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Dipyrone attenuates acute sickness response to lipopolysaccharide in mice

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ABSTRACT

Sickness behavior appears to be the expression of a central motivational state that reorganizes the organism's priorities to cope with infectious pathogens. To evaluate the effect of dipyrone in lipopolysaccharide (LPS)-induced sickness behavior, mice were subjected to the forced swim test (FST), tail suspension test (TST), dark–light box test, open field test, sucrose preference intake test and food intake test. LPS administration increased the immobility time in the TST, increased the time spent floating in the FST, and depressed locomotor activity in the open field test. Treatment with LPS decreased the total number of transitions made between the dark and light compartments of the apparatus and induced anhedonia and anorexia. Pre-treatment with dipyrone (10, 50, or 200 mg/kg) attenuated behavioral changes induced by LPS in the FST, TST, open field and light–dark box tests. In addition, dipyrone prevented anhedonia and anorexia in mice challenged with LPS. Considering that dipyrone attenuates LPS-induced behavioral changes, it is proposed that LPS-induced sickness behavior is dependent on the COX pathway.

1. Introduction

Sickness behavior is the expression of a motivational state triggered by an activation of the peripheral innate immune system and is characterized by depressive-like behavior, such as a reduction in locomotor activity and exploratory behavior, anorexia and anhedonia [7,17]. The mechanisms underlying sickness behavior have not been fully elucidated, but it has been suggested that cytokines and prostaglandins are involved [7,9,17,30]. Interleukin-1β, interleukin-6 and tumor necrosis factor-α (TNF-α) may be secreted in response to infections and endotoxemia [7,17]. Previous reports have demonstrated that LPS-induced depressive-like behavior appears to depend on the cyclooxygenase (COX) pathway as the use of a non-steroidal anti-inflammatory drug (NSAID) (indomethacin and nimesulide) has been shown to attenuate the behavioral changes induced by LPS [9]. COX is the key enzyme in the synthesis of prostaglandins from arachidonic acid. While COX-1 is a constitutive enzyme, COX-2 is induced by several stimuli, and the biosynthesis of both is inhibited by NSAIDs [31]. The pyrazolone derivative dipyrone, also known as metamizole, is an NSAID that acts as an effective analgesic and antipyretic and has been demonstrated to inhibit COX [25].

In contrast to classical NSAIDs, dipyrone produces analgesic effects associated with a less potent anti-inflammatory action in different animal models [15]. There are four major metabolites of dipyrone, but only the main metabolites, 4-methylamino-antipyrine (MAA) and 4-amino-antipyrine (AA), alter the biochemical properties of COX [25]. Considering the difference in the mechanism of action between dipyrone and the nonsteroidal anti-inflammatory drugs and the popularity of dipyrone as an analgesic and antipyretic drug during infective illnesses in many countries, the aim of the present study was to investigate the effects of pre-treatment with dipyrone on LPS-induced sickness behavior.

2. Materials and methods

2.1. Animals

Adult male Swiss mice (25–30 g), obtained from the Central Facility of the Federal University of Alfenas, were individually housed, for at least a week, in a room controlled temperature (24 ± 1 °C), humidity (40–60%) and a 12:12 h light–dark cycle (lights off at 6:00 pm). Standard rodent chow and tap water were provided *ad libitum* throughout the experiments except where indicated. Different animals were used for each experiment. Immediately after the end of the experiments, the animals were euthanized with halothane overdose so as to avoid any suffering.

All procedures were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of

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Alfenas-MG (protocol number: 167/2008). All behavioral tests were conducted by an experimenter blind to the treatment groups.

2.2. Depressive-like and exploratory behavior

Mice were pre-treated with dipyrone (10, 50 or 200 mg/kg, i.p.) or vehicle (sterile saline, 0.9% NaCl, 1 ml/kg) 30 min before injection of LPS extracted from *Escherichia coli* serotype 026:B6 (200 μg/kg, i.p.; at 12:00 pm) or sterile saline (0.9% NaCl). The behavioral tests included the forced swimming test (FST), tail suspension test (TST), light–dark box test and open field test all of which were performed 120 min after LPS administration (for details, see Ref. [9]). The experiments were recorded using a video camera.

2.2.1. Forced swimming test (FST)

Mice ($n = 12$ per group) were placed in a vertical glass cylinder (26 cm high, 12 cm in diameter) filled with 25 °C water to a depth of 16 cm. For testing, 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 [9] from the film.

2.2.2. Tail suspension test (TST)

The mice ($n = 8$ per group) were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. The procedure was modified from Dunn and Swiergiel [10].

2.2.3. Light–dark box test

The apparatus consisted of a rectangular Plexiglas box (48 cm long × 24 cm wide × 24 cm high) divided into a dark and light region (both 24 cm long). The light and dark regions were separated by an opening (8 cm × 8 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 with a 60 W light positioned directly over it. The mice ($n = 10$ per group) were placed in the light compartment and allowed to move freely between the two compartments. Behavior was recorded for a total of 5 min and scored for latency to the first transition and the number of transitions between the light and dark compartments [9].

2.2.4. Open field behavioral test

Locomotor activity was recorded for 5 min in an open field consisting of a 60 cm × 60 cm white Plexiglas box with its floor divided into 16 squares. 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 exactly in the center of the box, and 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 [9,10].

2.3. Sucrose preference intake test

Animals received food and water ad libitum and had access to 10% sucrose for 2 h every day (2:00–4:00 pm) for five days (sucrose intake training). After this period, to establish LPS doses that could produce a pronounced anhedonic behavior, doses of LPS (200, 500 or 1000 μg/kg, i.p.; $n = 10$ animals per group) or saline were injected at 12:00 pm. At 2:00 pm, mice had access to water and 10% sucrose for 24 h. This experiment was designed to assess the effect of LPS on the preference for a palatable solution using a two-bottle paradigm in which mice could choose between a bottle of water and a bottle containing a sucrose solution. The fluid intake was measured by weighing water and sucrose bottles at 2 and 24 h. Another set of mice ($n = 10$ animals per group) received injections of dipyrone or saline 30 min prior to LPS (1000 μg/kg) both administered at 12:00 pm. At 2:00 pm, mice had access to water and 10% sucrose for 24 h. Fluid intake was measured by weighing the water and sucrose bottles at 2 and 24 h. Sucrose preference was determined using the following equation: sucrose intake/total fluid intake (water + sucrose intake) × 100 [18].

2.4. Food intake

Mice were weighed and assigned arbitrarily to body weight-matched groups and were deprived of food for 24 h but still had free access to tap water. On the following day, the animals received injections of dipyrone ($n = 6$ animals) or saline (i.p., $n = 13$ animals) and 30 min later (around to 12:00 pm) were administered an injection of LPS (100 μg/kg, i.p.) or saline (i.p). Immediately after, pre-weighed chow pellets were offered to the animals. Food intake was measured at 2, 4, 6 and 24 h by weighing the remaining food pellets along with any spillage into the cage.

2.5. Statistical analysis

The results are reported as the mean ± S.E.M. Analysis of variance (ANOVA) followed by the Newman–Keuls test was used for comparisons. Differences were considered significant at $p < 0.05$.

3. Results

There was an increase in the immobility period 120 min after the administration of LPS in the FST ($F_{4,39} = 27.3$; $p < 0.001$; Fig. 1A) and TST ($F_{4,39} = 19.4$; $p < 0.001$; Fig. 1B) in mice pre-treated with vehicle. Pre-treatment with dipyrone significantly reversed the LPS-mediated increase in the immobility period in both the FST (at doses of 10, 50 and 200 mg/kg) and TST (at doses of 50 and 200 mg/kg). Furthermore, there was a significant reduction in the number of transitions between the light and dark compartments after LPS administration. The pretreatment with dipyrone (50 and 200 mg/kg) prior to LPS administration caused a greater number of transitions between the compartments compared to the vehicle plus LPS group ($F_{4,39} = 89.6$; $p < 0.001$; Fig. 1C). In addition, LPS significantly decreased the number of line crossings in the center and periphery as well as the total number of line crossings. *Post hoc* analyses indicated that pre-treatment with dipyrone significantly reversed LPS-induced decreases in the number of central ($F_{4,39} = 129.7$; $p < 0.001$; Fig. 1D) and peripheral line crossings ($F_{4,39} = 143.2$; $p < 0.001$; Fig. 1E) as well as the total number of line crossings ($F_{4,39} = 224.5$; $p < 0.001$; Fig. 1F).

The 1000 μg/kg dose of LPS reduced sucrose preference at 2 h ($F_{3,35} = 3.44$; $p < 0.05$) and 24 h ($F_{3,35} = 6.71$; $p < 0.0012$) when compared to the control group (Fig. 2). After 2 h ($F_{4,55} = 2.68$; $p < 0.041$) and 24 h ($F_{4,55} = 7.49$; $p < 0.001$), the dipyrone plus LPS group reestablished the sucrose preference as observed in the control group (Fig. 3).

Compared to the control group, LPS significantly depressed food intake 2, 4, and 6 h after injection as well as overnight food intake (Fig. 4). Pre-treatment with dipyrone (at doses of 10, 50 and 200 mg/kg) significantly reversed the anorexic effect induced by LPS at 2 h ($F_{4,39} = 7.3$; $p < 0.002$), 4 h ($F_{4,39} = 13.84$; $p < 0.001$), 6 h ($F_{4,39} = 13.89$; $p < 0.001$) and 24 h ($F_{4,39} = 7.17$; $p < 0.001$).

The animals that received dipyrone plus vehicle (saline), regardless of dose, showed no significant differences in food intake or sucrose preference in relation to the control group (data not shown).