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Agmatine attenuates stress- and lipopolysaccharide-induced fever in rats

Feyza Aricioglu[a,b], Soundar Regunathan[b,*]

[a]*University of Marmara, Faculty of Pharmacy, Department of Pharmacology, Haydarpasa, Istanbul, Turkey*
[b]*University of Mississippi Medical Center, Department of Psychiatry, Division of Neurobiology and Behavior Research, 2500 North State Street, Jackson, MS, USA*

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Abstract

Physiological stress evokes a number of responses, including a rise in body temperature, which has been suggested to be the result of an elevation in the thermoregulatory set point. This response seems to share similar mechanisms with infectious fever. The aim of the present study was to investigate the effect of agmatine on different models of stressors [(restraint and lipopolysaccaride (LPS)] on body temperature. Rats were either restrained for 4 h or injected with LPS, both of these stressors caused an increase in body temperature. While agmatine itself had no effect on body temperature, treatment with agmatine (20, 40, 80 mg/kg intraperitoneally) dose dependently inhibited stress- and LPS-induced hyperthermia. When agmatine (80 mg/kg) was administered 30 min later than LPS (500 μg/kg) it also inhibited LPS-induced hyperthermia although the effect became significant only at later time points and lower maximal response compared to simultaneous administration. To determine if the decrease in body temperature is associated with an anti-inflammatory effect of agmatine, the nitrite/nitrate levels in plasma was measured. Agmatine treatment inhibited LPS-induced production of nitrates dose dependently. As an endogenous molecule, agmatine has the capacity to inhibit stress- and LPS-induced increases in body temperature.

Keywords: Agmatine; Restraint stress; Lipopolysaccharide; Nitric oxide; Arginine; Nitrites

1. Introduction

Agmatine (l-amino-4-guanidinobutane), is an endogenous amine synthesized from the decarboxylation of arginine. Agmatine has been identified in nearly all of the organs of the rat including brain and plasma [20]. Agmatine exerts a wide range of biological activities on several organ systems, including the central nervous system, where it has been proposed to act as a neurotransmitter [22,23]. Agmatine interacts with the imidazoline receptors, alpha2-adrenoceptors, nicotinic cholinergic and 5-HT3 receptors [13,19,23]. It selectively modulates the NMDA subclass of glutamate receptors in rat hippocampal neurons [24] and has neuroprotective properties, presumably mediated by NMDA blockade [5,8,12].

Fever is an adaptive and nearly universal response among vertebrates to systemic inflammation. It is the most important non-specific systemic reaction designed to combat the delirious effects of invading pathogens to restore health to the afflicted host. Fever is an integrated response of the body, involving the release of endogenous pyrogens (interleukin-1, interleukin-6, tumor necrosis factor-α, etc.) by immune cells, the transfer of these immune signals to the brain, coordinated response of several brain regions to increase the thermoregulatory set point and consequently body temperature [5]. Stress-induced hyperthermia appears a consistent physiological phenomenon when organism is confronted with a stressor, either physical or psychological. Although the evoking stimuli differ, the response of the animal regarding autonomical parameters like body temperature seems to be similar. While the precise mechanism for stress-induced hyperthermia is not clear, the roles of inflammatory cytokines and NOS have been widely reported [25,30]. Agmatine has anti-depressant like effects in rat and mice models of depression [32,33] and cold

* Corresponding author. University of Mississippi Medical Center Department of Psychiatry and Human Behavior, 2500 North State Street, Jackson, MS, 39216. Tel.: +601 984 57 41; fax: +601 984-5899.
E-mail address: sregunathan@psychiatry.umsmed.edu (S. Regunathan).

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immobilization stress increases endogenous production of agmatine in plasma and cortex of rats [2]. It has also been shown that agmatine suppresses the lipopolysaccharide (LPS)-induced NO production in cultured microglia [1], inducible nitric oxide synthase (iNOS) expression in macrophages and astrocytes [21] as well as in rat kidney [29]. These actions of agmatine result in the reversal low blood pressure and abnormal renal function and increases the survival after LPS treatment in mice [29].

Thus, agmatine, acting like an inhibitor of NOS and NMDA receptors, has the ability to reduce the effect of stress and inflammation. The present study was designed to investigate whether agmatine modulates the increase in body temperature after acute restraint stress and after LPS treatment and that this effect is associated with the anti-inflammatory effect of agmatine as measured by plasma nitrite/nitrate levels.

2. Materials and methods

2.1. Animals

All procedures in this study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA) and the declaration of Helsinki. Adult male Spraque Dawley rats (270–300 g) were housed in a quiet room with temperature (20±2 °C) and humidity (60±3%) control, and 12/12 h light–dark cycle was maintained (07:00–19:00 hours light). All experiments were conducted from 9 AM until the end of the experimental time period. The rats were fed standard lab chow and tap water ad lib during the study.

2.2. Measurement of body temperature

Rats from each cage were randomly numbered 1–10 (handling) 1 day prior to the temperature measurement. The temperature of all ten rats was measured sequentially by inserting a thermistor probe for a length of 4 cm into the rectum of the rat. Digital recordings of temperature were made with an accuracy of 0.1 °C by means of a Sensortek (Model BAT-12) digital thermometer. The probe, dipped into silicone oil before inserting, was held in the rectum until a stable rectal temperature was measured for 20 s. Basal temperatures were measured every 10 min for 90 min before each experiment in order to minimize the effect of handling stress on body temperature.

2.3. Restraint stress

Rats were taken out of their home cage, weighed and then colonorectal temperatures were measured. When the body temperature reached steady-state level, rat was gently placed in a Plexiglas cylinder that was designed to restrain the rat from moving or turning. Rats were remained in this position for 4 h and colonorectal temperatures were measured every 30 min until the end of the experiment.

2.4. Substances and injections

Purified lyophilized extract of *Escherichia coli* LPS and agmatine sulfate were purchased from Sigma (St. Louis, MO). LPS was dissolved in pyrogen-free 0.9% sodium chloride at a concentration of 1 mg/ml and stored −20 °C as a stock solution. On the day of experiment, LPS was diluted to 100, 200 and 500 μg/ml in saline and injected intraperitoneally. Agmatine was dissolved in pyrogen-free saline and injected (20, 40, 80 mg/kg) intraperitoneally just before LPS injection. In one experiment, agmatine was injected 30 min after LPS injection. Control rats were injected intraperitoneally with equal volume of saline. Colonorectal temperatures were recorded in all rats for 8 h. Rats were sacrificed immediately after the temperature measurements and blood was collected for the measurement of nitrite/nitrate levels.

2.5. Nitrite/nitrate determination in plasma

Trunk blood samples were collected and centrifuged at 1500 rpm for 10 min to remove red blood cells. The plasma supernatant was stored at −80 °C until analysis for nitrate using the Griess reagent. Briefly, nitrate was converted to nitrite in the presence of NADPH as enzyme cofactor and nitrate reductase. One hundred microliter of plasma was incubated for 30 min at room temperature with nitrate reductase (50 mU/100 μl sample; Sigma) and with NADPH (final concentration=80 μmol/l; Sigma) diluted in 20 mmol/l Tris buffer (pH 7.6). After incubation, samples were again centrifuged (10,000 rpm, 10 min, 4 °C) and the supernatants were used for analysis. The samples were transferred into 96-well microtiter plates, Griess reagent was added and the absorbance was measured at 540 nm using a microplate reader. Standard curve was prepared using sodium nitrite and the concentration in plasma was calculated from the standard curve.

2.6. Statistical analysis

All data are reported as means±SD. Statistical analysis of the data was made by analysis of variance (ANOVA) of repeated measures and for significance, ANOVA values were compared by Tukey's test for multiple comparisons. A probability level of less than 0.05 was accepted as significant difference.

3. Results

3.1. Effect of agmatine on body temperature

In the initial experiment, we tested whether agmatine alone had any effect on body temperature in normal rats. As