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ANTIDEPRESSANT-LIKE EFFECT OF NITRIC OXIDE SYNTHASE INHIBITORS AND SILDENAFIL AGAINST LIPOPOLYSACCHARIDE-INDUCED DEPRESSIVE-LIKE BEHAVIOR IN MICE

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Abstract—Inflammation, oxidative and nitrosative stress underlie depression being assessed in rodents by the systemic administration of lipopolysacharide (LPS). There is an increasing body of evidence of an involvement of nitric oxide (NO) pathway in depression, but this issue was not investigated in LPS-induced model. Thus, herein we evaluated the effects of NO-pathway-modulating drugs, named aminoguanidine, L-NAME, sildenafil and L-arginine, on the behavioral (forced swimming test [FST], sucrose preference [SPT] and prepulse inhibition [PPI] of the startle) and neurochemical (glutathione [GSH], lipid peroxidation, IL-1β) alterations in the prefrontal cortex, hippocampus and striatum as well as in BDNF levels in the hippocampus 24 h after LPS (0.5 mg/kg, i.p.) administration, a time-point related to depressive-like behavior. Twenty-four hours post LPS there was an increase in immobility time in the FST, decrease in sucrose preference and PPI levels accompanied by a decrease in GSH levels and an increase in lipid peroxidation, IL-1β and hippocampal BDNF levels suggestive of a depressive-like state. The pretreatment with the NOS inhibitors, L-NAME and aminoguanidine as well as sildenafil prevented the behavioral and neurochemical alterations induced by LPS, although sildenafil and L-NAME were not able to prevent the increase in hippocampal BDNF levels induced by LPS. The iNOS inhibitor, aminoguanidine, and imipramine prevented all behavioral and neurochemical alterations induced by LPS. L-arginine did not prevent the alterations in immobility time, sucrose preference and GSH induced by LPS. Taken together our results show that the NO-cGMP pathway is important in the modulation of the depressive-like alterations induced by LPS.

Key words: depressive-like behavior, LPS, nitric oxide, oxidative stress, neuroinflammation, BDNF.

INTRODUCTION

Major depressive disorder (MDD) is a stress-related illness that affects 4.4–20% of general population (Bakish, 2001). Stress regulates proinflammatory cytokines (e.g. IL-1β, IL-6, and TNF-α), cyclooxygenase-2 and lipid peroxidation. Indeed, MDD patients present increased levels of proinflammatory cytokines, for instance, IL-6, IL-1β and TNF-α in the blood (Maes, 1999) and cerebrospinal fluid (Levine et al., 1999). In addition, these inflammatory cytokines can practically interact with every pathophysiologic target relevant to depression, including neurotransmitter metabolism, neuroendocrine function and synaptic plasticity (Maes, 1999; Tsigos and Chrousos, 2002) being correlated in humans, for instance IL-1β, with anhedonia (DellaGioia et al., 2013). Within this context, brain-derived neurotrophic factor (BDNF), a key factor in neuroplasticity, is decreased in MDD (McNally et al., 2008).

The poor control of the immune/inflammatory response is related to the absence of clinical therapeutic

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Abbreviations: AMINO, aminoguanidine; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; cGMP, cyclic guanosine monophosphate; DTNB, 5,5-dithiobis-(2-nitrobenzoic acid); eNOS, endothelial NOS; FST, forced swimming test; HC, hippocampus; IDO, indoleamine 2,3-dioxygenase; IL-1β, interleukin 1β; IMI, imipramine; iNOS, inducible NOS; L-Arg, L-arginine; L-NAME, (N^{ω}-nitro-L-arginine methyl ester hydrochloride); LPS, lipopolysacharide; MDA, malondialdehyde; MDD, major depressive disorder; NF-κB, nuclear factor-κB; NMDAR, *N*-methyl-D-aspartate receptor; nNOS, neuronal NOS; NO, nitric oxide; NOS, NO synthases; O&NS, oxidative and nitrosative stress; OFT, open field test; PDE5, phosphodiesterase 5; PFC, prefrontal cortex; PPI, prepulse inhibition; Sal, saline; SEM, standard errors of the mean; sGC, soluble guanylate cyclase; SIL, sildenafil citrate; SPT, sucrose preference test; ST, striatum; TBA, thiobarbituric acid; TBARS, thiobarbituric-acid reacting substances; TCA, trichloroacetic acid; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor α.

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benefit of antidepressants in patients with treatment-resistant depression (Maes et al., 1997; Carvalho et al., 2013). Currently, treatment resistant depression occurs in up to 40% of the patients diagnosed with MDD (Rush et al., 2012).

Based on the outlined role of cytokines in depression, the systemic administration of the endotoxin lipopolysaccharide (LPS) has been used to trigger depressive-like alterations in rodents (De La Garza Ii, 2005; Dantzer et al., 2008; Custodio et al., 2013) and depressive mood in humans (Grigoleit et al., 2011). Lipopolysacharide activates toll-like receptor 4 (TLR4) (Hoshino et al., 1999). The TLR4 signaling pathway activates nuclear factor-κB (NF-κB), which leads to the production of proinflammatory cytokines (Akira and Takeda, 2004). Recently, preclinical evidences pointed toward an activation of TLR4 signaling pathway in mice prefrontal cortex (PFC) after repeated restraint/acoustic stress exposure being responsible for triggering neuroinflammation at PFC level and regulating gut barrier function/permeability (Garate et al., 2011).

The behavioral alterations induced by LPS are time-related. In this regard, the depressive-like behavior induced by this endotoxin occurs 24 h after administration in rodents (Custodio et al., 2013; Ohgi et al., 2013) and was prevented by antidepressants such as fluoxetine, paroxetine (Ohgi et al., 2013) and imipramine (Ferreira Mello et al., 2013).

Aside from inflammation, increased oxidative and nitrosative stress (O&NS) (Maes et al., 2011; Leonard and Maes, 2012) are related to MDD. The occurrence of nitrosative insult *in vivo* is observed in inflammatory processes, neurotoxicity and ischemia (Sayre et al., 2007; Şeneş et al., 2007; Leonard and Maes, 2012) as well as during neurotransmission through N-methyl-D-aspartate receptor (NMDAR) activation (Calabrese et al., 2007). Inflammation together with excessive excitatory neurotransmission and alterations in NMDAR subunits are important features of MDD (Zarate et al., 2006; Feyissa et al., 2009; Leonard and Maes, 2012).

Nitric oxide production in mammalian cells is a result of the enzymatic oxidation of L-arginine by NO synthases (NOS). This gas is regarded as a ubiquitous, janus-faced signaling molecule in the regulation of key functions in the immune, cardiovascular and nervous system (Calabrese et al., 2007).

Nitric oxide synthases comprise a family of three related proteins that modulate diverse biological processes such as neurotransmission, vascular homeostasis and immunological surveillance (Alderton et al., 2001). These enzymes namely endothelial (eNOS), neuronal (nNOS) and inducible NOS (iNOS) present different functions and subcellular distribution (Alderton et al., 2001). In this regard, nNOS and eNOS are constitutive enzymes being responsible for the production of low quantities of NO. In contrast, iNOS mediates neurotoxic events due to the overproduction of NO (Ainscough and Brodie, 1995; Calabrese et al., 2007).

Recently, NO-pathway-modulating drugs are gaining increasing relevance in the study of depression because NOS inhibitors, e.g. L-NAME (a nonspecific NOS inhibitor), aminoguanidine (a specific iNOS inhibitor) and sildenafil (a phosphodiesterase 5 – PDE5 inhibitor) are presenting antidepressant-like activity in rodents (Bettio et al., 2012; Montezuma et al., 2012; Zhang et al., 2013) that was reversed by L-arginine, a NO precursor (Joca and Guimarães, 2006). By contrast, a recent study showed that NOS inhibitors potentiated LPS-induced sickness behavior (Ribeiro et al., 2013). Thus, the issue regarding the role of NO in LPS-induced behavioral alterations needs to be clarified.

To our best knowledge there are no reports exploring the role of NO on LPS-induced depressive-like symptoms, with the exception of two recent studies of our research group that demonstrated, 24 h after the LPS challenge, a decrease in nitrite levels in the PFC, hippocampus (HC) and striatum (ST) (Custodio et al., 2013) that was restored by imipramine (Ferreira Mello et al., 2013).

Thus, based on the putative role of NO in depression and the absence of studies exploring the effects of this gas on the model of depressive-like behavior induced by immune challenge we decided to test the hypothesis that NO-pathway-modulating drugs, i.e. the NOS inhibitors L-NAME and aminoguanidine as well as sildenafil could present antidepressant-like effect against LPS-induced depressive-like behavior in mice. Furthermore, we assessed whether the effects were associated with alterations in oxidative stress parameters (i.e. reduced glutathione – GSH and lipid peroxidation), IL-1β and BDNF levels in discrete areas named prefrontal cortex, hippocampus and striatum of the mice brain following the immune challenge with LPS.

EXPERIMENTAL PROCEDURES

Animals

Adult male Swiss mice (8 weeks) weighing 20–30 g were housed eight per cage in standard polycarbonate cages (42 × 20.5 × 20 cm) with standard environmental conditions (22 ± 1 °C, humidity 60 ± 5% and 12-h light–dark cycle) and food and water *ad libitum*. The animals were obtained from the Central Animal Facility of the Federal University of Ceara. The experimental procedures were performed in the period from 8:00 to 14:00 h. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals, from the US Department of Health and Human Services (Resources, 1996) and adhered to the Brazilian legislation on animal experimentation (law n° 11.794 of 10/08/2008). The experimental protocol was approved by the Federal University of Ceara Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs

Lipopolysaccharide (LPS) from *Escherichia coli*, strain 055:B5, aminoguanidine hydrochloride, L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochloride), L-arginine and sildenafil citrate from Sigma–Aldrich