

# Agmatine Potentiates the Analgesic Effect of Morphine by an $\alpha_2$ -Adrenoceptor-Mediated Mechanism in Mice

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*The effects of agmatine, which is an endogenous polyamine metabolite formed by decarboxylation of L-arginine, and a combination of agmatine and morphine on tail-flick test have been investigated in mice. Adult male Swiss-Webster mice were used in the study. Agmatine (10, 20 and 40 mg/kg), clonidine (0.15 mg/kg), yohimbine (0.625 and 1.25 mg/kg), or saline were injected into mice intraperitoneally. Morphine (1 and 2 mg/kg) was given subcutaneously. Agmatine alone did not produce any significant change on radiant tail-flick latencies, but it potentiated significantly and dose-dependently morphine-induced (1 mg/kg) analgesia. The potentiating effect of agmatine (40 mg/kg) on morphine-induced analgesia was blocked completely by yohimbine (0.625 mg/kg), a selective  $\alpha_2$ -adrenoceptor antagonist, pretreatment. Clonidine (0.15*

*mg/kg), an  $\alpha_2$ -adrenergic receptor agonist, caused a significant increase of the tail-flick latencies of the mice. Yohimbine (0.625 mg/kg) also blocked clonidine-induced analgesia. In addition, yohimbine (0.625 mg/kg) was ineffective on the tail-flick test and did not produce any significant change on the morphine-induced analgesia. Our results indicate that cotreatment of agmatine with morphine produces antinociceptive enhancement via an  $\alpha_2$ -adrenergic receptor-mediated mechanism and agmatine-morphine combination may be an effective therapeutic strategy for medical treatment of pain.*

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Agmatine is a cationic amine formed by decarboxylation of arginine by the enzyme arginine decarboxylase (Tabor and Tabor 1984). It is a biological active substance (Lortie et al. 1996) and binds with high affinity to both imidazoline and  $\alpha_2$ -adrenergic receptors of all subclasses (Li et al.

1994; Piletz et al. 1995; Regunathan and Reis 1996; Reis and Regunathan 1998a). Recently, it has been suggested that agmatine meets many criteria for a neurotransmitter in brain. It is synthesised, stored, and released in brain; is contained in neurones and axon terminals; interacts with cell-specific receptors; and elicits biological actions within the central nervous system (Reis and Regunathan 1998a,b). Thus, results of some recent studies indicate the modulatory effects of agmatine in central nervous system. For example, agmatine selectively inhibits nitric oxide synthase (Galea et al. 1996), an enzyme converting L-arginine to nitric oxide, and the NMDA subclass of glutamate receptor channels (Yang and Reis 1999) in rat brain. Several reports indicated that an important role of NMDA receptors and nitric oxide in development of the opioid (Trujillo and Akil 1991; Adams et al. 1993; Thorat et al. 1994; Vaupel et al. 1997) and ethanol (Rossetti and

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Carboni 1995; Adams et al. 1995; Kotlinska and Liljequist 1996; Uzbay et al. 1997) physical dependence. Agmatine also attenuates naloxone-induced morphine withdrawal signs in morphine-dependent mice (Aricioglu-Kartal and Uzbay 1997) and many signs of ethanol withdrawal syndrome in ethanol-dependent rats (Uzbay et al. 2000).

The drugs that have selective agonistic activity on imidazoline/ $\alpha_2$ -adrenergic receptors such as clonidine, xylazine, and moxonidine produce antinociceptive responses in rodents (Paalzow 1974; Browning et al. 1982; Ossipov et al. 1989; Fairbanks et al. 2000). The role of adrenoceptors, particularly the  $\alpha_2$  subtype, in the potentiation of opioid-induced analgesia is also well known (Wigdor and Wilcox 1987; Dambisya et al. 1991). In the light of these reports, it would be expected that agmatine, another agent binding imidazoline/ $\alpha_2$ -receptors, should also have antinociceptive activity. Thus, some recent studies indicate modulatory effects of agmatine on opioid analgesia in mice (Kolesnikov et al. 1996; Fairbanks and Wilcox 1997) and rats (Horváth et al. 1999). It was reported that intrathecal administration of agmatine potentiated morphine-induced  $\delta$ -opioid receptor-mediated analgesia ninefold without affecting pain thresholds and chronic administration prevented  $\kappa_1$ -opioid receptor-mediated tolerance in mice (Kolesnikov et al. 1996; Bradley and Headley 1997). However, the mechanisms of these effects remain to be fully understood. It is not certain whether these effects are mediated by  $\alpha_2$ -adrenergic or imidazoline receptors. On the other hand, there are limited studies investigating the antinociceptive effect of peripheral agmatine administration in rodents.

The main objective of the present study was to investigate the possible effects of agmatine and an agmatine-morphine combination on nociception by using the tail-flick method, a thermal analgesic test in mice. We also evaluated the mechanism of the potentiating effect of agmatine on morphine-induced analgesia by pretreatment with yohimbine, a selective  $\alpha_2$ -adrenergic receptor blocker.

## MATERIALS AND METHODS

### Animals and Laboratory

All experiments were performed at the same time every day and in the light period (10:30 – 13:00 AM). The experiments performed in this study have been carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by National Institutes of Health (USA) and the Declaration of Helsinki. Adult male albino Swiss-Webster mice (22–30 g) were subjects in our study. They were placed in a quiet and temperature- and humidity-controlled room ( $22 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$ , respectively) in which a 12/12 hour light-dark cycle was maintained (07 AM – 07 PM light).

### Drugs Used

Agmatine sulphate, morphine sulphate, clonidine HCl, and yohimbine HCl were purchased from Sigma Chemical (St. Louis, MO) and dissolved in saline. The drugs or saline, except morphine, which was given subcutaneously, were injected intraperitoneally in to mice at a volume of 0.5 ml/100 g.

### Measurement of Nociception and Procedure

Nociceptive effects were assessed using the tail-flick test, which is a thermal analgesia measurement method for rodents (D'Amour and Smith 1941). Tail-flick latencies were measured by a tail-flick test apparatus (Columbus, OH, Type 812). The mean of the tail-flick latencies measured in three predrug trials represented the individual baseline. Animals showing tail-flick latencies ranging from 3 to 4.5 s before treatments were only used in the experiments. Immediately after baseline assessment, drugs or saline were injected in separately grouped mice according to the protocols in Table 1. Tail-flick latencies were measured 30 min after administration of the last treatments. During the combination treatments, agmatine was injected 15 min before mor-

**Table 1.** Groups and Injection Protocols

Group No.	Injection
1	Saline ( $n = 10$ )
2	Agmatine 10 mg/kg ( $n = 10$ )
3	Agmatine 20 mg/kg ( $n = 10$ )
4	Agmatine 40 mg/kg ( $n = 10$ )
5	Morphine 1 mg/kg ( $n = 10$ )
6	Morphine 2 mg/kg ( $n = 10$ )
7	Yohimbine 0.625 mg/kg ( $n = 10$ )
8	Yohimbine 1.25 mg/kg ( $n = 10$ )
9	Saline + saline ( $n = 10$ )
10	Saline + morphine 1 mg/kg ( $n = 10$ )
11	Saline + morphine 2 mg/kg ( $n = 6$ )
12	Agmatine 10 mg/kg + morphine 1 mg/kg ( $n = 10$ )
13	Agmatine 20 mg/kg + morphine 1 mg/kg ( $n = 10$ )
14	Agmatine 40 mg/kg + morphine 1 mg/kg ( $n = 10$ )
15	Yohimbine 0.625 mg/kg + morphine 1 mg/kg ( $n = 8$ )
16	Yohimbine 0.625 mg/kg + morphine 2 mg/kg ( $n = 6$ )
17	Saline + clonidine 0.15 mg/kg ( $n = 6$ )
18	Yohimbine 0.625 mg/kg + clonidine 0.15 mg/kg ( $n = 6$ )
19	Saline + saline + saline ( $n = 8$ )
20	Saline + agmatine 40 mg/kg + morphine 1 mg/kg ( $n = 8$ )
21	Yohimbine 0.625 mg/kg + agmatine 40 mg/kg + Morphine 1 mg/kg ( $n = 8$ )

phine, and yohimbine was injected 15 min before agmatine or clonidine, and the tail-flick latencies were assessed 30 min after the morphine, clonidine, or saline injections. The timing of the drug injections was adjusted according to the previous studies investigating the effect of agmatine and the role of  $\alpha$ -adrenoceptors on opioid antinociception in rodents (Browning et al. 1982; Ossipov et al. 1989; Dambisya et al. 1991; Kolesnikov et al. 1996). Cut-off time was 12 s. The degree of analgesia was calculated as the percentage of the maximum possible effect according to the following formula (Akil and Mayer 1972; Uzbay et al. 1999) [Eq. (1)]:

% Analgesic effect =

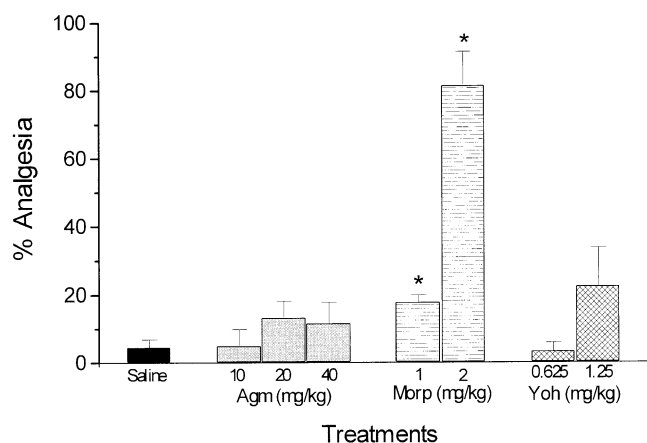
$$100 \times \frac{[(\text{postdrug reaction time}) - (\text{predrug reaction time})]}{[(\text{Cut-off value}) - (\text{predrug reaction time})]} \quad (1)$$

### Statistical Analysis

Results on antinociception were expressed as mean  $\pm$  SEM. Analysis of variance (one-way ANOVA) followed by Dunnett's test were used in evaluation of the effects of agmatine, morphine, or yohimbine alone and combination treatments on the nociception. Changes in nociceptive responses after clonidine alone, and combination treatments with yohimbine were analyzed by unpaired (between groups) Student's *t*-test. The level of statistical significance was set at  $p < .05$  level.

## RESULTS

The effects of agmatine, morphine, yohimbine and combinations of all doses of agmatine with low doses of morphine on tail-flick test are shown in Figures 1 and 2,



**Figure 1.** Effects of agmatine, morphine, and yohimbine on tail-flick latencies of the mice (\* $p < .05$  significantly different from saline treatment; Agm = agmatine; Morp = morphine; Yoh = yohimbine).

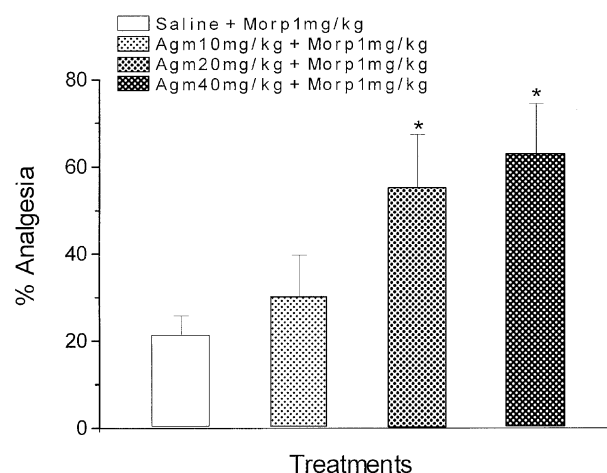
respectively. Morphine (1 and 2 mg/kg) prolonged the tail-flick latency of the mice significantly [ $F(2,27) = 41.19$ ;  $p = .0006$ ] and dose dependently, as compared to mice treated with saline. Yohimbine (0.625 and 1.25 mg/kg), a selective  $\alpha_2$ -adrenergic receptor antagonist, alone did not produce any significant change [ $F(2,27) = 1.58$ ;  $p = .223$ ] on the tail-flick latencies (Figure 1). Agmatine (10–40 mg/kg) did not produce any significant change [ $F(3,36) = 0.92$ ;  $p = .439$ ] on the tail-flick latencies (Figure 1), but it potentiated the analgesic effect of morphine (1 mg/kg) significantly [ $F(3,36) = 4.59$ ;  $p = .008$ ] and dose dependently (Figure 2).

The effect of yohimbine on the potentiating effect of agmatine on morphine-induced analgesia is shown in Figure 3. Yohimbine (0.625 mg/kg) pretreatment completely and significantly blocked the potentiating effect of agmatine (40 mg/kg) on morphine (1 mg/kg) analgesia.

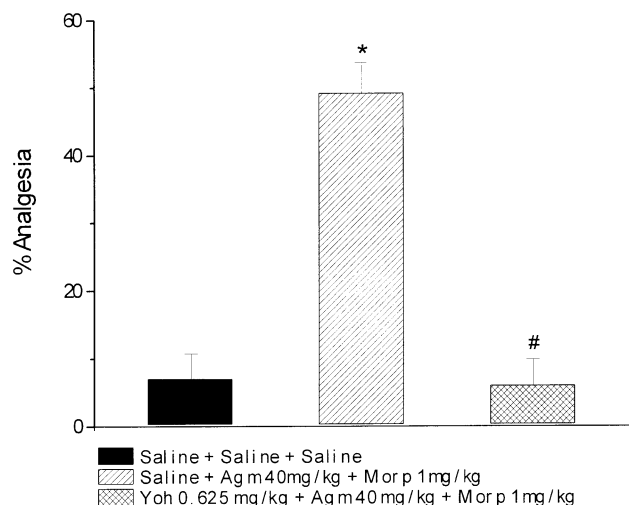
The effects of clonidine, morphine, and combinations of clonidine and morphine with yohimbine on tail-flick latencies of the mice are shown in Figure 4. Both clonidine (0.15 mg/kg) and morphine (1 and 2 mg/kg) caused significant increases in radiant tail-flick latencies of the mice. Although yohimbine (0.625 mg/kg) antagonized the analgesic effect of clonidine, it was ineffective on morphine-induced analgesia.

## DISCUSSION

The present study demonstrates that agmatine, an arginine metabolite, did not produce any analgesic effect itself, but it potentiated morphine-induced analgesia in mice. Because the agmatine-induced potentiation of morphine analgesia was blocked completely by yohim-



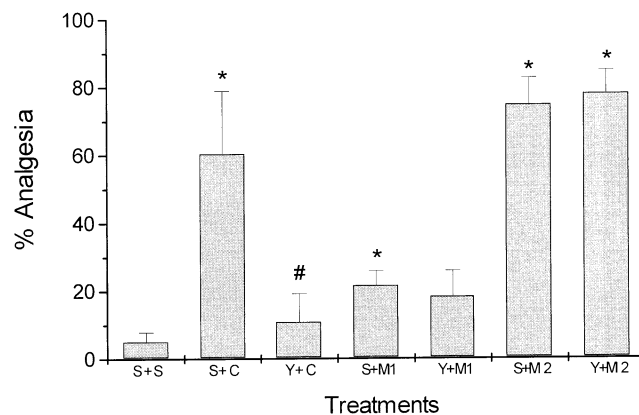
**Figure 2.** Effects of agmatine on morphine-induced analgesia (\* $p < .05$  significantly different from morphine plus saline treatment; Agm: agmatine; Morp: morphine).



**Figure 3.** Antagonism of potentiating effect of agmatine on morphine-induced analgesia by yohimbine (\* $p < .05$  significantly different from the group treated with saline plus saline plus saline; # $p < .05$  significantly different from group treated saline plus agmatine plus morphine; Agm = agmatine; Morp = morphine; Yoh = yohimbine).

bine, a selective  $\alpha_2$ -adrenergic receptor antagonist, without producing any significant effect on tail-flick latencies of the mice and causing any change on morphine-induced analgesia, it is obvious that the augmenting effect of agmatine may be related to  $\alpha_2$ -adrenergic receptor-mediated mechanisms.

Our results indicating that agmatine (10 mg/kg) did not produce any significant effect on the tail-flick latency are consistent with a previous study showing that doses of agmatine up to 10 mg/kg had no effect on the tail-flick latency in mice (Kolesnikov et al. 1996; Fairbanks and Wilcox 1997). In addition, we did not observe any prominent effects with higher doses of agmatine up to 40 mg/kg on the tail-flick test in mice. Although these doses of agmatine increased the tail-flick latency of the mice slightly, these increases did not reach a statistically significant level. This finding does not support the hypothesis that agmatine itself could have an antinociceptive activity like other imidazoline/ $\alpha_2$ -adrenergic receptor agonists (Paalzow 1974; Browning et al. 1982; Ossipov et al. 1989; Fairbanks et al. 2000). Some conflicting studies on the role of  $\alpha_2$ -adrenergic receptors in agmatine effects have been published. Although agmatine can bind to  $\alpha_2$ -adrenergic receptors, it does not activate them (Pinthong et al. 1995a). Agmatine has no activity at  $\alpha_2$ -adrenoceptors modulating the firing rate of locus coeruleus neurones (Pineda et al. 1996) and, thus, agmatine alone is unlikely to account for all of the biological activity of clonidine-displacing substance (Piletz et al. 1995; Pinthong et al. 1995 a,b). Furthermore, the systemic administration of agmatine alone did not affect



**Figure 4.** Effects of clonidine, morphine, and combination treatments of yohimbine with clonidine and morphine on tail-flick latencies of the mice (S+S = saline plus saline; S+C = saline plus clonidine 0.15 mg/kg; Y+C = yohimbine 0.625 mg/kg plus clonidine 0.15 mg/kg; S+M1 = saline plus morphine 1 mg/kg; Y+M1 = yohimbine 0.625 mg/kg plus morphine 1 mg/kg; S+M2 = saline plus morphine 2 mg/kg; Y+M2 = yohimbine 0.625 mg/kg plus morphine 2 mg/kg; \* $p < .05$  significantly different from the group treated with saline plus saline; # $p < .05$  significantly different from saline plus clonidine).

the nociceptive reflexes evoked by mechanical and electrical stimuli until very high doses (200 mg/kg, IV) were reached. The high dose of agmatine also caused complex cardiovascular disturbances, and atipemazole, a specific  $\alpha_2$ -adrenoceptor antagonist, did not influence these effects (Bradley and Headley 1997). In contrast to the results of these studies, agmatine has been shown to have agonist activity at prejunctional  $\alpha_2$ -adrenoceptors in the rat tail artery (Gonzalez et al. 1996) and multiple effects on sympathetic neurotransmission in rat vas deferens (Jurkewicz et al. 1996). Using different animal species, doses, administration routes, and in vivo or in vitro techniques may be responsible for the discrepancies between the studies. We selected the dose range (10–40 mg/kg) to test the effects of agmatine on tail-flick latency, because these doses were very effective on naloxone-precipitated abstinence signs without influencing motor coordination in morphine-naïve rats (Aricioglu-Kartal and Uzbay 1997).

In the present study, co-administration of agmatine with morphine produced a significant and dose-dependent antinociceptive enhancement. This finding is consistent with many studies that have demonstrated the ability of systemically co-administered imidazoline/ $\alpha_2$ -adrenoceptor agonists and morphine to produce antinociceptive enhancement in mice when measured by the substance P nociceptive test (Roerig et al. 1992; Fairbanks et al. 2000), the radiant heat tail-flick test (Roerig 1995), and the warm water immersion tail-flick test (Fairbanks and Wilcox 1999).

It has been reported that systemic administration of yohimbine (2–5 mg/kg) blocks various behavioral parameters induced by  $\alpha_2$ -receptor agonists (Currie and Wilson 1992; Denizbasi et al. 1999). We observed significant changes in the tail-flick reaction time by higher doses of yohimbine than 1.25 mg/kg (data not shown). In addition, Kihara and Kaneto (1986) showed that yohimbine (1–5 mg/kg) antagonised morphine-induced analgesia in mice. Thus, in the present study, we used yohimbine at a lower dose than 1 mg/kg during combination treatments. The dose of yohimbine (0.625 mg/kg) was ineffective on the tail-flick reaction time of the naive mice. However, it blocked completely the agmatine-induced potentiation of the morphine analgesia. Moreover, yohimbine pretreatment (0.625 mg/kg) did not produce any significant change on the morphine (1 and 2 mg/kg)-induced analgesia. In addition, we tested the effect of yohimbine pretreatment on clonidine-induced analgesia. Yohimbine (0.625 mg/kg) also blocked the antinociceptive effect of clonidine, an  $\alpha_2$ -adrenoceptor agonist that has antinociceptive activity in rodents (Paalzow 1974; Browning et al. 1982; Ossipov et al. 1989). This observation confirms that the dose of yohimbine used in the present study effectively blocked  $\alpha_2$ -adrenergic receptors. These findings show that the augmenting effects of agmatine on morphine analgesia may be mediated by an  $\alpha_2$ -adrenoceptor modulated mechanism. Our observations also support the results of the previous report suggesting systemic administration of agmatine potentiates morphine-induced analgesia in mice (Kolesnikov et al. 1996). In a recent study, Horváth et al. (1999) also reported that intrathecal agmatine pretreatment potentiated the effect of intrathecal morphine by attenuating inflammation-induced thermal hyperalgesia in rats. Our findings are also distinguished from that of Kolesnikov et al. (1996), where they showed that systemically administered agmatine potentiated morphine analgesia in an idazoxan-dependent manner. As different from idazoxan, yohimbine, a selective  $\alpha_2$ -adrenoceptor antagonist, does not bear an imidazoline ring. Therefore, our data may provide more direct evidence that the agmatine-enhancing effect is mediated by  $\alpha_2$ -adrenergic receptors rather than imidazoline receptors.

In conclusion, our results demonstrate that agmatine combination with morphine produces an antinociceptive enhancement and this effect seems to be mediated via  $\alpha_2$ -adrenergic receptors. Administration of agmatine-opioid combinations may also provide an effective therapeutic strategy for future medical treatment of pain.

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