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Agmatine produces antinociception in tonic pain in mice

Aytül Önal*, Necdet Soykan

Department of Pharmacology, Faculty of Medicine, Ege University, 35100 İzmir, Turkey

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Abstract

Agmatine is an endogenous polyamine metabolite formed by decarboxylation of L-arginine. In this study, the effect of agmatine on tonic pain was compared to its effect on phasic pain by using the formalin and tail-flick (TF) tests in mice. When administered intraperitoneally (ip), agmatine (37.5–300 mg/kg) exhibited a decrease in nociceptive behaviours in the first and second phase of the formalin test, which is a tonic pain model. The α_2 adrenoceptor antagonist yohimbine blocked the effect of agmatine in Phase 2 but did not change its effect in Phase 1. In the TF test, there was no significant change in the behaviour of agmatine-administered (75–300 mg/kg) animals. As a result, agmatine appears to have an analgesic effect on tonic rather than phasic pain, and α_2 receptors seem partly to have a role in the antinociceptive effect of agmatine on tonic pain.

Keywords: Agmatine; Tonic; Pain; Mice

1. Introduction

Agmatine is a newly identified amine in mammals that is widely distributed in a number of tissues including brain, stomach, intestine and aorta (Delbarre et al., 1995; Li et al., 1994; Raasch et al., 1995). It is synthesized from L-arginine by the enzyme arginine decarboxylase (ADC) (Cox and Boeker, 1987; Wu and Morris, 1998), and it has been suggested to be an endogenous clonidine-displacing substance (Li et al., 1994). In experimental studies, clonidine was shown to have an α_2 receptor-mediated antinociceptive effect (Bernard et al., 1995; Minor et al., 1989; Waterman et al., 1988). Agmatine, like clonidine, was demonstrated to bind to α_2 receptors, though it is not clear how it affects these receptors (Pinthong et al., 1995).

Nitric oxide (NO) is a free radical gas synthesized from the semiessential amino acid L-arginine by NO synthase (NOS). NO is an endogenous stimulator of guanylate cylase that has a role in the formation of cyclic guanosine monophosphate (GMP) and acts as a messenger molecule in many systems such as white blood cells, blood vessels and nervous system (Moncada et al., 1991; Moncada and Higgs, 1993; Rang et al., 1995). The relationship between pain and L-arginine has been shown in several investigations (Duarte et al., 1990; Dumka et al., 1996; Kawabata et al., 1994; Kitto et al., 1992; Meller and Gebhart, 1993).

In recent years, there have been very few studies investigating the relationship between agmatine and nociception (Bradley and Headley, 1997; Horvath et al., 1999; Kolesnikov et al., 1996; Li et al., 1999a). These studies were performed mostly using phasic pain models. Tonic (persistent) pain is believed to be processed differently than phasic (short-term or acute) in the central nervous system (CNS) (Tjolsen et al., 1992).

In our study, we studied the effect of agmatine on nociception in mice by comparing its effects on tonic pain in the formalin test and phasic pain in the tail-flick (TF) test. In addition, we investigated the possible role of α_2 receptors in the agmatine–nociception relationship.

2. Materials and methods

2.1. Animals and laboratory

Locally bred male albino mice (23–29 g) were used in this study. Animals were housed in groups of 8–10 under a standard 12 h light/12 h dark cycle in a room maintained at

* Corresponding author. Tel.: +90-232-3882862; fax: +90-232-3422142.

E-mail address: aytul@med.ege.edu.tr (A. Önal).

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22±3°C. All experiments were carried out between 0900 and 1200 h. Each animal was used only once.

2.2. Sensorimotor performance

To evaluate sensorimotor performance, the mice were tested on a rotarod device (Rosland et al., 1990) before beginning the experiment. Briefly, the rotarod apparatus consists of a rod 30 cm long and 3 cm in diameter that is subdivided into five compartments by discs 24 cm in diameter. The rod rotates at a constant speed of 16 rpm. The mice with insufficient sensorimotor performance were not included in the experiment. The rotarod test, which was done before administration of any drug, was repeated 15 min after the administration of saline or agmatine. Results were expressed as the duration which the animals could stand on the rod (cut-off time was 120 s.).

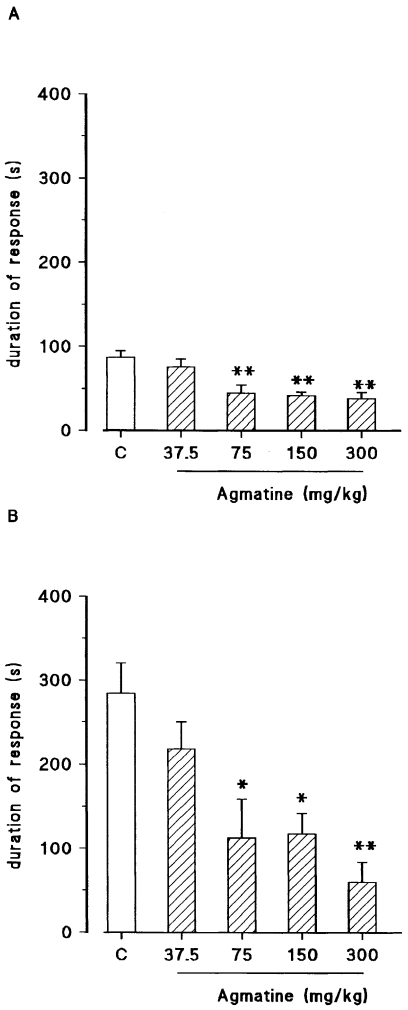

Fig. 1. Effect of ip administered agmatine (37.5–300 mg/kg) on Phase 1 (A) and Phase 2 (B) of formalin-induced nociception in mice. (C) represents control results obtained in saline-administered animals. The data are presented as the mean±S.E.M. from six mice, * $P<.05$ and ** $P<.01$ versus control.

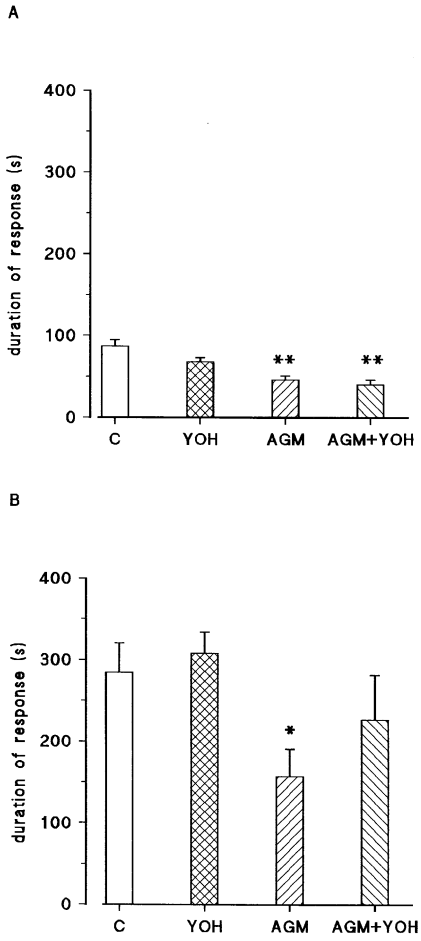

Fig. 2. Effect of ip administered yohimbine (YOH) (1 mg/kg)+agmatine (AGM) (162 mg/kg, ED_{50} dose of Phase 1) on Phase 1 (A) and yohimbine (1 mg/kg)+agmatine (85 mg/kg, ED_{50} dose of Phase2) on Phase 2 (B) of formalin-induced nociception in mice. (C) represents control results obtained in saline-administered animals. The data are presented as the mean±S.E.M. from five to six mice, * $P<.05$ and ** $P<.01$ versus control.

2.3. Nociceptive tests

Nociceptive responses were determined using the formalin and TF tests. In the formalin test, mice were acclimated to individual cylindrical shape Plexiglas observation chambers (height: 25 cm; diameter: 30 cm) for at least 1 h prior to testing. According to the method of Dubuisson and Dennis (1977), modified by Shibata et al. (1989), after the administration of formalin (5%; 25 μl sc) to the right hindpaw of the mice, the duration of licking, biting and flinching of the injected paw was recorded using a digital stopwatch (0.01-s accuracy) for each observation period. The nociceptive responses to formalin were assessed by observing the animals in chambers and quantitating the above behaviours every 5 min for a total period of 45 min. Results were expressed as mean nociceptive response time (s) ±S.E.M. in the first (0–5 min) and second (15–45 min) phases. Agmatine (37.5–300 mg/kg), alone and in combination with the α_2 adrenoceptor

Table 1
Effects of ip administered agmatine in the mice TF test

	Percent maximum effect (at the post injection time)		
	15 min	30 min	60 min
Control	−3.77±5.47	−6.12±6.01	−7.23±6.72
Agmatine (mg/kg)			
75	4.33±5.00	5.86±5.86	−7.93±5.95
150	6.11±2.04	2.18±5.68	6.41±2.90
300	10.75±6.47	20.63±11.07	19.15±12.60

TF responses were measured 15, 30 and 60 min after drug administration. The data are presented as the mean±S.E.M ($n=6$).

antagonist yohimbine (1 mg/kg) (ED_{50} dose of agmatine in Phases 1 and 2), and saline were administered 15 min before the formalin injection.

The nociceptive TF reflex was determined using a TF apparatus (May, 9604, Ankara). Briefly, the TF apparatus consists of a beam of focused radiant light, which is focused on the dorsal surface of the tail at one of five sites 8–10 mm apart (D'Amour and Smith, 1941). The latency to reflexive removal of the tail from the heat was measured by a photocell timing circuit to the nearest 0.1 s. The intensity of heat stimulus was adjusted so that the mouse flicked its tail after 2–4 s. Cut-off time of 10 s was imposed to minimize damage to the skin of the tail. Each mouse was tested twice before saline and drug administration, and reaction times were averaged to obtain a baseline. The TF test was repeated 15, 30 and 60 min after agmatine (75–300 mg/kg) and saline administration. TF data were expressed as percent maximum possible effect (MPE), which was calculated by using the formula (postdrug TF latency − predrug TF latency/cut-off TF latency − predrug TF latency × 100) and stated as a percentage.

2.4. Drugs

Agmatine (Sigma, St. Louis, MO) and yohimbine (Sigma) were dissolved in saline. The drugs and saline were injected intraperitoneally (ip) at a volume of 0.1 ml/10 g bodyweight. Drug stocks were prepared fresh the morning of each experiment.

2.5. Statistical analysis

All data were presented as the mean±S.E.M. Formalin latencies were analyzed by Kruskal–Wallis followed by Mann–Whitney *U* tests. TF statistical comparisons were made using an analysis of variance with repeated measures. Sensorimotor performance results were compared using the Student's *t* test. All groups were compared with the saline-administered group and *P* values less than 0.05 were considered statistically significant. The effective dose-50 (ED_{50}) with 95% confidence limits was determined according to the Litchfield and Wilcoxon test (Litchfield and Wilcoxon, 1949) using Pharma/PCS software.

3. Results

3.1. Formalin-induced nociception test

In the saline administered mice, a highly significant increase was observed in the nociceptive behaviours within 5 min of the subcutaneous injection of formalin. While a decrease was detected in the nociceptive behaviours within 5–15 min, an increase was observed between 15 and 30 min. Nociceptive responses began to decrease after 30 min and kept decreasing until the end of the test at 45 min. The periods 0–5 and 15–45 min were considered as Phases 1 and 2, respectively.

Agmatine (decarboxylated arginine), in the dose range of 37.5–300 mg/kg, reduced nociceptive activity at 75, 150 and 300 mg/kg in the first phase ($P<.01$ for all doses) (Fig. 1A) and in the second phase ($P<.05$ for 75 and 150 mg/kg doses, $P<.01$ for 300 mg/kg dose) (Fig. 1B).

While the adrenoceptor antagonist yohimbine (1 mg/kg) did not change the Phase 1 response of agmatine at the dose of 162 mg/kg (ED_{50} dose of Phase 1), it changed the Phase 2 response of agmatine to control values at the dose of 85 mg/kg (ED_{50} dose of Phase 2) (Fig. 2A and B).

3.2. Tail-flick test

Although the TF responses decreased slightly at 300 mg/kg, there was no significant change at any dose in the agmatine administered animals. $F(1, 12)=4.09$, $P>.05$ (Table 1).

3.3. Sensorimotor performance

In the agmatine-administered mice, sensorimotor performance did not change at the lowest dose (37.5 mg/kg). While a slight decrease, which was not statistically signifi-

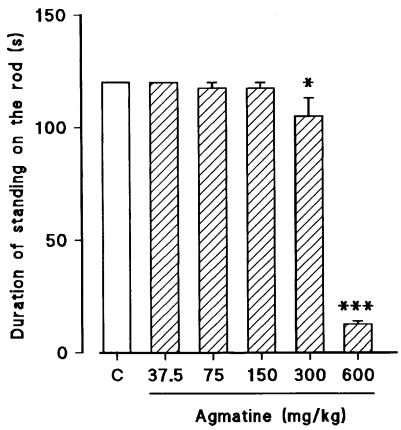

Fig. 3. Effects of ip administered agmatine (37.5–600 mg/kg) on sensorimotor performance in the rotarod test. C represents control results obtained in saline-administered animals. The data are presented as the mean+S.E.M of duration (second) that the animals could stand on the rod (cut-off time was 120 s.). *$P<.05$ and **$P<.01$ versus control.

cant was observed at middle doses (75 and 150 mg/kg), sensorimotor activity decreased with the increasing dose (300 mg/kg, $P < .05$). No mouse stayed on the rod more than 15 s at 600 mg/kg ($P < .0001$).

Sensorimotor performance values are shown in Fig. 3.

4. Discussion

In the present study, agmatine reduced Phase 1 and 2 responses with increasing doses in the formalin test, which is considered to be a tonic pain model. However, agmatine did not produce a significant change in the TF test, which is a phasic pain model. So it may be suggested that agmatine played a role in tonic rather than phasic pain. A study made by Horvath et al. (1999) showed that agmatine produced an antihyperalgesic effect in the carrageenan-treated paw, and no analgesic effect in thermal stimuli applied to the non-inflamed paw in rats. Another study by Li et al. (1999a) demonstrated that agmatine did not significantly change TF latency in mice, but did attenuate acetic acid-induced writhing in mice.

Nociceptive tests such as the TF and hot-plate tests conventionally depend on high intensity phasic (acute) stimuli. These are short-period tests and it is not possible to get any information about the mechanism of the events in the time period following the stimulus. It is believed that tonic (continuous) pain has been organized in a different way from the pain that is formed by the short-period stimuli in the CNS (Tjolsen et al., 1992).

N-methyl-D-aspartate (NMDA) receptors play an important role in the formation of tonic pain. In the tonic pain models, while the facilitation of responses to noxious stimuli can be blocked by NMDA receptor antagonists, NMDA receptor agonists increase this facilitation (Meller and Gebhart, 1993). Studies have shown that NMDA antagonists block formalin-induced pain (Vaccarino et al., 1993) that may be related to a blockade of central neural plasticity in this model (Coderre et al., 1993). Recently, it has been reported that agmatine selectively blocks the NMDA subclass of glutamate receptor channels in rat hippocampal neurons (Yang and Reis, 1999). Blockade of CNS plasticity via NMDA receptors may play a role in the antinociceptive effect of agmatine in the formalin test. NO, which is released via NMDA receptor activation, is an effective mediator in hyperalgesia (Kitto et al., 1992; Meller and Gebhart, 1993; Moore et al., 1991). Recent studies have demonstrated that agmatine is a competitive NOS (Auguet et al., 1995; Galea et al., 1996). A decrease in NO produced by NMDA activation may play a role in the blockade of CNS plasticity formed by agmatine.

Agmatine, like clonidine, has been shown to bind to α_2 receptors (Pinthong et al., 1995), but it is not clear how it affects these receptors (Raasch et al., 1995). In experiments performed on animals and humans, clonidine was shown to have an α_2 receptor-mediated antinociceptive and sedative effect where the latter appeared at increasing doses (Bernard et al., 1995; Delbarre et al., 1995; Waterman et al., 1988). These data suggested that agmatine, identified as a "clonidine-displacing substance," reduced nociceptive behaviours perhaps via the activation of α_2 receptors. In our study, the α_2 adrenoceptor antagonist yohimbine blocked the effect of agmatine in Phase 2 rather than Phase 1 in the formalin test. While the stimulus for the early phase is a direct chemical stimulation of the nociceptors, the late phase involves inflammation in the formalin test. In addition, the response in the late phase depends on changes in processing of the information in the spinal cord due to the afferent barrage during the early phase (Coderre et al., 1990). Our data indicate that α_2 receptors might have a role in the effect of agmatine on the formation of spinal sensitivity and inflammation. α_2 Adrenoceptors have been shown to be involved in the periaqueductal gray-induced inhibition of dorsal horn cell activity in rats (Peng et al., 1996). Dumka et al. (1996) showed that clonidine, which is an α_2 adrenoceptor agonist, suppressed formaldehyde-induced inflammation producing a decrease in edema volume and an increase in pain threshold. The authors suggested that the results of the study indicated that central noradrenaline exerted an inhibitory effect on peripheral edema and pain. Yohimbine did not change the effect of agmatine in Phase 1 in our study, implying that there could be other mechanisms responsible for the effect of agmatine on direct chemical stimulation of the nociceptors. Besides the actions of agmatine at α_2 adrenoceptors, agmatine also binds to imidazoline receptors (Reis et al., 1995). Perhaps imidazoline receptors play a role on the effect of agmatine in the first phase. Li et al. (1999b) suggested that imidazoline receptors are at least partly involved in agmatine's effect on naloxone-precipitated withdrawal jumps.

The sedative effect of agmatine at the highest doses seems to be independent from its antinociceptive effect at the lower doses. The decrease in the sensorimotor activity that was detected at the highest doses of agmatine seems to be a clue for future investigations related to the sedative effect of this drug.

As a result, our observations indicate that agmatine decreased nociceptive behaviours in the formalin test but not in the TF test, suggesting that agmatine seems to have an analgesic effect on tonic pain rather than on phasic pain. We also suggest that α_2 receptors play a role in the antinociceptive effect of agmatine on tonic pain.

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