Research Report

Behavioral effects of lipopolysaccharide in rats: involvement of endogenous opioids

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Abstract

Activation of the immune system in response to either infection or lipopolysaccharide (LPS) produces neurophysiological, neuroendocrine and behavioral changes. Some of the physiological consequences of LPS are mediated by endogenous opioid peptides. The following studies were designed to characterize the effects of LPS in several behavioral paradigms, and to determine the role of opioids in mediating these effects. The effects of LPS on locomotor and self-care activity were assessed in the open field test. Rats were injected with either saline or a dose of LPS (25, 50, 100, or 1000 μg/kg). 4h later, the animals were placed in an open field and the numbers of line crossings, rearings and grooming episodes were counted. LPS significantly suppressed the three open field behaviors in a dose-related manner. The effect of LPS on sensitivity to pain was determined using the hot-plate and tail-flick tests. Administration of LPS (200 μg/kg) increased pain sensitivity in the hot plate test 30 min after drug administration, but produced a significant analgesic response 4 h after drug administration in both tests. Further characterization of LPS-induced analgesia demonstrated that it began about 2 h after and disappeared 30 h after the administration of LPS. Administration of naltrexone completely blocked the analgesic effects of LPS 4 h after its administration, but had no effect on LPS-induced suppression of activity in the open field. The effect of LPS on body temperature was biphasic, producing hypothenmia at 2 h and hyperthermia at 8–30 h after its administration. Naltrexone had no effect on the body temperature changes induced by LPS. These results suggest that endogenous opioids mediate the analgesic effects of LPS, but they are involved neither in mediating LPS-induced suppression of locomotor and self care behaviors nor in alterations of body temperature.

Key words: Sickness behavior; Lipopolysaccharide; Opiate; Interleukin-1; Infection; Open field; Analgesia; Naltrexone; Fever; Rat

1. Introduction

Activation of the immune system in response to infection produces neural, neuroendocrine, and behavioral effects. Infectious agents, and cytokines secreted by activated immune cells, such as interleukin-1 (IL-1), alter neuronal activity [5,34,35], change central neurotransmitter levels [9,14,43], regulate pituitary hormone release [3,4,36,37], and produce behavioral changes such as depression of motor activity and mood, and reduced ingestive, exploratory, and self care behaviors [11,19,20,27,30,33,38,42]. These behavioral symptoms, which were collectively termed ‘sickness behavior’, may be an adaptive response to an infectious agent attack, rather than secondary to the disease process itself and the fever that accompanies it [17,21].

Symptoms similar to those observed during sickness are produced in animals by administering lipopolysaccharide (LPS), which is a product of the cell wall of Gram-negative bacteria. Administration of LPS to animals is known to activate immune functions and to produce fever [22]. It is further known that LPS-induced fever depends on the release of several cytokines from cells of the immune system, particularly IL-1 and IL-6 [22]. Administration of LPS has also been shown to have several behavioral effects, including general malaise [41], anorexia and decreased body
weight [6,42], depression of locomotion, exploration and social exploration [6,13], and altered pain sensitivity [25,26,29].

Several lines of evidence indicate that endogenous opioid peptides mediate some of the physiological consequences of LPS administration: (1) The levels of opioid peptides are higher in the plasma of LPS-treated animals [10]; (2) mononuclear cells, particularly B cells synthesize large quantities of opioids upon exposure to LPS [2,16], and these B-lymphocyte-derived opioids mediate some of the pathophysiological effects of LPS [15]; (3) administration of naloxone attenuates the effects of LPS on blood pressure and increases overall survival rates in LPS-treated rats [18]; (4) the shock-producing effects of LPS can be mimicked by exogenous administration of the δ-agonist DADLE [18].

Based on the above mentioned findings regarding the involvement of endogenous opioids in mediating the physiological effects of LPS, we hypothesized that endogenous opioids may also be involved in some of the behavioral effects of LPS. The present study was designed to characterize the effects of LPS on motor activity and pain sensitivity and to investigate the role of opioids in mediating these effects. We report that LPS administration decreased locomotor and self-care (grooming) activity in the open field test and produced a long-lasting analgesia. Only the latter response was attenuated by the opiate antagonist naltrexone, indicating that LPS-induced analgesia is mediated by endogenous opioids.

2. Materials and methods

2.1. Subjects

Subjects were Fischer 344 male rats, 10–12 weeks old (Harlan-Sprague-Dawley, Indianapolis, USA). Rats were housed 4–5/cage with free access to food and water. Experiments were conducted during the first half of the dark phase of a 12:12 h light-dark cycle under dim illumination.

The first experiment was designed to examine the effects of LPS on motor activity in the Open Field test. Rats were divided into five groups (n = 9–10) administered either saline or a dose of LPS (25, 50, 100 or 1000 μg/kg) from E. coli (O55:BS, Difco Labs, Detroit, USA, in a volume of 1 ml/kg). 4 h after the injection, each rat was placed in the corner of an open field (95×95×60 cm) divided into 35 identical squares. The incidence of line crossings with both hind paws, rearing and grooming episodes was recorded by an observer blind to the treatment received by the animal, over a period of 3 min.

The second experiment was designed to characterize the effects of LPS on pain sensitivity using the hot-plate and the tail-flick tests. In one part of this experiment, rats were divided into two groups (n = 14–16), injected either with LPS (200 μg/kg) or saline. Fifteen min later, pain sensitivity was tested using the hot-plate method. Rats were placed on a 52.5°C metal surface covered with an insulating cardboard. The rats were allowed to habituate to the apparatus for 1 min, and then the cardboard was removed and the latency to hind-paw lick was measured. To avoid tissue damage, animals that did not lick their hind paw were removed from the plate after 60 s. Measurements of hot-plate latency were taken again in the same animals 30 and 240 min after drug administration. In another part of this experiment, the effects of LPS were examined in the tail-flick test using the D'Amour and Smith [12] method. A 'cut-off' time of 0.3 s was used for automatic termination of a tail-flick trial. Rats were habituated to handling and to being inserted into plastic cylindrical tubes on four occasions before the experimental day. At the beginning of the experiment, baseline tail flick latency (TFL) was measured until a stable baseline was obtained. The rats were then divided into two groups (n = 12–13) matched for mean baseline TFL and injected with either saline or 200 μg/kg LPS. Tail-flick latency was measured again 30 and 240 min after the injection. A percent of the maximum possible analgesic effect (MPAE) was computed at each of these time points according to the formula: percent MPAE = [TF - BL]/(9.0 - BL)×100, where TF is the TFL at any one time point after injection, and BL is the mean of the last two baseline TFL measurements.

A third experiment was designed to further characterize the dose response and time course of LPS-induced analgesia. Rats were divided into five groups (n = 9–10) administered either saline or a dose of LPS (25, 50, 100 or 1000 μg/kg). 2 h later, rats were tested for pain sensitivity using the hot-plate method as described above. Measurements of hot-plate latency were taken again in the same animals 4, 8, 12, 20, and 30 h after drug administration.

The fourth experiment was designed to test the involvement of endogenous opioids in mediating the motor and analgesic effects of

![Fig 1](image-url). The effect of LPS dosage on the behavior of rats in the open field test. Rats were injected with either saline or a dose of LPS (25–1000 μg/kg), and 4 h later, the number of line crossings (A), rearing (B) and grooming episodes (C) was recorded over a 3 min period. * Significantly different from saline-injected rats (P < 0.05), ** Significantly different from rats injected with saline, 25 or 50 μg/kg LPS (P < 0.05).
LPS. In this experiment half of the rats were injected with naloxone (10 mg/kg); the others with saline. 20 min later, half of the rats within each group (n = 7–8) were injected with LPS (1000 µg/kg); the others with saline. 4 h later all animals were examined with the open-field test, followed, 15 min later, by the hot-plate test, as described above.

The fifth experiment was designed to test the involvement of endogenous opioids in LPS-induced body temperature mediation. Rectal temperatures were determined with a Tele-thermometer (Yellow Springs Instruments Co., Yellow Springs, OH) at an environmental temperature of 22 ± 1°C. Baseline temperature was measured in all rats, followed, 20 min later, by an injection of either naloxone (10 mg/kg) or saline. 40 min later, baseline temperature was measured again, and then half of the rats within each group (n = 11) were injected with LPS (1000 µg/kg); the others with saline. Rectal temperature was measured again in the same animals 2, 4, 8, 12, 20 and 30 h after the second drug administration.

The results were analysed by ANOVAs followed by post-hoc tests with the Fisher PLSD procedure (P < 0.05).

3. Results

The results of the experiment on the effects of various doses of LPS on pain behavior in the open field test revealed significant differences among the groups in line crossings, rearings and grooming episodes (F(4,47) = 8.93, 5.38, and 5.02, respectively, all significant at the P < 0.01 level) (Fig. 1). Post-hoc comparisons showed that each one of the groups administered with LPS manifested significantly less line crossings and rearings than saline-injected controls. Animals injected with the high dose of LPS crossed significantly less lines than animals injected with 25 or 50 µg/kg LPS. Finally, administration of 100 and 1000 µg/kg LPS significantly reduced the number of grooming episodes.

Analysis of the results of the first part of the second experiment, which examined the effects of LPS on pain sensitivity in the hot-plate test, revealed a significant injection × time interaction (F(2,50) = 7.48, P < 0.005), reflecting the differential effect of LPS at various time points after its administration (Fig. 2). Post-hoc comparisons showed significant differences between the groups at 30 and 240 min, but not at 15 min, after drug administration. The results of the tail-flick experiment revealed a significant effect of the injection (F(1,23) = 6.13, P < 0.05), the time (F(1,23) = 10.97, P < 0.005), and the interaction between the injection and time (F(1,23) = 11.21, P < 0.01) (Fig. 3). Post-hoc comparisons showed that the analgesic effect of LPS at 240 min post-administration was statistically significant, whereas the difference between the groups at 20 min after the injection did not reach statistical significance.

Analysis of the results of the third experiment, which determined the time course and dose response relationship of LPS administration on hot-plate latency, revealed a significant group effect (F(4,42) = 6.92, P < 0.002), reflecting the overall difference in hot-plate latency among groups receiving various doses of LPS, significant time effect (F(6,42) = 17.07, P < 0.001), reflecting the increase of hot-plate latency after LPS administration, and a significant group × time interaction (F(24,252) = 2.34, P < 0.001), reflecting the greater increase in hot-plate latency after administration of high, but not low, doses of LPS (Fig. 4). Post-hoc comparisons showed that rats injected with 1000 µg/kg LPS had significantly higher hot-plate latencies than rats injected with either saline, 25 or 50 µg/kg LPS at the 2, 4, 8, 12, and 20 h hot-plate tests. Administration of 100 µg/kg LPS also slightly increased hot-plate latency, producing significant analgesia compared to saline-injected rats 8 h after drug administration. There were no significant differences among the groups at baseline or at 30 h after drug administration.

Analysis of the results of the fourth experiment, which examined the effects of naloxone on LPS-induced changes in the open field and hot-plate tests, revealed a significant effect of LPS on line crossings (F(1,25) = 18.9, P < 0.001) (Fig. 5A), rearings (F(1,25) = 13.4, P < 0.005) (Fig. 5B), and grooming episodes.

Fig. 2. Effects of LPS on pain sensitivity in the hot-plate test. Rats were injected with either saline or LPS (200 µg/kg) and the latency to hind paw lick was measured 15, 30 and 240 min later. * Significantly different from saline-injected rats (P < 0.05).

Fig. 3. Effects of LPS on pain sensitivity in the tail-flick test. After establishing a stable baseline, rats were injected with either saline or LPS (300 µg/kg), and the latency to removal of the tail from a radiant heat source was measured 30 and 240 min later. The results presented are the Percent of Maximal Possible Analgesic Effect (MPAE). * Significantly different from saline-injected rats (P < 0.05).
(F(1,25) = 15.2, P < 0.001) (Fig. 5C). There were no overall or group-specific effects of naltrexone. Post-hoc analysis showed that line crossings, rearing and grooming in rats treated with saline or naltrexone followed by LPS (Sal-LPS and Nalt-LPS groups) was significantly lower than in rats treated with saline or naltrexone followed by saline (Sal-Sal and Nalt-Sal groups). An ANOVA on the hot-plate latency results revealed a significant second injection effect (F(1,25) = 4.6, P < 0.05), reflecting an overall elevation of hot-plate latency by LPS, and a significant first by second-injection interaction (F(1,25) = 6.9, P < 0.05), reflecting a block of LPS-induced analgesia by naltrexone (Fig. 5D). Post-hoc tests revealed that hot-plate latency in Sal-LPS rats was significantly higher than in the other three groups. There were no significant differences in hot-plate latency among the latter three groups.

Analysis of the results of the fifth experiment, which examined the effect of LPS and its interaction with naltrexone on body temperature, revealed a significant second-injection effect (LPS vs. saline) (F(1,40) = 40.3, P < 0.0001), as well as a second-injection by time interaction (F(6,240) = 32.8, 0 < 0.0001), reflecting the differences between saline- and LPS-injected animals at different times (Fig. 6). Naltrexone had no overall or group-specific effects on body temperature. Post-hoc analysis showed that there were no differences between the groups at the two baseline measurements. 2 h after the second injection, rats treated with saline or naltrexone followed by LPS (Sal-LPS and Nalt-LPS groups) had significantly lower body temperature than rats treated with saline or naltrexone followed by saline (Sal-Sal and Nalt-Sal groups). 4 h after the second injection there were no significant differences in body temperature among the groups. At all other times (8, 12, 20 and 30 h after the second injection) the two groups treated with LPS (Sal-LPS and Nalt-LPS groups)
had significantly higher body temperature than the two groups not treated with LPS (Sal-Sal and Nalt-Sal groups).

4. Discussion

The results indicate that administration of LPS affects the behavior of animals in the open-field test. The effects of LPS on line crossings and rearings may be viewed as a general depressive effect on locomotion as well as suppression of exploratory behavior. These results are consistent with a recent report that LPS decreases locomotor and exploratory activity in a different paradigm, the multicomartment chamber [13]. The effects of LPS on grooming represents the reduction in self-care behaviors associated with sickness. Reduced grooming has been anecdotally documented [17], but to our knowledge has not been quantitatively demonstrated before.

LPS-induced depression of motor, exploratory, and self-care behaviors may be a model for the adaptive behavioral response to infection and sickness [17,21]. Reduced locomotor and exploratory behaviors save body energy reserves that are required for the increased metabolic costs of fever, and reduces heat loss that occurs from exposure of body surface during locomotion. The effect of LPS on grooming may be secondary to its effect on general motor activity. Alternatively, it has been suggested that reduced grooming may be specifically adaptive during illness since it prevents heat loss from exposure of skin surface and loss of water through saliva used in grooming [17].

The mechanism by which LPS produces its effects on behavior in the open-field test could involve the production of cytokines, such as IL-1, IL-6 and TNF, and/or the production of other immune- or neural-derived factors, such as endogenous opioids. Indeed, exogenous administration of IL-1 was found to decrease exploratory behavior in a multicomartment chamber [13,38], as well as exploration of a juvenile conspecific, and food-motivated operant behavior [11,20]. Moreover, several recent studies have demonstrated that some of the behavioral effects of LPS are mediated by IL-1 secretion. For example, administration of the IL-1 receptor antagonist (IL-1ra) was found to block the effects of LPS (250 μg/kg) on social exploration, and body weight [6,20,21]. Although in experiments using the multicompartment chamber acute IL-1 administration did not reduce locomotor activity [13,38], it has been reported that continuous administration of IL-1α did reduce horizontal locomotor activity and rearings [30]. We have also demonstrated that IL-1β significantly reduced the number of line crossings and rearings in the open field test [1]. These findings suggest that the effects of LPS on rats in the open field test are at least partly mediated by IL-1.

A previous study reported that the effect of IL-1α on exploratory behavior of mice in the multicomartment chamber was blocked by naloxone, suggesting the involvement of endogenous opioids in mediating IL-1's effects in that paradigm. Thus, if the response to LPS in the open field is indeed mediated by IL-1, as suggested above, it could have been expected that it would be attenuated by naloxone. However, the findings of the fourth experiment, which showed no effect of naloxone on LPS-induced suppression of locomotion, rearing and grooming suggest that these effects are not mediated by LPS-induced modulation of endogenous opioid systems. Differences in the species (mice vs. rats) and the behavioral paradigms (multicompartment chamber vs. open field) may explain these differential effects of opiate antagonists.

Our results indicate that LPS produced time-dependent changes in pain sensitivity in both the hot-plate and tail flick tests. These findings are consistent with previous studies that demonstrated a hyperalgesic response to LPS in the tail-flick test [25,26], as well as an analgesic effect of LPS in the phencyclidine-induced writhing test [29]. In the present study, LPS produced an initial increase in pain sensitivity in the hot-plate test (compared to saline-injected controls), followed by a later decrease in pain sensitivity in both the hot-plate and tail flick tests. The lack of a clear and significant LPS-induced hyperalgesia in the tail-flick test, which has been recently reported by others [25,26], may be due to differences in the LPS and the rat strains that were used.

The previous finding that administration of the IL-1 antagonist IL-1ra blocked the hyperalgesia produced by LPS, indicates that IL-1 is involved in mediating LPS-induced hyperalgesia [25]. Interestingly, after administration of IL-1ra to LPS-injected animals, pain sensitivity was decreased even more than in control
animals, suggesting that following LPS administration there is a parallel action of an hyperalgesic mediator (supposedly IL-1) and an analgesic substance [25]. Taken together with the result of the present study, these findings suggest that after LPS administration, the balance between the pain modulatory substances shifts from an initial predominance of the pain facilitatory influence to a later predominance of the pain inhibitory influence. It may be suggested that the initial LPS-induced hyperalgesic response is related to the somatic aches and pains that are frequently reported by humans during febrile illness, whereas the analgesic response serves as a feedback mechanism that terminates the hyperalgesic period.

The finding that 4 h after its administration LPS induced analgesia that was completely blocked by naltraxone suggest its mediation by endogenous opioids. LPS-induced opioid release could be similar to the release of opioids by other stressful stimuli, either by the brain or the pituitary [34,35], or by peripheral tissues, e.g., the adrenal gland [23]. Moreover, since LPS administration increases blood levels of opioids [10] and induces the production of endorphins in lymphocytes [16], and since B-lymphocyte-derived endorphins have been suggested to mediate some of the pathophysiological effects of endotoxic shock [15], it is possible that the analgesic effect of LPS is produced by immune-derived opioids. In support of this hypothesis, it was recently demonstrated that subcutaneous injections of various doses of LPS induced an intense and transient expression of the proenkephalin (PEA) gene in macrophages located within the peripheral and mesenteric lymph nodes [2]. Proenkephalin mRNA in LPS-treated rats accumulated to concentrations that are 15–60-fold higher than those observed in saline-injected control animals. Actually, per gram tissue, the total PEA expression in lymph nodes is higher than that expressed constitutively in the brain [2]. Administration of LPS also had a profound effect on PEA expression in the adrenal gland (500-fold induction), while it had no effect on the constitutive PEA expression in the pituitary gland and in the hypothalamus. Moreover, the expression of PEA peaks at 4 h and 17 h after LPS administration in the lymph nodes and adrenal gland, respectively [2]. Interestingly, in the present study the analgesic effect of LPS also showed a biphasic pattern, peaking 4 h and 20 h after drug administration. This correlation between the effects of LPS on PEA expression and analgesia may reflect the involvement of opioids from these two peripheral sources in the modulation of pain sensitivity. The findings that enkephalins produced by monocytes interact with receptors on sensory nerves to inhibit nociception at inflamed tissues [39,40] further suggest that LPS-induced analgesia depends on activation of peripheral opioid systems.

The analgesic effect of LPS probably did not result from a general motor impairment. This conclusion is based on two of the present findings: (1) 4 h after LPS administration there was no good correlation between the motor and analgesic effects of LPS; whereas motor depression was seen with all LPS doses, only the highest dose produced analgesia. (2) While naltraxone completely blocked LPS-induced analgesia, it had no effect on locomotor depression. The effect of LPS on body temperature was biphasic, producing hyperthermia at 2 h and hypothermia at 8–30 h after its administration. This finding is consistent with a previous study, showing a similar biphasic effect of LPS on body temperature [8]. A role for opioids in body temperature regulation has been suggested, as the concentration of opioids in the brain and serum increases during the course of the normal fever response [10,11], with exogenous administration of β-endorphin increases core temperature [31], and naloxone attenuates the hyperthermic effect of antigen challenge in sleep-deprived rats [7]. However, in the present study naltraxone had no effect on the core temperature changes induced by LPS, which suggest that opioids are not involved in LPS-induced body temperature alterations. The results of the present study also suggest that the motor and the analgesic effects of LPS are not related to the effect of LPS on body temperature. This conclusion is based on the findings that 4 h after its administration, LPS significantly altered locomotion and pain sensitivity but not body temperature, and on the finding that naltraxone significantly attenuated the analgesic effects of LPS but it had no effect on body temperature.

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References


