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Inhibition of nitric oxide synthase accentuates endotoxin-induced sickness behavior in mice

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ABSTRACT

Sickness behavior appears to be the expression of a central motivational state that reorganizes an organism's priorities to cope with infectious pathogens. To evaluate the possible participation of nitric oxide (NO) in lipopolysaccharide-induced sickness behaviors, mice were submitted to the forced swim test (FST), open field test and dark–light box test. Food intake and corticosterone plasma levels were evaluated. Lipopolysaccharide (LPS, 100 μg/kg; i.p.) administration increased the time spent floating in the FST and decreased locomotor activity in the open field. Indeed, treatment with LPS decreased the total number of transitions between the dark and light compartments of the apparatus. In addition, LPS reduced food intake and increased corticosterone levels. Pretreatment with L-NAME (30 mg/kg; i.p.) or aminoguanidine (50 mg/kg; i.p.) accentuated the behavioral changes induced by LPS in the FST, open field and light–dark box tests as well as induced an increment in hypophagia and in corticosterone levels. These findings confirm previous observations that have reported LPS-induced sickness behaviors. In addition, they provide evidence that the synthesis of NO modulates changes in depressive-like and exploratory behaviors in mice, which is supported by the fact that NO synthase inhibitors also attenuate LPS-induced behavioral changes. In addition, the present study suggests that NO may have a protective role, acting in an inhibitory feedback manner to limit LPS-induced sickness behavior.

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1. Introduction

Sickness behavior is an expression of a motivational state triggered by activation of the peripheral innate immune system whereby an organism reorganizes its priorities to fight infection (Parnet et al., 2002; Dantzer et al., 2008; Dantzer, 2009). Sickness behavior is easily induced in laboratory animals by the administration of endotoxins, which are the main active components of the outer membrane of gram-negative bacteria. Peripheral administration of endotoxin activates the immune system, resulting in the release of pro-inflammatory cytokines and prostaglandins (Long et al., 1990; Kelley et al., 2003; Engblom et al., 2002; Rorato et al., 2009; de Paiva et al., 2010). These peripherally produced cytokines gain access to the brain and produce the classical symptoms of sickness behavior, including reduction in locomotor activity and exploratory behaviors, anorexia and anhedonia (Engblom et al., 2002; Frenois et al., 2007). In addition, it has been demonstrated that upon stimulation by endotoxin and cytokines, nitric oxide synthase (NOS) becomes expressed, leading to the production of large amounts of nitric oxide (NO) in peripheral tissues and in the brain (Moncada et al., 1991; Giusti-Paiva et al., 2005; Mollace et al., 2005).

NO is produced from L-arginine by three different isoforms of NOS, two of which are expressed constitutively, endothelial (eNOS) and neuronal (nNOS) (Mollace et al., 2005). Endotoxin induces the expression of another calcium-independent inducible isoform of NOS (iNOS) in a number of tissues including the brain (Moncada et al., 1991; Mollace et al., 2005; Cauwels, 2007). Pharmacological inhibition of NOS can be used to elucidate the contribution of NO, and previous reports support both a detrimental and beneficial role of NO during immune challenges. An increase in the amount of NO produced by iNOS might play a pivotal role in the pathophysiology of sepsis (Thiemermann, 1997; Szabo, 1996; Vincent et al., 2000; Cauwels, 2007).

Although a significant volume of literature indicates that cytokines and prostaglandins are important mediators of sickness behavior (Dunn and Swiergiel, 2005; Dantzer et al., 2008; de Paiva et al., 2010), there is a lack of data on the role of NO in endotoxin-induced sickness behavior. To evaluate the possible participation of NO in LPS-induced sickness behaviors, mice were submitted to well-accepted tests to evaluate depressive-like and exploratory behaviors, including the forced swim test (FST), open field test and dark–light box test.

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2. Materials and methods

2.1. Animals

Adult male Swiss mice (22–30 g) were obtained from the Central Animal Facility of the Federal University of Alfenas. Animals were housed under controlled light (12:12 h light–dark cycle; lights on at 06:00 am) and temperature conditions (23 ± 1 °C) with access to water and food *ad libitum*. Animals were allowed to habituate to the housing facilities for at least 1 week before the experiments began. Behavioral studies were conducted in a quiet room between 09:00 and 11:00 a.m. to avoid circadian variation. All experiments were conducted in accordance with the Declaration of Helsinki addressing the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas (protocol #0178/2008).

2.2. Experimental procedures

In the animal room, the mice were pretreated with non-selective NOS inhibitor (Nϖ-L-nitro-arginine methyl ester; L-NAME, 30 mg/kg), or a relatively selective iNOS inhibitor (aminoguanidine, 50 mg/kg), or vehicle (0.9% NaCl) 30 min before injections of lipopolysaccharide (LPS) serotype 026:B6 (100 μg/kg; i.p.) or saline (0.9% NaCl). The behavioral tests were performed 120 min after the LPS treatment. These time points were chosen on the basis of previous behavioral, endocrine and neurochemical studies (Dunn and Swiergiel, 2005; Rorato et al., 2009; de Paiva et al., 2010). The doses of L-NAME and aminoguanidine used in the present study are in agreement with the doses commonly used in other reports that injected these compounds peripherally (Giusti-Paiva et al., 2004). All drugs were purchased from Sigma-Aldrich Co. and dissolved in sterile isotonic saline.

2.2.1. Forced swim test

This test was performed according to the method developed by Porsolt et al. for mice. Mice (n = 12 per group) were placed in a vertical glass cylinder (26 cm high, 12 cm diameter) filled with 25 °C water to a depth of 16 cm. Water depth was chosen to ensure that animals swam or floated without their hind limbs or tail touching the bottom. Each mouse was placed in the cylinder for 6 min, and the duration of floating (i.e., the time during which mice made only the smallest movements necessary to keep their heads above water) was scored. As suggested by Porsolt et al. (1977), only the data scored during the last 4 min were analyzed and presented (Porsolt et al., 1977; Dunn and Swiergiel, 2005; de Paiva et al., 2010).

2.2.2. Open field behavioral test

Locomotor activity was quantified for 5 min in an open field box, consisting of white Plexiglas 60 × 60 cm in diameter with a floor divided into 16 squares. Previous studies have indicated that this time-period was sufficient to produce differences between treatment groups. Furthermore, after 5 min, the mice habituate to the apparatus, thereby decreasing the differences between groups. Four squares were defined as the center and the 12 squares along the walls were considered the periphery. Each mouse (n = 10 per group) was gently placed in the exact center of the box. Activity was scored as a line crossing when a mouse removed all four paws from one square and entered another. Line crossings among the central four squares or among the peripheral 12 squares of the open field were counted separately (Dunn and Swiergiel, 2005; de Paiva et al., 2010).

2.2.3. Light–dark box test

The apparatus consisted of a Plexiglas rectangular box (48 cm long × 24 cm wide × 24 cm high) divided into a dark region (24 cm long) and a light region (24 cm long). The light and dark regions were separated by an opening (8.0 × 8.0 cm) that allowed the animals to move between the two compartments. The dark region was made of black Plexiglas and covered with a black lid. The light portion was made of white Plexiglas, and a 60 W light was positioned directly over it. On the day of testing, each mouse was transported individually from the housing room to the testing room. Each mouse (n = 10 per group) was placed in the light compartment and allowed to move freely between the two compartments. The behavior was video-recorded for a total of 5 min, and the videotapes were scored for latency to the first transition and the number of transitions between the light and dark compartments (Lacosta et al., 1999; de Paiva et al., 2010).

2.2.4. Feeding behavior

The animals (n = 10 per group) fasted for 12 h before receiving injections. Immediately after injections, a fresh supply of pre-weighed food was given. Food intake was calculated at 2, 4, 6 and 24 h after the injection by measuring the difference between the pre-weighed standard chow available and the weight of chow and spilled crumbs at each time point. Changes in body weight were measured by weighing the animals at the beginning of the experiment as well as before and after an experimental day (Rorato et al., 2009; Soncini et al., 2012).

2.2.5. Plasma corticosterone assay

Trunk blood was collected from animals 2 h after LPS or saline injections in chilled heparinized tubes and was centrifuged at 3000 rpm for 15 min at 4 °C. Plasma was collected and stored at −20 °C. Plasma corticosterone was measured by radioimmunoassay.

2.2.6. Statistical analysis

The data obtained were analyzed using the GraphPad software program Version 4.0 and expressed as the mean ± S.E.M. Statistically significant differences among groups were calculated by the application of an analysis of variance (ANOVA) followed by the Newman–Keuls test. *P*-values less than 0.05 ($p<0.05$) were considered significant.

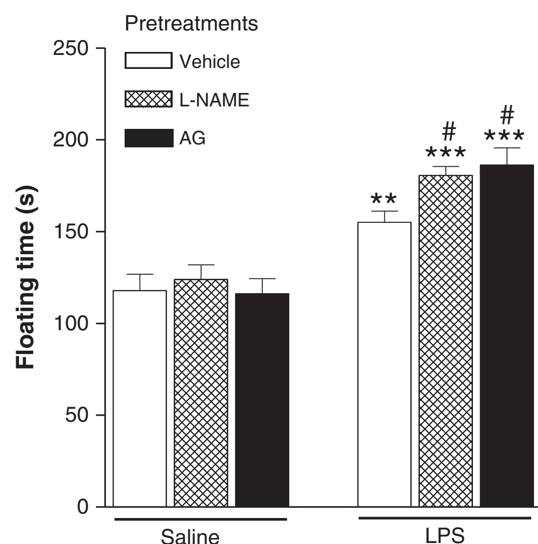

Fig. 1. Effects of pretreatment with vehicle, L-NAME (30 mg/kg) or aminoguanidine (AG, 50 mg/kg) on time spent floating in the forced swim test (n = 12 animals per group), which were measured 2 h after administration of either lipopolysaccharide (LPS) or saline. Each column represents the mean with S.E.M. The symbols denote significance levels: **$p<0.01$; ***$p<0.001$ when compared with the control groups; # $p<0.05$ when compared with the vehicle plus LPS group.

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