to any change in the thermal withdrawal threshold in the contralateral non-inflamed paw. Agmatine pretreatment was found to dose-dependently attenuate the thermal hyperalgesia induced by intraplantar carrageenan. The effect of 100 μg agmatine was completely lost by 60 min, whereas the effect of 50 μg was of similar magnitude but exhibited a longer duration. Agmatine posttreatment had a slighter effect. Agmatine pretreatment (100 μg) together with 1 μg morphine (subeffective dose) has significantly higher anti-hyperalgesic effect then the individual compounds by themselves. These are the first data demonstrating the behavioral and anti-hyperalgesic effects of intrathecal agmatine. The results reveal important interactions between intrathecal agmatine and opioids in thermal hyperalgesia.

Keywords: Hyperalgesia; Intrathecal; Agmatine; α2-Adrenoceptor; Imidazoline binding site

1. Introduction

It has recently been discovered that agmatine (decarboxylated arginine), its biosynthetic enzyme (arginine decarboxylase) and agmatinase (the enzyme that hydrolyzes agmatine) are present in various mammalian organs, including the bovine and the rat brain (Li et al., 1994; Raasch et al., 1995, Sastre et al., 1996). Until recently, agmatine was considered to be only a precursor of putrescine and other polyamines (Tabor and Tabor, 1984). It is now known that agmatine binds to α_2-adrenoceptor and imidazoline binding sites, but it has a higher affinity for the imidazoline binding sites in the rat cerebral cortex (Li et al., 1994).

Several widely-used α_2-adrenoceptor drugs, including clonidine and idazoxan, also bind with high affinity to nonadrenergic sites, which have been designated imidazoline binding sites (Ernsberger et al., 1987). These binding sites are not part of the α_2-adrenoceptor family as they have very low affinities for the catecholamines epinephrine and norepinephrine. The presence of imidazoline binding sites in various tissues of several species is now well established (Hieble and Ruffolo, 1992). Imidazoline binding sites are expressed along the entire extent of the central nervous system from the spinal cord to the hippocampal cortex (Ruggiero et al., 1995; Ruggiero et al., 1998). To date, two major subclasses of imidazoline binding sites have been identified on the basis of their high (imidazoline$_1$) or low (imidazoline$_2$) affinity for clonidine (Ernsberger, 1992). Convergent studies attribute a role in blood pressure regulation to the imidazoline$_1$ binding sites (Bousquet et al., 1992). In contrast, the biological effects mediated by imidazoline$_2$ binding sites appear to be less abundant. Some studies have shown that imidazoline$_2$ binding sites are located on monoamino oxidases, and the

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fact that they are inhibitory in nature suggests that the imidazoline$_2$ binding site might be a previously unknown monoamino oxidase regulatory site (Carpéné et al., 1995; Tesson et al., 1995).

In the whole brain, the concentration of agmatine is comparable to that of norepinephrine or dopamine (Li et al., 1994) and the central distribution of agmatine (Otake et al., 1998), raising the possibility that agmatine may be a biologically active molecule in its own right. In support of this are older observations that agmatine can block the nicotinic cation channel in both the retina and sympathetic ganglion cells (Loring, 1990), similarly to imidazoline (Molderings et al., 1995), and can stimulate the release of insulin and increase the uptake of calcium in pancreatic islet cells (Sener et al., 1989). It is still uncertain whether agmatine has an effect on noradrenergic nerve terminals. The main indication to date of a functional role for agmatine in the sympathetic system is its ability to release catecholamines from chromaffin cells (Li et al., 1994), in addition to its complex cardiovascular effects, increasing or decreasing the blood pressure and/or noradrenaline overflow (Gao et al., 1995; Sun et al., 1995; Szabo et al., 1995).

Numerous studies have provided evidence that α_2-adrenoceptor mediated events contribute to the inhibition of the generation of central hyperalgesia in animal models and humans (Maze and Tranquilli, 1991; Eisenach et al., 1996). There have been few published reports on the analgesic properties of agmatine after systemic administration. It has been shown that systemic administration of agmatine enhances morphine analgesia and also blocks tolerance to opioids in mice (Kolesnikov et al., 1996), and it attenuates all the signs of the morphine abstinence syndrome in rats (Aricioglu-Kartal and Uzbay, 1997). No reports are available on its analgesic properties after intrathecal administration. The objective of the present investigation was to assess the effects of agmatine and its effects on morphine-induced anti-hyperalgesia after intrathecal administration during carrageenan-induced hyperalgesia.

2. Materials and methods

2.1. Intrathecal catheterization

After approval had been obtained from the Animal Care Committee of Albert Szent-Györgyi Medical University, male Wistar rats weighing 200–300 g were studied. The rats were surgically prepared under ketamine–xylazine anesthesia (87 and 13 mg/kg intraperitoneally, respectively). An intrathecal catheter (PE-10 tubing) was inserted through a small opening in the cisterna magna and passed 8.5 cm caudally into the intrathecal space. After surgery, the rats were housed individually with free access to food and water and allowed to recover for at least 3 days before use. Rats exhibiting postoperative neurologic deficits were not used. All experiments were performed on freely-moving animals during the same period of the day (8:00–13:00 h) to exclude diurnal variations in pharmacological effects. The animals were randomly assigned to treatment groups and the observer was blind to the treatment administered.

2.2. Drugs and their administration

The following drugs were administered in this study: ketamine (Ketalar, Parke-Davis, Austria), xylazine (Rompun, Bayer, Germany), carrageenan (Sigma–Aldrich Kft, Budapest, Hungary), agmatine (Sigma–Aldrich Kft) and morphine HCl (Alkaloida, Tiszavasvar, Hungary). All intrathecally applied drugs were dissolved in sterile physiological saline, freshly prepared on the day of the experiment, and were injected intrathecally over 30 s in a volume of 5 μl, followed by a 10 μl flush of physiological saline.

2.3. Inflammatory pain test (paw withdrawal test)

The rats were placed in a plastic chamber on a glass surface and allowed to acclimatize to their environment for 15–30 min before testing. Baseline hindpaw withdrawal latencies (pre-carrageenan values at −180 min) were obtained. The heat stimulus was directed onto the plantar surface of each hindpaw. A detailed description of this method has been published elsewhere (Hargreaves et al., 1988). Unilateral inflammation was induced by intraplantar injection of 3 mg carrageenan in 0.1 ml physiological saline into the right hindpaw. The paw withdrawal latencies were determined again 3 h after carrageenan injection (post-carrageenan baseline values at 0 min), and then after several periods of time, depending on the series of experiments (the different treatments).

2.4. Series of experiments

2.4.1. Agmatine treatment alone

2.4.1.1. Agmatine pretreatment. In a preliminary experiment, we observed the behavioral effects of different doses of agmatine (1–500 μg). Doses larger than 250 μg caused extreme behavioral changes, involving: vocalization, agitation and fighting behavior lasting for several hours in all animals, together with a decrease in withdrawal latency of the normal paw. Accordingly, in the further pain studies, we used lower doses. Agmatine (1, 10, 50 or 100 μg) or saline ($n = 7–12$/group) was administered after the baseline latency determination, but before the carrageenan injection. The paw withdrawal latencies were obtained 3 h later (0 min), and then a further 30, 60 or 90 min later.

2.4.1.2. Agmatine posttreatment. Agmatine (50 or 100 μg) or saline ($n = 8–12$/group) was administered after determination of the post-carrageenan baseline values. The paw

withdrawal latencies were obtained 3 h later (0 min), and then a further 30, 60 or 90 min later.

2.4.2. Agmatine–morphine coadministration

2.4.2.1. Agmatine pretreatment. Agmatine (50 or 100 μg) or saline ($n = 8–10$/group) was administered after the baseline latency determination, but before the carrageenan injection. Morphine was administered in cumulative doses (1, 5, 10 μg) after determination of the post-carrageenan baseline value. The paw withdrawal latencies were obtained 3 h later (0 min), and then 10 and 30 min after the drug (morphine) injections. The values obtained at 10 and 30 min after each dose were averaged for the value for that dose.

2.4.2.2. Agmatine posttreatment. Agmatine (50 or 100 μg) or saline ($n = 9–10$/group) was administered after determination of the post-carrageenan baseline value, together with the first dose of morphine (1 μg). Morphine was administered in cumulative doses (1, 5, 10 μg). The paw withdrawal latencies were obtained 3 h later (0 min), and then 10 and 30 min after the drug (morphine) injections. The values obtained at 10 and 30 min after each dose were averaged for the value for that dose.

2.5. Data analysis

Data are presented as means ± S.E.M. Analysis of variance (ANOVA) of data for repeated measures was used for overall effects, with the Newman–Keuls test for post hoc comparison for differences between means. Comparisons within groups required paired analysis. A probability level $P < 0.05$ was considered significant. In addition to the raw data analysis, the data were analyzed as percentages of the pre-carrageenan values (percentage score = postcarrageenan value × 100/precarrageenan value, and it was calculated for each rat).

3. Results

3.1. Thermal hyperalgesia

There was no significant difference in paw withdrawal response to noxious thermal stimuli between the right and left hindpaws prior to the intraplantar injection of carrageenan. The overall mean paw withdrawal times for the ipsilateral and contralateral paws were 9.2 ± 0.17 and 9.6 ± 0.16, respectively ($n = 108$). Carrageenan injection induced inflammation of the injected hindpaw, as evidenced by edema and erythema. Fifty-nine rats from 108 did not receive any pretreatment (non-agmatine pretreated animals). The paw withdrawal latency of the carrageenan-injected paw was significantly reduced from 9.2 ± 0.24 to 3.0 ± 0.18 s ($P < 0.001$) in these animals after 3 h of the carrageenan administration. A slight but significant decrease in paw withdrawal latency was also observed on the

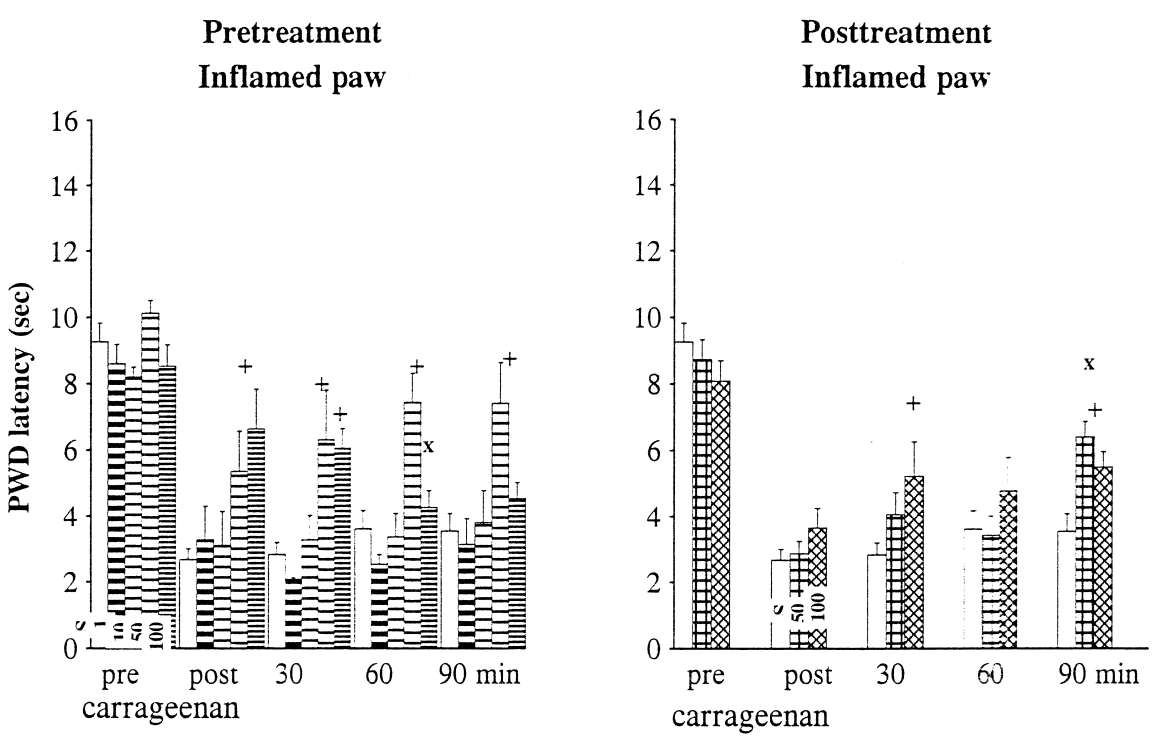

Fig. 1. Effects of pretreatment (left sides) or posttreatment (right side) with different doses (the doses [μg] are shown in the bars; S = saline-treated group) of agmatine on inflamed paws. The results are expressed as means ± S.E.M. $^{x}P < 0.05$ compared to post-carrageenan baseline values. $^{+}P < 0.05$ compared to saline-treated control group.

Table 1
Magnitude of agmatine-induced attenuation in thermal hyperalgesia as a percentage score (percentage score = postcarrageenan value × 100/precarrageenan value, and it was calculated for each rat)

Treatment	Dose (μg)	At 0 min	At 60 min
Saline		29.98 ± 3.10	39.9 ± 4.51
Pre-agmatine	1	35.4 ± 7.53	30.8 ± 4.65
	10	37.8 ± 11.85	40.4 ± 7.63
	50	53.1 ± 11.59	72.8 ± 7.60[a]
	100	76.9 ± 12.29[a]	50.7 ± 5.46
Post-agmatine	50	34.5 ± 4.94	41.5 ± 7.43
	100	46.8 ± 7.97	59.9 ± 12.05

[a] $P < 0.05$ compared to saline-treated group.

non-inflamed side (from 9.6 ± 0.25 to 8.8 ± 0.25 s, $P < 0.01$). Thus, thermal hyperalgesia was consistently produced in rats with carrageenan-induced inflammation. The hyperalgesia did not change significantly in time in the saline-treated (control) group, showing that neither learning nor the repeated injections influenced the carrageenan-induced hyperalgesia.

3.2. Effects of intrathecal agmatine pre- or posttreatment alone on normal and inflamed paws

The effects of agmatine injected into the subarachnoid space were evaluated during the peak hyperalgesic response 3–6 h after carrageenan administration (Fig. 1). Neither pre- nor posttreatment influenced the paw withdrawal latency of the noninjected paws. Agmatine pretreatment significantly and dose-dependently attenuated the carrageenan-induced hyperalgesia at 0 min. The maximal attenuation in the thermal hyperalgesia was observed after the 100 μg pretreatment at 0 min. Interestingly, the effect of 100 μg agmatine was completely lost by 60 min, whereas the effect of 50 μg was of similar magnitude but exhibited a longer duration (Fig. 1, left side). Agmatine posttreatment had a slighter effect (Fig. 1, right side). Table 1 details percentage attenuation of the thermal hyperalgesia produced by carrageenan in the presence or absence of agmatine pre- or posttreatment at 0 and 60 min.

3.3. Effects of intrathecal agmatine–morphine coadministration on paw withdrawal latencies of normal and inflamed paws

Morphine alone significantly increased the paw withdrawal latency in the normal paw as compared to the post-carrageenan values, but not to the pre-carrageenan values (Fig. 2, right side). Furthermore, morphine dose-dependently decreased the carrageenan-induced hyperalgesia in the inflamed paw (Fig. 2, left side and Fig. 4). At the highest dose (10 μg cumulative), the paw withdrawal

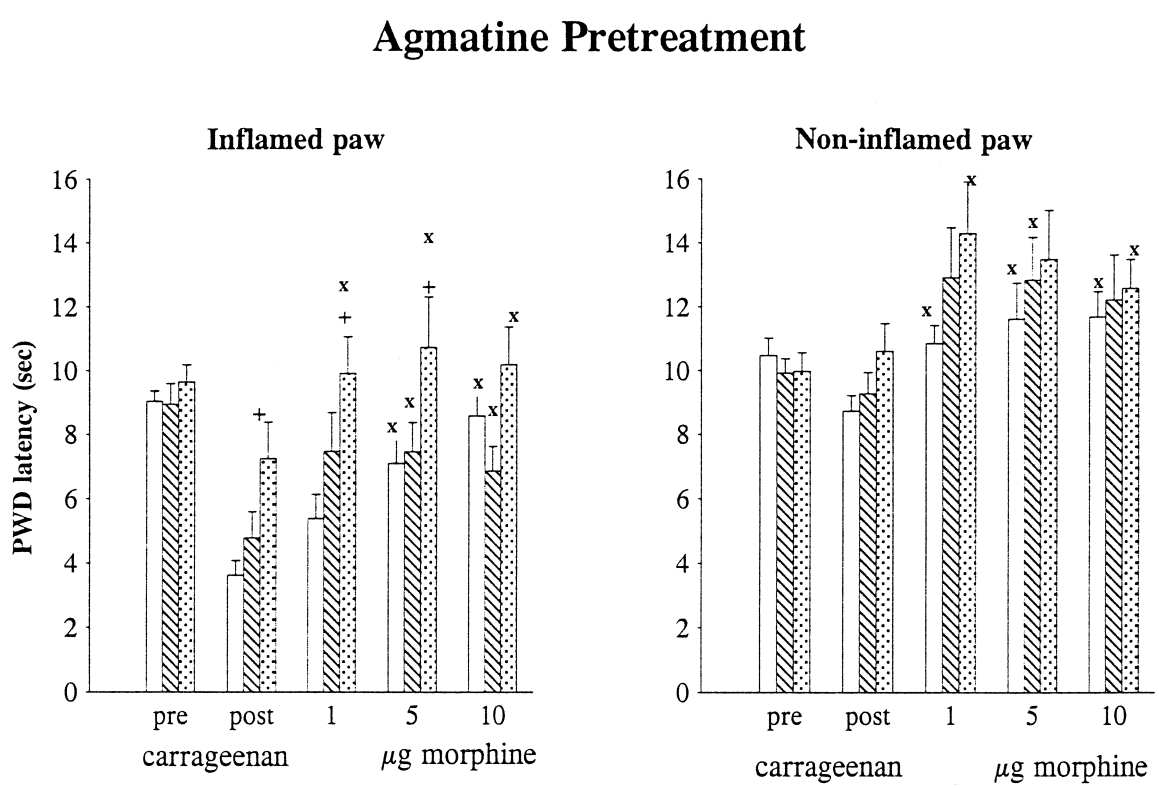

Fig. 2. Effects of pretreatment (saline-pretreated rats: open bars, 50 μg agmatine-pretreated rats: hatched bars, 100 μg agmatine-pretreated rats: dotted bars) with different doses of agmatine on morphine-induced antinoception (1, 5, 10 μg cumulative) in normal (right side) and inflamed paws (left side). The results are expressed as means ± S.E.M. $^{x}P < 0.05$ compared to post-carrageenan baseline values. $^{+}P < 0.05$ compared to saline-treated control group.

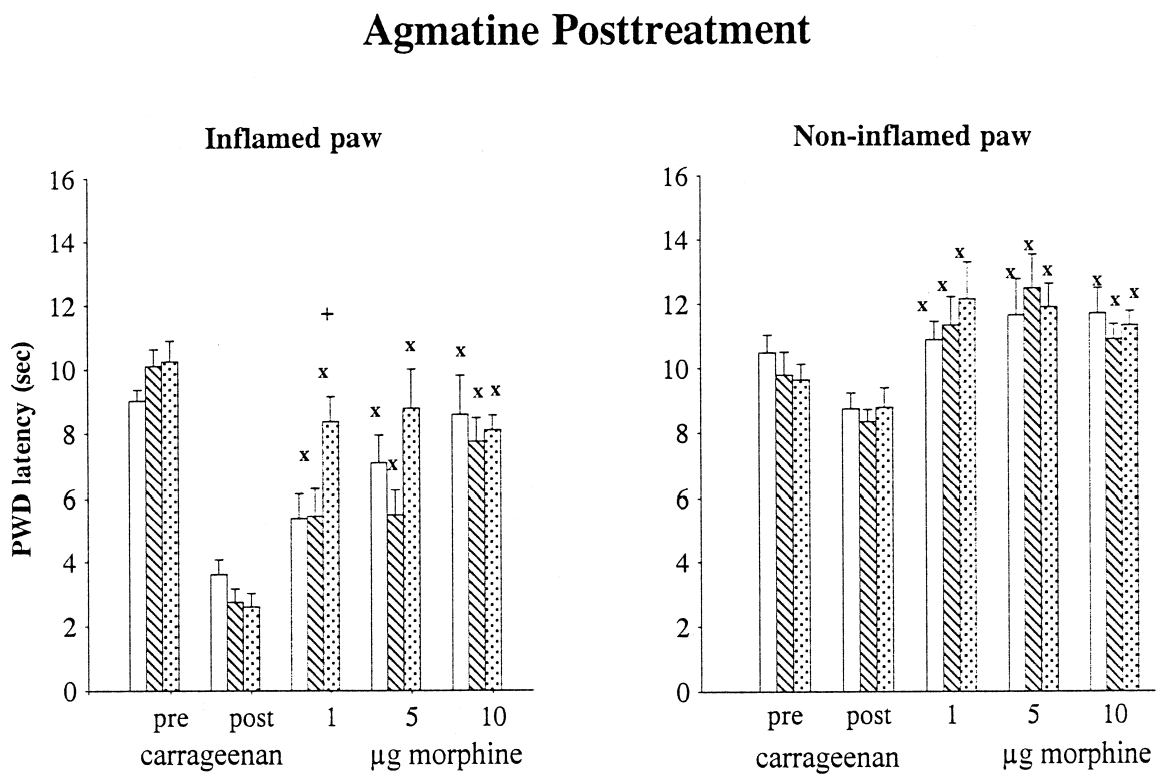

Fig. 3. Effects of posttreatment (saline-pretreated rats: open bars, 50 μg agmatine-pretreated rats: hatched bars, 100 μg agmatine-pretreated rats: dotted bars) with different doses of agmatine on morphine-induced antinoception (1, 5, 10 μg cumulative) in normal (right side) and inflamed paws (left side). The results are expressed as means ± S.E.M. $^{x}P < 0.05$ compared to post-carrageenan baseline values. $^{+}P < 0.05$ compared to saline-treated control group.

latency reached the pre-carrageenan value. Similarly, agmatine pre- or posttreatment plus morphine coadministration increased the paw withdrawal latency on the normal side (Figs. 2 and 3, right side). Agmatine pretreatment dose-dependently increased the morphine (1 μg) anti-hyperalgesic effect on the inflamed paw, and the latency attained the pre-carrageenan value. Furthermore, morphine prevented the reappearance of hyperalgesia produced by 100 μg agmatine pretreatment (Figs. 2 and 3, left side). As regards the posttreatment, only 100 μg agmatine coadministered with 1 μg morphine was effective, but the latency did not achieve the pre-carrageenan value. Table 2 reports the magnitudes of the agmatine-produced percentage attenuation of the thermal hyperalgesia with or without morphine. Fig. 4 depicts the percentage attenuation resulting

Table 2
Potentiating effect of agmatine on anti-hyperalgesic effect of morphine hyperalgesia as a percentage score (percentage score = postcarrageenan value × 100/precarrageenan value, and it was calculated for each rat)

Treatment	Dose (μg)	At 0 min	At 30 min
Morphine	1	40.6 ± 5.32	60.1 ± 8.40
Pre-agmatine	50	53.2 ± 11.59	61.4 ± 13.46
Pre-agmatine–morphine	50–1	54.3 ± 9.00	89.8 ± 19.38
Pre-agmatine	100–1	76.9 ± 12.29	72.1 ± 6.49
Pre-agmatine–morphine	100–1	74.0 ± 9.97[a]	103.0 ± 10.16[a,b]
Post-agmatine	50	34.5 ± 4.94	51.1 ± 10.43
Post-agmatine–morphine	50–1	28.6 ± 5.07	56.0 ± 9.21
Post-agmatine	100	46.8 ± 7.97	64.8 ± 11.42
Post-agmatine–morphine	100–1	25.6 ± 3.36	83.6 ± 8.15

[a] $P < 0.05$ compared to morphine-treated group.
[b] $P < 0.05$ compared to agmatine-treated group.

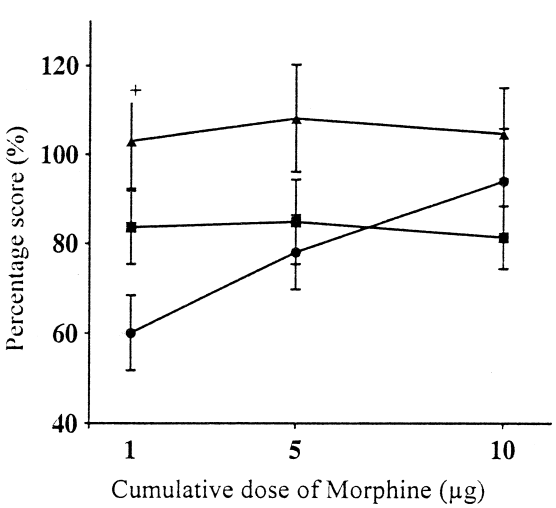

Fig. 4. Potentiating effect of agmatine on anti-hyperalgesic effect of morphine (percentages of pre-carrageenan values). Morphine alone: circle, 100 μg agmatine-pretreatment + morphine: triangle, 100 μg agmatine-posttreatment + morphine: square. $* P < 0.05$ compared to agmatine-pretreated group without morphine.

from morphine or morphine-agmatine coadministration (100 μg pre- or posttreatment) in thermal hyperalgesia.

4. Discussion

This is the first study of the anti-hyperalgesic potency of intrathecally administered agmatine on carrageenan-induced hyperalgesia. Intrathecal pre- or posttreatment with agmatine does not lead to any change in thermal withdrawal threshold in the contralateral paw. Agmatine pretreatment significantly attenuates the thermal hyperalgesia produced by intraplantar carrageenan, while agmatine posttreatment has lower efficacy. Both agmatine pre- and posttreatment potentiated the effect of intrathecal morphine in attenuating the thermal hyperalgesia induced by intraplantar carrageenan. Doses larger than 250 μg (cumulative) also caused extreme behavioral changes, involving agitation and vocalization as observed earlier in rabbits (Szabo et al., 1995): vocalization and fighting lasting for several hours in all animals together, with a decrease in withdrawal latency of the normal paw.

The combined results of the binding and functional experiments indicate that agmatine exerts little activity on the α_2-adrenoceptor (Bylund, 1995). The action of agmatine is of particular interest since it is the only native ligand for imidazoline binding sites yet identified. In addition, agmatine has been found to be synthesized and stored in astrocytes in tissue cultures (Regunathan et al., 1995). Despite binding like clonidine to α_2-adrenoceptor and imidazoline binding sites of both classes, agmatine does not replicate any central or peripheral actions of clonidine. Similarly to clonidine (Lal and Shearman, 1981; Gellai and Ruffolo, 1987), agmatine enhances food intake in satiated rats (Prasad and Prasad, 1996), and increases the urine flow rate and osmolar clearance (Smyth et al., 1996). However, unlike clonidine (Lal and Shearman, 1981), intracerebroventricularly administered agmatine increases the sympathetic nerve activity and arterial pressure (Sun et al., 1995). In contrast with clonidine (Reiner, 1985, 1986; Williams et al., 1985; Aghajanian and Wang, 1986), local application of agmatine in vivo causes a slight and short-lasting increase in locus coeruleus cell firing rate (Pineda et al., 1996), which suggests a possible involvement of imidazoline binding sites (Pineda et al., 1993). Our results demonstrate a further difference between the two drugs, e.g., while clonidine or the more specific α_2-adrenoceptor agonist dexmedetomidine in higher doses caused sedation and anesthesia (Horvath et al., 1994), agmatine administration evoked excitation. Interestingly, 100 μg agmatine pretreatment gives rise to a less sustained anti-hyperalgesic effect than does 50 μg, and the hyperalgesia reappears after the higher dose. There are several possibilities to explain the 'bell-shaped' phenomenon and the excitation. First, we could not exclude the idea that intrathecal agmatine may reach supraspinal centers and inhibit the activation of the descending inhibitory pathway, causing anti-analgesia (Horvath et al., 1994). This possibility agrees with the observation of a hyperalgesic effect of supraspinally administered clonidine (Fujimoto and Arts, 1990). Secondly, agmatine could increase the release of endogenous anti-analgesic substances, e.g., dynorphin A or CCK (Arts et al., 1992). These effects could cause excitation by larger doses, which could be manifested by repeated pain stimulation. The distributions of imidazoline binding sites and α_2-adrenoceptors, though overlapping to an appreciable extent, still differ substantially. Immunostained fibers were detected in the superficial laminae of the dorsal horn, where they were heavily concentrated in laminae I and II, a terminal field of primary nociceptive and visceral afferents, suggesting a role of imidazoline binding sites in specific neuronal functions, particularly the processing of primary somatic and visceral afferents, autonomic and neuroendocrine functions, chemoreception, and the integration of emotional behavior (Ruggiero et al., 1995, 1998). Thus, the high-affinity imidazoline$_2$ binding site ligand reduced the nociceptive responses of dorsal horn neurons in anesthetized rats (Diaz et al., 1997). However, one study suggested that the spinal action of clonidine is mediated solely by α_2-adrenoceptors (Monroe et al., 1995).

A recent study found that systemically administered agmatine did not express any antinociceptive effects itself, but it enhanced μ- and δ-opioid induced analgesia in the tail-flick test in mice (Kolesnikov et al., 1996). Furthermore, the systemic administration of agmatine did not affect the nociceptive reflexes evoked by mechanical and electrical stimuli until very high doses were reached (Bradley and Headley, 1997). A high dose also caused complex cardiovascular disturbances, and atipamezole, a specific α_2-adrenoceptor agonist, did not influence these effects (Bradley and Headley, 1997). Our findings are in line with these results after intrathecal agmatine administration, e.g., agmatine did not influence the normal paw latency. Similarly, Bradley and Headley (1997) demonstrated that systemic administration of agmatine did not influence the antinociceptive effect of the μ-opioid receptor agonist fentanyl in an acute pain test; we observed that intrathecal agmatine potentiated the anti-hyperalgesic effect of intrathecal morphine. One of the reasons for this difference might be that they did not use an inflammatory stimulus to produce hyperalgesia, and another may be the systemic route of drug administration. Our data suggest that this potentiation occurs only in the presence of an inflammatory stimulus and/or when there is hyperalgesia present. Naturally, we could not determine the exact type of interaction from these data and we could not exclude the possibility that the interaction between morphine and agmatine is additive. It is a fact that the lowest dose of morphine, which itself causes no significant decrease in the thermal hyperalgesia, suppressed the hyperalgesia entirely when applied together with agmatine. It is an addi-

tional advantage that morphine inhibits the excitatory effects of agmatine. Agmatine has the further favorable effect that it does not suppress pain responses in the non-inflamed paw; it acts only in a hyperalgesic state. The potentiating effect of agmatine on the anti-hyperalgesic effect of intrathecal morphine may be related to several mechanisms: first, agmatine binds to α_2-adrenoceptors (Li et al., 1994), and drugs such as clonidine which bind to these receptors also have prominent potentiating effects on morphine-induced antinociception (Lal and Shearman, 1981; Horvath et al., 1990) These data suggest that agmatine may increase the effect of morphine by a clonidine-like effect. However, unlike clonidine, agmatine has not been shown to have agonist activity at α_2-adrenoceptors (Pinthong et al., 1995). Thus, it is unlikely that an α_2-mechanism explains the present results. Alternatively, its action as an agonist at imidazoline binding sites could be responsible for the beneficial effects of agmatine on morphine-induced anti-hyperalgesia.

A further possibility may be central nitric oxide synthase inhibition by agmatine. It has been shown that nitric oxide has a significant role in the development and maintenance of the thermal hyperalgesia produced by intraplantar injection of carrageenan in the rat (Meller et al., 1994a). Furthermore, inducible nitric oxide synthase can be induced in the spinal cord following either a peripheral or a central stimulus that results in thermal hyperalgesia, and intrathecal treatment with inducible nitric oxide synthase inhibitors reduces the thermal hyperalgesia produced by intraplantar zymosan (Meller et al., 1994b; Grzybicki et al., 1996; Wu et al., 1998). The fact that agmatine is synthesized from arginine in endothelial cells, likewise the site of production of nitric oxide from arginine (Moncada et al., 1991), may have important implications. This pathway for the arginine metabolism in endothelial cells, besides forming agmatine, could also regulate the synthesis of nitric oxide by limiting the availability of arginine. Some data suggest that agmatine may act as an endogenous inhibitor of inducible nitric oxide synthase (Auguet et al., 1995). It has recently been observed that agmatine may additionally inhibit the activity of constitutive nitric oxide synthase (Galea et al., 1996). These interesting ramifications of this study necessitate further investigations.

The current study has demonstrated that agmatine does not suppress pain responses in the non-inflamed paw; it acts only in a hyperalgesic state. Our studies indicate that intrathecally administered agmatine can influence the analgesic actions of specific opioid receptor agonists. The combination of agmatine in relatively small doses with other analgesics is particularly appealing because of the potential for this drug to produce side-effects. With combined low doses of agmatine plus morphine that produced substantial analgesic effects, no side-effects were seen. The potential clinical use of spinal agmatine, following the appropriate preclinical toxicological studies, appears to gain at least theoretical support from the present studies.

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