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Role of nitric oxide in additive anticonvulsant effects of agmatine and morphine

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HIGHLIGHTS

- Agmatine shows anticonvulsive properties on seizure susceptibility in male NMRI mice.
- Administration of low doses of morphine augments anticonvulsant effect of Agmatine.
- This additive effect of morphine and Agmatine is mediated via nitric oxide system.

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ABSTRACT

The anticonvulsant effects of agmatine, an endogenous polyamine and a metabolite of L-arginine, have been shown in various experimental seizure models. Agmatine also potentiates the anti-seizure activity of morphine. The present study aimed to investigate a possible involvement of nitric oxide (NO) pathway in the protection by agmatine and morphine co-administration against pentylenetetrazole (PTZ) –induced seizure in male mice. To this end, the thresholds for the clonic seizures induced by the intravenous administration of PTZ, a GABA antagonist, were assessed. Intraperitoneal administration of morphine at lower dose (1 mg/kg) increased the seizure threshold. Also intraperitoneal administration of agmatine (5 and 10 mg/kg) increased the seizure threshold significantly. Combination of subeffective doses of morphine and agmatine led to potent anticonvulsant effects. Non-effective doses of morphine (0.1 and 0.5 mg/kg) were able to induce anticonvulsant effects in mice pretreated with agmatine (3 mg/kg). Concomitant administration of either the non-selective nitric oxide synthase (NOS) inhibitor L-NAME (1, 5 mg/kg, i.p.) or the selective NOS inhibitor 7-NI (15, 30 mg/kg, i.p.), with an ineffective combination of morphine (0.1 mg/kg) plus agmatine (1 mg/kg) produced significant anticonvulsant impacts. Moreover, the NO precursor, L-arginine (30, 60 mg/kg, i.p.), inhibited the anticonvulsant action of agmatine (3 mg/kg) plus morphine (0.5 mg/kg) co-administration. Our results indicate that pretreatment of animals with agmatine enhances the anticonvulsant effects of morphine via a mechanism which may involve the NO pathway.

1. Introduction

Agmatine, a polycationic amine, is an endogenous ligand/modulator of imidazoline receptors and alpha 2-adrenoceptors as well as N-methyl-D-aspartate (NMDA) receptor channels [1–4].

Agmatine, synthesized from L-arginine by the enzyme arginine decarboxylase (ADC), has been shown to exert neuromodulatory functions in the central nervous system (CNS) [5]; it enhances the analgesic effects of morphine, prevents morphine tolerance, and attenuates all behavioural signs of the morphine abstinence syndrome in rats [6,7]. Growing body of evidence indicates that agmatine may alter the action of L-glutamate. The association between agmatine and NMDA receptor function has been confirmed by showing that agmatine selectively modulates the NMDA subclass of glutamate receptors in the hippocampal neurons [4,8].

In our recent study, agmatine displayed a dose-dependent anticonvulsant effect on pentylenetetrazole induced seizure which was mediated by α_2-adrenoceptors and the nitric oxide pathway [9].

Other studies have also shown the anticonvulsant effects of agmatine in several experimental seizure models [10,11]. Although mechanisms by which agmatine modulates seizure threshold are not fully understood, these reports suggest that agmatine has crosstalk with other protective systems that raise the seizure threshold. The endogenous opioid system constitutes one such example that contributes to anti-seizure activity in stressful conditions and post-ictal phases

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[12,13]. Exogenously, a biphasic modulatory effect on seizure threshold was characterized for morphine in our lab where morphine proved anticonvulsant at low doses while proconvulsant at high doses [14]. Interestingly, several lines of evidence support neuromodulatory interplay between agmatine and opioids such that agmatine potentiates certain opioid-induced effects including spinal and supraspinal antinociception [6,15,16].

Riazi et al. have reported that agmatine potentiates the anticonvulsant effects of morphine through α_2-adrenoceptors [13]. Although agmatine can interact with α_2-adrenergic and imidazoline receptors, it has been suggested that some of the pharmacological aspects of agmatine, such as morphine tolerance prevention, may be due to the inhibition of neuronal nitric oxide synthase (nNOS) [17]. It has been reported that agmatine causes a time- and concentration-dependent irreversible inactivation of nNOS [18]. The involvement of NO in other central effects of agmatine and in its interaction with opioids has been also reported [17,19,20]. Notably, we have reported that NO is differentially involved in modulation of seizure susceptibility by exogenous and endogenous opioids [14].

Thus, following our previous study that revealed the additive anticonvulsant effects of morphine and agmatine [13], the present study aimed to investigate another important and logical pathway for this additive anticonvulsant effect; L-arginine/nitric oxide pathway. Regarding to the role of NO pathway in both agmatine and morphine pharmacologic actions as well as its known roles in seizure paradigm, it seems logical to examine this combination.

Using L-arginine (a NO precursor), L-NAME (a nonspecific NOS inhibitor) or 7-NI (a selective neuronal NOS (nNOS) inhibitor) in the PTZ-induced clonic seizure model in mice, we investigated the possible involvement of the NO system in the agmatine/morphine interaction.

2. Materials and methods

2.1. Chemicals

The following compounds were used throughout the study: Pentylenetetrazole (PTZ), morphine sulfate (Sigma, UK), L-arginine, N^GL-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI), and agmatine sulfate (Sigma, St Louis, MO, USA). Morphine sulfate, L-arginine, L-NAME, 7-NI, and agmatine were administered intraperitoneally. 7-NI was suspended in a 1% aqueous solution of Tween 80, and all other drugs were dissolved in normal saline. To induce clonic seizures, PTZ was administered intravenously (0.5%, iv).The doses were chosen based on our previously published data [21,22] and pilot experiments. In experiments with sequential treatments, the interval between administration of NOS inhibitors or L-arginine and agmatine was 15 min so that an effective blockade of enzymes by inhibitors was allowed. Morphine was injected 15 min after agmatine and 30 min before performing the test. The results suggested that the selected time intervals allowed effective activation or blockade of receptors.

2.2. Experimental animals

Male NMRI mice weighing 23–30 g (Razi Institute, Karadj, Iran) were used in the study. The animals were housed in standard polycarbonate cages in groups of 4–5 and kept in a temperature-controlled room (22 C) with 12 h light/12 h dark cycle. Animals were acclimated at least 2 days before experiments with free access to food and water. The experiments were conducted between 09:00 and 13:00. All procedures were carried out in accordance with institutional guidelines for animal care and use. Groups consisted of at least eight animals and each animal was used only once. Additionally, efforts were made to reduce animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.3. Seizure paradigm

The clonic seizure threshold was determined by inserting a 30-gauge dental needle into the lateral tail vein of mouse [23]. The needle was then secured to the tail by a narrow piece of adhesive tape. With mouse moving freely, the PTZ solution(0.5%) was slowly infused into the tail vein at a constant rate of 1 ml/min using an infusion pump (NE 1000, new era pump system, Inc), which was connected to the dental needle by polyethylene tubing. Infusion was halted when general clonus (forelimb clonus followed by full clonus of the body) was observed. Minimal dose of PTZ (mg/kg of mice weight) needed to induce general clonus was recorded as an index of clonic seizure threshold. As such, seizure threshold is dependent on PTZ dose administered and time-related.

2.4. Statistical analysis

Seizure thresholds are expressed as the mean ± SEM of clonic seizure threshold in each experimental group. One-way analysis of variance (ANOVA) followed by Tukey's post hoc for multiple comparisons was used for data analysis. Two-way ANOVA was employed to analyze the effects of agmatine co-administration with various doses of morphine. In all experiments, a P value less than 0.05 was considered as statistically significant.

3. Results

3.1. Effect of different doses of morphine on seizure threshold

Fig. 1 illustrates the effect of acute intraperitoneal administration of escalating doses of morphine (0.1, 0.5, 1, 10, and 15 mg/kg, i.p) on PTZ-induced clonic seizure threshold. The seizure threshold was determined 30 min after morphine administration. One-way ANOVA revealed a significant effect for morphine ($F[5,41] = 9.61$, $P < 0.001$). Statistical analysis showed a significant anticonvulsant effect for morphine at 1 mg/kg compared with saline-treated control animals. Based on this experiment, the doses 0.1 and 0.5cih mg/kg were selected as subeffective doses of morphine.

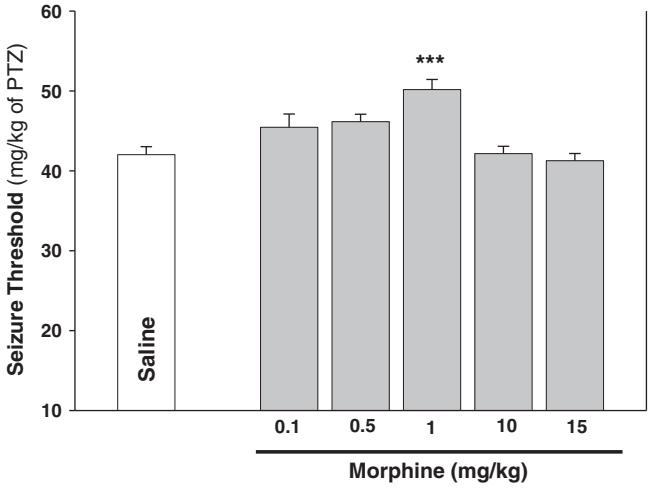

Fig. 1. Effects of administration of different doses of morphine sulfate (0.1, 0.5, 1, 10, and 15 mg/kg, i.p.) on threshold to PTZ-induced seizures in mice. Morphine sulfate was administered 30 min before determination of PTZ seizure threshold. Data are expressed as the mean ± SEM of seizure threshold in each group. Each group consisted of at least eight mice. ***P < 0.001, compared with saline control group.

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