Research report

Antidepressant-like effect of agmatine is not mediated by serotonin

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Abstract

The aim of this study was to characterize the behavioral effects of systemically administered agmatine in animal models predictive of antidepressant- and anxiolytic-like activity and clarify whether the effects of agmatine depend on the intact serotonergic system. Only the highest dose of agmatine tested (50 mg/kg) decreased immobility of mice in the forced swimming test. The magnitude of the effect was slightly smaller than that of the tricyclic antidepressant imipramine (15 mg/kg). Agmatine did not change the locomotion of mice in the open field. Pretreatment with the tryptophane hydroxylase inhibitor PCPA for 3 days resulted in more than 70% drop in the tissue levels of 5-HT and 5-HIIA but did not counteract the antidepressant-like effect of agmatine.

The administration of agmatine did not modify behavior of animals in the light–dark compartment test of anxiety. We conclude that the antidepressant-like effect of agmatine seems not to be mediated by the serotonergic system. We failed to confirm the reported anxiolytic-like activity of agmatine.

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The implication of the NMDA/l-arginine/NO pathway in the regulation of anxiety and depression has been demonstrated in numerous preclinical studies [8,17,18]. Interestingly, another metabolite of arginine, agmatine (1-amino-4-guanidinobutane), has also been suggested to serve as a putative neurotransmitter in the brain [15]. Administration of agmatine affects pain threshold, memory functions, and other behavioral parameters in animals. Several potential targets have been suggested for agmatine, including the NMDA receptor, imidazoline binding sites, and nitric oxide synthase [15].

Systemic administration of agmatine has been reported to produce antidepressant- and anxiolytic-like effects. Thus, Lavinsky et al. showed that agmatine induces an anxiolytic-like effect in the rat elevated-plus maze test [10]. Other groups have described the antidepressant-like effects of agmatine in the mouse forced swimming and tail suspension tests and the rat forced swimming test [11,21]. However, the minimal effective dose in these studies varied from 0.001 mg to 50 mg/kg.

The aim of this study was to characterize the behavioral effects of agmatine in animal models predictive of antidepressant- and anxiolytic-like activity and to evaluate the involvement of serotonin (5-HT) in these effects.

1. Materials and methods

1.1. Animals

Male C57Bl/6Bkl mice (Scanbur BK, AB Sweden) weighing 20–25 g were used. Mice were kept 10 per cage (21 cm × 37 cm × 15 cm) in an animal house at 20°C in a 12 h light/dark cycle (light on at 7.00 a.m.). Tap water and food pellets were available ad libitum. The animals were kept for at least 2 weeks in the animal colony before entering experiments. All animal procedures were accepted by the University’s Committee for Ethics in Animal Experimentation and complied with “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985).

1.2. Forced swimming test

The forced swimming test was performed as described by Porsolt et al. [13]. A glass cylinder with a diameter of 12 cm was filled with 8 cm water at 25°C. An
animal was put in the water and behavior videotaped during 6 min. An observer blind to treatment protocol counted the immobility time during the last 4 min of the 6 min test.

1.3. Mouse light–dark compartment test

The exploratory model first described by Crawley and Goodwin [3] was used. The apparatus consisted of two compartments (20 cm × 15 cm × 20 cm and 20 cm × 30 cm × 20 cm) connected by a 7.5 cm × 7.5 cm opening in the wall. The smaller compartment was painted black and covered with a roof. The other compartment had no roof and was brightly illuminated by a 60 W bulb located 25 cm above the box. An animal was placed in the dark compartment and number of transitions and time spent in the light compartment was recorded during 5 min.

1.4. Locomotor activity

Locomotor activity was measured using an automated system with six chambers (45 cm × 45 cm × 45 cm) made from transparent acrylic (MOTI, Technical & Scientific Equipment GMBH, Germany). The apparatus-naive mice were put into the chamber, and vertical and horizontal activity was counted during a 10 min test period.

1.5. Experimental design

The light–dark compartment test, measurement of locomotor activity, and forced swimming test were carried out consecutively 30, 40, and 50 min after treatment with agmatine, respectively [17]. Each group comprised eight animals.

1.6. Measurement of tissue 5-hydroxytryptophane levels

In all groups, the brains were rapidly removed and put on ice, the frontal corticis were dissected, weighted and frozen at −80° until day of analysis. At the day of analysis, brain pieces were homogenized (1:10, w/v) in ice-cold Tris–HCl buffer containing EDTA. After centrifugation, the supernatants were removed. To remove protein impurities before chromatographic analysis, 5 μl of 0.05 mM HClO₄ was added to 50 μl of supernatant, and centrifuged at 15,000 g for 15 min. High-pressure liquid chromatography (HPLC) with electrochemical detection was used for sample analysis (ESA Coulochem II with 5014B coulometric analytic cell, ESA Inc., MA, USA). The mobile phase was composed of 50 mM NaH₂PO₄, 740 μM 1-octanesulfonic acid, 108 μM Na-EDTA, 80 ml/l acetonitrile, and 100 μl/l triethylamine with pH adjusted to 3.5 using H₃PO₄.

1.7. Drugs

All chemicals were purchased from Sigma (St. Louis, USA). Imipramine and agmatine hydrochloride were dissolved in saline. PCPA (p-chlorophenylelenine methyl ester) was dissolved in saline using a few drops of Tween-80. All drugs were freshly prepared and given intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight of mice. Doses of drugs were selected according to behavioral and neurochemical studies showing the drugs to have the intended effect [10,20,21].

To test the possible involvement of 5-HT in the behavioral effects of agmatine, animals were pretreated with PCPA (100 mg/kg, i.p.) or saline, once a day, for four consecutive days. Animals were treated with agmatine 2 h after the last injection of PCPA or saline and tested 30 min later.

1.8. Statistics

Data were statistically treated using one-way analysis of variance (ANOVA). Post hoc comparisons between individual groups were performed by Duncan’s multiple range test. Data are expressed as the mean values ± S.E.M. Differences were considered statistically significant when P was less than 0.05.

2. Results

2.1. Effect of agmatine in forced swimming test

Treatment had a significant effect on immobility time in the forced swimming test as indicated by one-way ANOVA (F_{4,35} = 4.5, P < 0.005). The standard antidepressant

![Fig. 1. Effect of imipramine (15 mg/kg) and agmatine in the forced swimming test (n = 8 per group). Drugs were injected 50 min prior to testing. Results are expressed as mean ± S.E.M. *P < 0.05 vs. saline; **P < 0.01 vs. saline.](image1)

![Fig. 2. (A) Effect of imipramine (15 mg/kg) and agmatine in light–dark compartment test (n = 8 per group). Drugs were injected 30 min prior to testing, respectively. Results are expressed as mean ± S.E.M. *P < 0.05 vs. saline. (B) Effect of imipramine (15 mg/kg) and agmatine in the locomotor activity test (n = 8 per group). Drugs were injected 40 min prior to testing.](image2)
imipramine (15 mg/kg) significantly decreased the immobility time \( (P < 0.01, \text{Duncan’s test}) \). Only the highest dose of agmatine (50 mg/kg) had a significant effect \( (P < 0.05) \) on the immobility time of mice (Fig. 1).

2.2. Effect of agmatine in the light–dark compartment test

Results are shown in Fig. 2A. One-way ANOVA indicated treatment effect for the time spent in the light compartment \( (F_{4,35} = 3.53, P < 0.05) \), but not for the number of transitions \( (F_{4,35} = 1.1, P = 0.38) \). The Post hoc analysis revealed that agmatine did not change the animal behavior in the light–dark compartment test. Treatment with imipramine (15 mg/kg) increased the time spent in the light compartment. A similar effect has been shown previously in CD1 mice in the same paradigm [4].

2.3. Effect of agmatine on locomotion

Results are shown in Fig. 2B. One-way ANOVA did not indicate a significant treatment effect on distance \( (F_{4,35} = 1.1, P = 0.36) \).

2.4. Effect of PCPA on cortical 5-HT and 5-HIIA concentrations

Results are shown in Fig. 3. Treatment had a significant effect on both 5-HT \( (F_{2.20} = 12.4, P < 0.001) \) and 5-HIIA \( (F_{2.20} = 36.6, P < 0.0001) \) concentrations. Post hoc comparisons revealed that PCPA depleted 5-HT and 5-HIIA concentrations more than 70 and 80%, respectively. In a separate experiment, agmatine alone had no effect on the levels of 5-HT or 5-HIIA (97 ± 17% and 95 ± 11% of that of control group values, respectively).

2.5. Treatment with PCPA did not block the anti-immobility effect of agmatine

Results are shown in Fig. 4A. Treatment had a significant effect on immobility time \( (F_{2.24} = 12.7, P < 0.001) \) as indicated by one-way ANOVA.

Agmatine significantly decreased the immobility time compared to animals treated with PCPA alone \( (P < 0.05) \). Inter-
estingly, treatment with PCPA itself decreased immobility compared to control animals. While usually PCPA does not modify immobility time, a decrease in immobility time has been reported previously [5].

Neither pretreatment with PCPA alone nor in combination with agmatine changed the behavior of animals in the light–dark compartment test (time in light compartment $F_{2,24} = 1.51, P = 0.24$; Fig. 4B) or locomotion ($F_{2,23} = 0.73, P = 0.49$; Fig. 4C).

### 3. Discussion

The main finding of the current study was that agmatine had an antidepressant-like effect, presumably independent of the brain serotonergic system.

The magnitude of the antidepressant-like effect of agmatine was slightly weaker compared with that of the standard antidepressant imipramine (15 mg/kg). Previously, the antidepressant-like effect of agmatine has been reported after a dose of 0.01 mg/kg of agmatine in the mouse forced swimming test [21]. In contrast to this study, we found that a much higher dose was needed for the drug to be effective. Our data are consistent with the report of Li et al. where antidepressant-like effect appeared in the dose of 20 mg/kg when administered subcutaneously [11]. As reported previously [11,21], agmatine did not change the ambulation of animals, excluding the possibility that the drug effect in the forced swimming test was of non-specific origin.

Surprisingly, pretreatment with the tryptophane hydroxylase inhibitor PCPA did not antagonize the antidepressant-like effect of agmatine. At present, one study has reported the loss of effect of agmatine after PCPA treatment [20]. The study used the same pretreatment scheme but did not measure the effect of PCPA on 5-HT level. In our experiment, pretreatment with PCPA resulted in more than 70 and 80% of drop in tissue levels of 5-HT and 5-HIAA, respectively. However, the possibility that a more complete inhibition of 5-HT synthesis is needed to antagonize the antidepressant-like effect of agmatine cannot be completely excluded. Recent study has shown that ca 80% decrease of 5-HT levels in frontal cortex reverses the effects of fluoxetine and citalopram in the tail suspension test [12]. The other difference between the studies was the lower agmatine dose used in the first study, probably making it easier to counteract.

Interestingly, treatment with PCPA itself decreased immobility compared to control animals. While usually PCPA does not modify immobility time, a decrease in immobility time has been reported previously [5].

Taken together, our results show that 5-HT does not play a major role in the antidepressant-like effect of agmatine.

Agmatine did not induce an anxiolytic-like effect in our study. Few studies have reported agmatine to possess an anxiolytic-like activity in mice and rats [1,7,10]. It is noteworthy that agmatine has displayed bell-shaped dose-effects after acute dosing in the animal models of anxiety. Thus, in the mouse light–dark compartment test the only dose of agmatine affecting the number of transitions was 80 mg/kg [7]. Moreover, the classical anxiolytic drug diazepam increased both number of transitions and time spent in a light compartment, whereas agmatine only changed the former. Therefore, the anxiolytic-like effect of agmatine seems weak.

Agmatine has been proposed to act as a neurotransmitter or modulator in the central nervous system [15]. However, some studies have questioned whether any meaningful agmatine synthesis occurs under physiological conditions [2,9,16]. The tissue level of agmatine has been estimated to be 0.1–6 μM [6,14,19]. Treatment with 100 mg/kg of agmatine resulted in a more than 55 times increase in cortical agmatine concentration measured 3h after dosing [6]. Thus, one can speculate that at least the antidepressant-like effect of agmatine is more likely to be a pharmacological effect, possibly via inhibition of the NMDA receptor and/or nitric oxide synthase functioning.

We conclude that the antidepressant-like effect of agmatine seems not to be mediated by the serotonergic system. We failed to confirm the reported anxiolytic-like activity of agmatine.

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### References


