Pharmacology, Biochemistry and Behavior 149 (2016) 1–8

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Agmatine ameliorates lipopolysaccharide induced depressive-like behaviour in mice by targeting the underlying inflammatory and oxido-nitrosative mediators

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ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 28 June 2016
Accepted 20 July 2016
Available online 21 July 2016

Keywords:
Agmatine
BDNF
Depressive-like behaviour
Lipopolysaccharide
Oxidative stress
Pro-inflammatory cytokines

ABSTRACT

Experimental and clinical evidence indicates that pro-inflammatory cytokines, oxidative stress and brain-derived neurotrophic factor (BDNF) signalling mechanisms play a role in the pathophysiology of depression. Agmatine is a neurotransmitter and/or neuromodulator that has emerged as a potential agent to manage diverse central nervous system disorders. Agmatine has been shown to exert antidepressant-like effect. The present study investigated ability of agmatine to abolish the depressive-like behaviour induced by the administration of the lipopolysaccharide (LPS) in mice. Agmatine (20 and 40 mg/kg) was administered daily for 7 days, then the mice were challenged with saline or LPS (0.83 mg/kg; i.p.) on the 7th day. After 24 h of LPS administration we tested mice for depressive-like behaviour. LPS treated animals presented an increase in immobility time in the forced-swim test (FST), tail suspension test (TST) which was reversed by agmatine pre-treatment (20 and 40 mg/kg). Oxidative/nitrosative stress evoked by LPS was ameliorated by both doses of agmatine in hippocampus (HC) and prefrontal cortex (PFC). Administration of LPS caused an increase in interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), whereas BDNF was down regulated in the HC. Agmatine pre-treatment at 40 mg/kg ameliorated LPS-induced neuroinflammation by attenuating brain IL-1β and TNF-α level. In addition, agmatine pre-treatment also up-regulated the BDNF level in the HC. The present study shows that pre-treatment of agmatine is able to abolish the behavioural responses in the FST and TST elicited by the LPS-induced model of depression that may depend on the inhibition of pro-inflammatory mediators, reduction of oxidative stress as well as activation neuroplasticity-related signalling in mice, suggesting that agmatine may constitute an monotherapy/adjuvant for the management of depression associated with inflammation.

1. Introduction

Depression is one of the most prevalent and life-threatening forms of a mental illness that affects up to 20% of the population across the globe (Bromet et al., 2011). World Health Organization (WHO) has projected that by 2020, depression will be the second leading cause of disability (Murray and Lopez, 1996). Converging lines of evidences suggest that depression is accompanied by activation of immune-inflammatory pathways (Maes, 1995; Sluzewska et al., 1996). Elevated plasma concentration of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been found in depressed patients, suggesting a potential link between depressive illness and activation of the inflammatory response (Dowlati et al., 2010). Studies have shown that anti-inflammatory agents could constitute therapeutic approach in depression and some anti-inflammatory drugs such as aspirin (Mendlewicz et al., 2006), celecoxib (Muller et al., 2006) and etanercept (Tyring et al., 2006) have been used as adjunctive therapy in depression. However, the results have been conflicting and detrimental adverse effects may contraindicate the use of anti-inflammatory agents (Na et al., 2013). Therefore, there is a need to develop and discover new agents with high efficacy that can improve the pharmacotherapy of depressive disorder.

Currently majority of available antidepressant medications enhance or otherwise modulate monoaminergic neurotransmission (Li et al., 2012). Based on clinical and animal studies it has been suggested that brain-derived neurotropic factor (BDNF) has been implicated in the pathophysiology of depression (Lee and Kim, 2010; Guan and Fang,

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; DTNB, 5,5-dithiobis-(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; FST, forced swimming test; HC, hippocampus; IL-1β, interleukin 1β; IL-6, interleukin-6; IMI, imipramine; LPS, lipopolysaccharide; MDD, major depressive disorder; MDA, malondialdehyde; NO, nitric oxide; OFT, open field test; IO&NS, oxidative and nitrosative stress; PFC, prefrontal cortex; SEM, standard errors of the mean; TBARS, thiobarbituric-acid reactive substances; TST, tail suspension test; TNF-α, tumor necrosis factor-α.

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http://dx.doi.org/10.1016/j.pbb.2016.07.004
0091-3057/

2006). Treatment with antidepressants may normalize the lowered BDNF levels in blood and brain of depressed patients (Brunoni et al., 2008). It has even hypothesized that the clinical efficacy of antidepressants depends on BDNF levels in the hippocampus (Adachi et al., 2008). Disrupted neuroplasticity and low level of BDNF had been noted in patients with major depressive disorders, suggesting that BDNF is an important target in the therapeutic effect of antidepressants (Aydemir et al., 2006; Castrén and Rantamäki, 2010; Lee and Kim, 2010).

Multiple lines of evidence suggest that oxidative stress and nitrosative stress plays an important role in the pathophysiology of depression (Maes et al., 2011). Furthermore, altered levels of antioxidant defences, such as glutathione (GSH) (Gawryluk et al., 2011), malondialdehyde (MDA) (Kotan et al., 2011) and nitric oxide (Galecki et al., 2012) have been demonstrated in the post-mortem major depressive disorder (MDD) brain. Increased oxidative stress markers along with neuroinflammatory signature have repeatedly been reported in the blood of depressed patients (Lopresti et al., 2014). Therefore, targeting the oxidative stress and neuroinflammatory disturbances has been acknowledged as a potential avenue for the treatment of depression.

Agmatine is a polyamine that is synthesized after decarboxylation of L-arginine by arginine decarboxylase. Agmatine has been recognized as an important neuromodulator and/or neurotransmitter in the brain (Reis and Regunathan, 2000; Uzbay, 2012) which binds with high affinity to α_2-adrenoceptors, imidazoline binding sites, inhibits NMDA receptors and it competitively inhibits nitric oxide (NO) synthase (Reis and Regunathan, 2000; Halaris and Plietz, 2007). It has been demonstrated that agmatine shows prominent therapeutic role in mental disorders such as schizophrenia and depression (Kotagale et al., 2012; Zomkowski et al., 2002, 2004). A reduced plasma concentration of agmatine has been in depressive patients in comparison to healthy subjects (Halaris et al., 1999). In addition, agmatine has the ability to act as a free radical scavenger, thus protecting from oxidative stress-induced antioxidant imbalance in hippocampus (Freitas et al., 2014). Agmatine has been shown to exert neuroprotective/neurorescue effect, protection against neurodegeneration and cause cognitive enhancement (Gilad et al., 1996; Bhutada et al., 2012). Agmatine has also been reported to prevent glutamate and NMDA neurotoxicity in cerebellum and cultured hippocampal cell line (Zhu et al., 2003; Wang et al., 2006). Additionally it is known to exert antiepileptic (Aricioglu et al., 2003), anxiolytic (Gong et al., 2006), antitumor cell proliferative effects (Haenisch et al., 2011). In addition, agmatine also suppresses LPS induced hyperthermia and hepatic failure (Aricioglu and Regunathan, 2005; El-Agamy et al., 2014). Sastre et al., 1998 reported that LPS reduces endogenous agmatine levels by stimulating its degrading enzyme, agmatinase and/or inhibiting stimulatory enzyme arginine decarboxylase.

Considering the fact that agmatine has not been analyzed for its antidepressant-like effect in neuroinflammation mice model, the present study was undertaken to investigate the effects of agmatine in LPS-induced depressive behaviour in mice. To understand whether the antidepressant actions of agmatine are related to the alteration of pro-inflammatory cytokine, oxidative and nitrosative stress along with changes in BDNF level, we examined the effects of agmatine treatment on the production of oxidative stress marker in hippocampus (HC) and prefrontal cortex (PFC) while pro-inflammatory mediators (IL-1β, TNF-α) and the BDNF levels in the HC, which are the specific brain region implicated in pathophysiology of depression (Eyre and Baune, 2012).

2. Experimental procedure

2.1. Animals

Adult male Swiss albino mice weighing 20–24 g were group housed in opaque polypropylene cages (28 × 21 × 14 cm) and maintained at 25 ± 2 °C under 12:12 h light/dark cycle (07:00–19:00 h) with free access to rodent chow (Amrut rat and mice feed, Sangli, India) and water. The animals were acclimatized for 7 days before use in the experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naïve to drug treatment and experimentation at the beginning of all studies. Each experimental group comprised of eight mice. Experiments were carried out between 09.00 and 17.00 h to minimize circadian influences. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Chemicals

Agmatine, Imipramine, LPS from *Escherichia coli* (0127:B8) and phosphatase inhibitors were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Griess reagent, Thiobarbituric acid, 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), L-Reduced glutathione (GSH) and Tris-buffer were purchased from Himedia Laboratories, Mumbai, India. Mouse ELISA kit for IL-1β, TNF-α and BDNF were purchased from KrishgenBio Systems, Mumbai, India. All the drugs were dissolved in 0.9% w/v saline and administered intraperitoneally (i.p.). Drug solutions were prepared freshly in the morning. All other chemicals used in present study were of analytical grade.

2.3. Experimental design and antidepressant treatment

Animals were randomly divided into five experimental groups experimental groups, each group consisting of eight mice.

1. Group I was treated with normal saline for 7 days. This group served as a normal control group.
2. Group II was treated with normal saline for 7 days and, LPS (0.83 mg/kg, i.p.) was administered on the 7th day. This group served as the disease control group.
3. Group III was treated with agmatine (20 mg/kg, i.p.) for 7 days and LPS (0.83 mg/kg, i.p.) was administered on the 7th day.
4. Group IV was treated with agmatine (40 mg/kg, i.p.) for 7 days and LPS (0.83 mg/kg, i.p.) was administered on the 7th day.
5. Group V was treated with imipramine (10 mg/kg, i.p.) for 7 days and LPS (0.83 mg/kg, i.p.) was administered on the 7th day. This group served as the positive control group.

The study design has been diagrammatically illustrated in the Fig. 1. Agmatine was prepared freshly in saline every morning prior to the treatment. Different doses of agmatine (20 and 40 mg/kg) were selected based on the previous experimental study (Gawali et al., 2016; Zomkowski et al., 2002). Mice received an i.p. injection of vehicle or agmatine (20 and 40 mg/kg) for 7 consecutive days. The LPS dose (0.83 mg/kg, i.p.) was chosen based on previous studies evaluating depressive-like behavioural changes and neurochemical alterations in mice (O'Connor et al., 2009). LPS from *Escherichia coli* (serotype 0127:B8) was freshly dissolved in sterile endotoxin-free isotonic saline prior to injection. After 30 min agmatine administration on the 7th day, mice were also injected with saline or LPS (0.83 mg/kg) i.p. All behavioural tests were performed during the illuminated part of the cycle (between 9:00 AM and 17:00 PM), under conditions of dim light and low noise. Behaviour experiments were monitored by a trained observer blind to drug treatments.

After 24 h of LPS or saline administration depressive-like behaviour was tested, a period in which the occurrence of depressive-like behaviour is observed (Dantzer et al., 2008; Ohgi et al., 2013). Depressive-like behaviour was assessed by forced swim test (FST), open field test (OFT) and tail suspension test (TST) after 24, 27 and 29 h respectively after the LPS or saline challenge (Zhang et al., 2015). For cytokines and neurochemical determinations, mice were sacrificed by cervical dislocation. The PFC and HC were dissected and immediately stored at −80 °C until the assay was performed.