Pharmacology, Biochemistry and Behavior 132 (2015) 108–114

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Agmatine attenuates lipopolysaccharide induced anorexia and sickness behavior in rats

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ARTICLE INFO

Article history:
Received 19 June 2014
Received in revised form 12 February 2015
Accepted 16 February 2015
Available online 22 February 2015

Keywords:
Agmatine
Lipopolysaccharide
Sickness behavior
Anorexia
Depression
Anxiety

ABSTRACT

Sickness behavior is characterized by lethargy, reduced appetite, anhedonia and anxiety. It can be induced in experimental animals by bacterial endotoxin, lipopolysaccharide (LPS). We investigated the impact of intracerebroventricular agmatine injections (5–20 μg/rat, icv) on sickness behavior induced by LPS (100 μg/rat, ip) in rats. Rats challenged with LPS demonstrated hyperthermia, anorexia, anxiety, depression like phenomenon and reduction in body weights. Additionally, mediators of sickness behaviors, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) level in LPS treated rat serum were also increased. The present study revealed that these LPS induced symptoms of sickness behavior including anorexia were normalized by pretreatment with agmatine. The IL-6 and TNF-α serum levels were also normalized in agmatine pretreated rats. It is anticipated that agmatine may suppress LPS induced sickness behavior by inhibiting proinflammatory pathway and/or activity circuitry in brain. This study suggests that agmatine may be an important therapeutic target in the treatment of anorexia and other neurological abnormalities associated with bacterial infection.

1. Introduction

Agmatine, an endogenous amine is synthesized through decarboxylation of L-arginine by arginine decarboxylase (ADC) and widely distributed throughout the body including brain. It is a neurotransmitter and/or neuromodulator (Raasch et al., 1995; Reis and Regunathan, 2000) and exhibits several biological effects by interacting with certain receptors and neuronal pathways in CNS. Agmatine activates α_2-adrenoceptors and imidazoline receptors (Reis and Regunathan, 2000; Halaris and Plietz, 2007), and blocks N-methyl D-aspartate (NMDA) receptors (Yang and Reis, 1999), nicotinic receptors and 5-HT3 receptors. Additionally, it competitively inhibits nitric oxide (NO) synthase (Auguet et al., 1995). In experimental studies, agmatine showed a variety of pharmacological effects including anticonvulsant, anxiolytic, antinociceptive, antidepressant, antistress and neuroprotective effects (Reis and Regunathan, 2000; Halaris and Plietz, 2007; Gilad and Gilad, 2000; Gilad et al., 2005; Olmos et al., 1999; Wang et al., 2006; Zhu et al., 2003, 2008; Taksande et al., 2010, Taksande et al., 2013). In addition, it augments the release of insulin from pancreatic β-cells (Sener et al., 1989), leutinizing hormone-releasing hormone (LHRH) from the hypothalamus (Kalra et al., 1995) and gastrin secretion. Several reports indicated that agmatine may be a useful substance in the treatment of number of CNS disorders ranging from pain to substance abuse and dependence. Few studies have demonstrated its orexigenic activity (Taksande et al., 2011; Prasad and Prasad, 1996) and suggest that agmatine may be an additional regulator of feeding behavior (Taksande et al., 2011; Prasad and Prasad, 1996). However, the role of agmatine in infection associated anorexia and sickness behavior remains poorly investigated.

Sickness behavior is a behavioral complex induced typically by infections, inflammation, tissue injury or immune trauma and mediated by proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α. Its characteristic features include anxiety, anorexia, depressed activity, hyperthermia, loss of interest in usual activities and sleepiness etc. (Becskei et al., 2008). In experimental animals, sickness behavioral response can be induced by administration of gram negative bacterial component, lipopolysaccharide (LPS) released during sepsis or severe infection. Importantly, Sastre et al. (1998) reported that LPS reduces endogenous agmatine levels by stimulating its degrading enzyme, agmatinase and/or inhibiting stimulatory enzyme ADC. The results of recent studies that agmatine suppresses LPS induced hyperthermia, hepatic failure (Aricioglu and Regunathan, 2005; El-Agamy et al., 2014) and NO synthesis in cultured microglia (Abe et al., 2000) indicated its role in sickness behavior. Considering the presence of agmatine in brain system known to be involved in food consumption, inflammation, pain, anxiety and depressive behavior (Taksande et al., 2009, Taksande et al., 2010; Fairbanks et al., 2000) we hypothesized that agmatine would prevent responses to infection such as sickness behavior. This study investigated the effect of agmatine on various indicators of sickness behavior including anorexia, hyperthermia, anxiety, depression, and body weight changes following intraperitoneal (ip) injections of LPS in rats. We also quantified the levels of TNF

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http://dx.doi.org/10.1016/j.pbb.2015.02.013
0091-3057/

and IL-6 in serum samples to elucidate the central mechanism behind the attenuation of sickness behavior by agmatine.

2. Materials and methods

2.1. Subjects

Adult Sprague–Dawley rats (225–250 g) of either sex were procured from the National Institute of Nutrition, Hyderabad, India and group ($n = 5$) housed in acrylic cages (24 × 17 × 12 cm) at an ambient temperature (25 ± 2 °C), relative humidity (50 ± 5%), with a 12:12 h light–dark cycle (lights on at 0600 h). Animals had free access to standard pellet chow (Trimurti Feeds, Nagpur, India; provide 3.30 kcal/g with 23.4% protein, 4.5% fat and 72.1% carbohydrate, which is primarily in the form of complex polysaccharides) and drinking water. The study was approved by the Institutional Animal Ethics Committee and the work was carried out as per guidelines of CPCSEA (Committee for the purpose of control and supervision of experimental animals, New Delhi, India). Separate groups of animals were used for food intake study and for behavioral (anxiety and depression) testing.

2.2. Surgery

The detailed procedure of the stereotaxic cannulation has been described previously (Taksande et al., 2010). Briefly, rats were implanted under anesthesia (thiopental sodium: 45 mg/kg, ip; Abbott Pharmaceuticals Ltd., Mumbai, India) with an indwelling 24 gauge stainless steel guide cannula [C316G/Spc, internal diameter (id) 0.29 mm, outer diameter (od) 0.56 mm; Plastics One, Roanoke, VA, USA] directed towards right ventricles (Co-ordinates: 0.8 mm posterior, + 1.3 mm lateral to midline, and 3.5 mm ventral with respect to bregma) according Paxinos and Watson, (1998). A flush-fit dummy cannula or stylet (C316DC/Spc, wire od 0.25 mm; Plastics One) was placed into the guide cannula to prevent the blockage. Post operatively animals were treated with buprenorphine (0.05 mg/kg, Tidigesic®, Sun Pharmaceuticals, Vadodara, Gujarat, India) to provide analgesia and with cefotaxime sodium (50 mg/kg, Cefantral®, Lupin Pharmaceuticals, Ankhaleshwar, Gujarat, India) as an antimicrobial therapy. Following surgery, the rats were placed individually in cages and allowed to recover at least for 7 days before being subjected to any testing. Rats were then randomly assigned to different groups and habituated to the testing environment by transferring to experimental room and twice daily handling for 1 week. Post surgery and recovery those animals showing stable baseline food intake were selected and assigned to different treatment groups ($n = 6–8$). Icv injections (5 μl) were given over 1 min, through a 31 gauge internal cannula (C316I/Spc, id 0.12 mm, od 0.25 mm; Plastics One) connected to the 100 μl syringe (Hamilton, Nevada, USA). The internal cannula was projected 0.5 mm below the guide cannula. Following microinjection, the internal cannula was kept in place for an additional minute to promote diffusion and to prevent the back-flow of the fluid during removal of the injection cannula.

2.3. Drug administration

Following post operative recovery and habituation period, the animals were separated into different treatment groups ($n = 5$) namely LPS + saline, agmatine + saline, agmatine + LPS, and Vehicle (saline or aCSF) control. LPS (Sigma-Aldrich USA) was dissolved in saline (0.9% *w/v* NaCl) and administered to each animal (100 μg/rat, i.p.) to induce sickness behavior (Dantzer, 2009; Dantzer et al., 2008). Agmatine (5–20 μg) (Sigma-Aldrich USA) was dissolved in artificial cerebrospinal fluid (aCSF) (Composition: 140 mM NaCl, 3.35 mM KCl, 1.15 mM $MgCl_2$,1.26 mM $CaCl_2$, 1.2 mM Na_2HPO_4 and 0.3 mM NaH_2PO_4, pH 7.4) containing 0.1% BSA and injected by intracerebroventricular (icv) route 30 min prior to LPS or saline injections.

2.4. Food, water intake and body weights

Rats usually show a peak feeding activity during the dark phase (Kimura et al., 1970) and LPS effects (e.g. motor activity, food intake etc) are more prominent during this phase of the circadian cycle (O'Reilly et al., 1988). Hence, these studies were conducted during the active phase of animals and drug treatments were offered at the onset (18 h) of the dark phase.

Similarly, water intake was assessed by measuring the amount (ml) of water in water bottles (250 ml) before treatments and at 24 h post-treatments period. Only two points were selected in these tests to minimize the disruption of feeding behavior.

Immediately after different treatments animals were shifted to their individual cages containing pre-weighed quantity of food pellets (30 g) in the cage hopper. Food consumption was monitored (g) manually by weighing the leftover food at 4, 6, 12 and 24 h post treatments. Food spillage collected from the tray positioned beneath the grid floor was subtracted from the total food consumed (Kokare et al., 2006). The spillage by individual rats, across all the treatment groups, was found to be negligible and measured to the nearest 0.4 g after the 24 h time point. In the same study, body weight changes were monitored by weighing animals immediately before treatments and again 12 and 24 h post-treatments.

2.5. Body temperature

Unlike the food intake experiments, these studies were undertaken during the normal light cycle of light dark cycle when the animals were in their resting phase and did not show any changes in basal body temperature due to circadian influences. The animals were divided into same treatments group ($n = 5$) as discussed earlier. The treatments were given between 9:00 and 10:00 h.

The rat was placed in a Plexiglas cylinder restrainer and basal temperature of each rat was measured by inserting the thermistor probe (size 5 mm) of the telethermometer (ElectroLab® digital Eds) for a length of 5 cm into the rectum of the rat. The probe was lubricated with glycerin before inserting and was held in the rectum until stable rectal temperature was recorded for 30 s. Basal body temperature were measured every 10 min till the body temperature reached steady state level. Following the drug treatments body temperature (°C) was measured at 4, 6 and 24 h time points.

2.6. Behavioral parameters (anxiety and depressive behavior)

These both tests were conducted during normal light cycle between 8:00 and 15:00 h for the same reason stated in the temperature study.

2.6.1. Depressive behavior (forced swim test: FST)

The procedure was quite similar to that described earlier (Porsolt et al., 1977; Kokare et al., 2010). Twenty four h before start of any treatments all rats were subjected to a “15 min pretest session” to maintain consistency in the basal immobility time between different groups. Briefly, rats were placed individually in Plexiglas cylinders (46 cm tall × 20 cm in diameter) containing fresh water up to 30 cm having temperature 25 ± 1 °C and forced to swim for 15 min after which they were dried briefly with a towel and returned to their cage. Twenty four hours later, rats were pretreated with LPS, agmatine or vehicle as described earlier. After 4 h post LPS injections each rat was again forced to swim in a similar environment for the period of only 5 min in a “test session” and the duration of immobility was measured. Rat was judged to be immobile when it remained floating motionless in the water, making only necessary movements to keep its head above water. Animals were allowed to dry before returning to their home cages. An increase in the duration of immobility reflects despair or depression like behavior in animals.