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Research report

Lipopolysaccharide affects exploratory behaviors toward novel objects by impairing cognition and/or motivation in mice: Possible role of activation of the central amygdala

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ABSTRACT

Lipopolysaccharide (LPS) produces a series of systemic and psychiatric changes called sickness behavior. In the present study, we characterized the LPS-induced decrease in novel object exploratory behaviors in BALB/c mice. As already reported, LPS (0.3–5 μg/mouse) induced dose- and time-dependent decreases in locomotor activity, food intake, social interaction, and exploration for novel objects, and an increase in immobility in the forced-swim test. Although the decrease in locomotor activity was ameliorated by 10 h postinjection, novel object exploratory behaviors remained decreased at 24 h and were observed even with the lowest dose of LPS. In an object exploration test, LPS shortened object exploration time but did not affect moving time or the frequency of object exploration. Although pre-exposure to the same object markedly decreased the duration of exploration and LPS did not change this reduction, LPS significantly impaired the exploration of a novel object that replaced the familiar one. LPS did not affect anxiety-like behaviors in open-field and elevated plus-maze tests. An LPS-induced increase in the number of c-Fos–immunoreactive cells was observed in several brain regions within 6 h of LPS administration, but the number of cells quickly returned to control levels, except in the central amygdala where the increase continued for 24 h. These results suggest that LPS most prominently affects object exploratory behaviors by impairing cognition and/or motivation including continuous attention and curiosity toward objects, and that this may be associated with activation of brain nuclei such as the central amygdala.

1. Introduction

In mammals, even low doses of bacterial endotoxin (lipopolysaccharide, LPS), which do not induce sepsis, cause a set of psychiatric changes called sickness behavior including fatigue, anorexia, anhedonia, depressed mood and apathy (i.e. loss of interest in a social environment) [1,2]. These behavioral changes seem to be phenotypes shared with depressive disorder [1,3], and are also observed in other inflammation-related states, such as cancer (with chemotherapy), autoimmune disease and diabetes in addition to most cases of infection [1,4,5]. Because these phenotypes reflect changes in brain function, studies on the underlying mechanisms contribute to our understanding of immune system-to-brain communication and the development of a therapy for psychiatric disturbance [1,6].

A number of papers have demonstrated decreases in locomotor activity, food intake and social interaction behaviors in rodents intraperitoneally (i.p.) administered LPS, an animal model of sickness behavior [7–14], while others have reported depressive-like behaviors such as decreased escape behaviors (increase of immobility) in the forced-swim test (FST) and tail-suspension test (TST), decreased sucrose preference [3,13,15], and deficits in several types

Abbreviations: LPS, lipopolysaccharide; FST, forced-swim test; TST, tail suspension test; NTS, nucleus of the solitary tract; BST, bed nucleus of the stria terminalis; CeA, central amygdala; PVN, paraventricular nucleus of the hypothalamus.

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and processes of memory function [16–19]. In most studies, these behavioral changes were evaluated within 6 h of LPS administration, when a profound decrease in locomotor activity by LPS was still observed. However, a recent study has shown that LPS induces depressive-like behaviors both at 6 and 24 h after injection, despite the decrease in locomotor activity no longer being observed at 24 h postinjection [13]. In addition, LPS is known to affect the consolidation of hippocampal-dependent memory in a process that is generally regarded to be independent of its effect on locomotor activity [16]. Thus, LPS has been suggested to affect emotional and cognitive behaviors independently of the decrease in locomotor activity [13,16].

On the other hand, the decrease in social interaction is clinically important because it is a well-known measure in other rodent models, having predictive and face validity for several psychiatric disorders including depression, autism and schizophrenia [20,21]. To understand the specific defects in social interaction, comprehensive examinations of not only social interaction but also "object exploration" were performed [22,23]. No papers have demonstrated the changes in exploratory behaviors for an "object" in rodents administered LPS i.p., although one study showed that intracerebroventricular injection of LPS caused significant decreases in both object and social investigations [24].

With this background, we hypothesized that mice i.p.-injected with LPS would also show decreased novel object exploration, and that there would be appropriate conditions (time point, dose and strain of mice) under which to specifically evaluate changes in novel-object exploration. To test these hypotheses, we first determined the time- and dose-dependent expression profiles of five forms of sickness behaviors, including novel-object exploration in BALB/c mice, and tried to characterize the specific effects of LPS on novel object exploratory behaviors. Because sickness behavior has been suggested to be associated with neuronal activation in the nucleus of the solitary tract (NTS) and limbic structures such as the bed nucleus of the stria terminalis (BST), the central amygdala (CeA) and the paraventricular nucleus (PVN) of the hypothalamus through LPS-induced activation of afferent fibers of the vagal nerve in this model [25,26], we also examined temporal changes in the expression of c-Fos, a marker of neuronal activation, in these brain regions, to understand the neuroanatomical basis of the behavioral changes in LPS-treated mice.

2. Materials and methods

2.1. Animals and reagents

All animal care and handling procedures were performed according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the Animal Care and Use Committee of Graduate School of Pharmaceutical Sciences, Osaka University. Male 7-week-old BALB/c (BALB/cCrSlc) mice were purchased from Shimizu Laboratory Supplies, Kyoto, Japan and were group-housed (4–5 per cage) with a 12-h light–dark cycle (light on at 8:00 am) at controlled room temperature (22 ± 1 °C) for 1 or 5 weeks in our animal facility. Each mouse was housed individually for one day before experiments, except for the experiment assessing the curiosity-driven exploration of a novel object (Fig. 3A). Pelleted food (CMF, Oriental Yeast, Osaka, Japan) and water were available ad libitum. LPS (*Escherichia coli* serotype 0127:B8) was purchased from Sigma–Aldrich (Tokyo, Japan), dissolved in sterile saline, and injected intraperitoneally (0.3, 0.5, 1.0, 3.0, or 5.0 μg per mouse).

2.2. Behavioral analysis

Each behavioral study was performed using a separate cohort of mice. Locomotor activity was measured for 30 min immediately after each mouse (time course study, n = 7–10 per group; dose–response study, n = 8–11 per group) was placed in an observation cage (28 cm length × 20 cm width × 12 cm height) using a digital counter system with an infrared sensor (Supermex, Muromachi Kikai Co., Tokyo, Japan). Food intake was measured every 8 h or at 24 h after injection of LPS by subtracting the weight of any uneaten pellets (which remained on the cage lid and fell into the cage) from the premeasured weight of pellets (time course study, n = 7–8 per group; dose–response study, n = 9–10 per group). A FST was performed as previously described [27,28], with minor modifications. Mice were forced to swim individually in a vertical glass cylinder (height 30 cm, diameter 18.5 cm) filled with water maintained at 24–26 °C to a depth of 13 cm (time course study, n = 10–11 per group; dose–response study, n = 8–9 per group). Six minutes after testing in the water, mice were removed and allowed to dry in a heated enclosure. The duration of immobility (making only minimal movements to keep the head above water or floating) in the FST was measured from videotapes by a trained observer blinded to the treatment. For social interaction and object exploration tests, each mouse was first placed in an observation cage (28 cm × 20 cm × 12 cm) and allowed 15 min of habituation under dim light (40 lx). After the habituation period, a male 3-week-old BALB/c mouse (BALB/cCrSlc, obtained from Shimizu Laboratory Supplies and housed in our animal facility for more than 1 week before the experiment) or a novel object (a wooden ball; diameter 5 cm) was placed in the center of the cage, and behaviors were videotaped for 5 min. We chose this observation period because the duration of object exploration in saline-treated mice was high during the first 5 min and dropped thereafter (data not shown), suggesting that this duration reflects brain function including novel object-evoked curiosity, and because the response could be easily evaluated during this period. Duration of social interaction behaviors (sniffing, licking or following the juvenile) was measured from videotapes by a trained, blinded observer (time course study: n = 8–11 per group; dose–response study: n = 7–8 per group). Durations of object exploratory behaviors to an object (sniffing or licking) and non-object exploratory behaviors (ambulation or rearings), and overall exploration duration (time not engaged in resting), were measured in the same way (time course study: n = 7–8 per group; dose–response study: n = 8–9 per group). To assess curiosity-driven exploration of a novel object, we investigated the behaviors of mice exposed to the same or different objects in the test session, as shown in Fig. 3A (n = 7–8 per group). In this experiment, we first housed mice with the wooden ball (the same object) or a metal object (7 cm × 3 cm × 2 cm, a different object) for 24 h; then, after LPS injection, the objects were removed from the home cage and mice from each group were subjected to the object exploration test using the wooden ball at 10 h postinjection. When both objects were simultaneously presented to naïve BALB/c mice for 5 min, mice explored the metal object for 67.0 ± 3.4 s and the wooden object for 64.8 ± 6.5 s (n = 3, P = 0.789, Student's t-test), suggesting that they exhibited almost equal interest in both objects. Open-field and elevated plus maze tests were performed at 10 h after injection (open field experiment: n = 4 per group; elevated plus maze experiment: n = 6 per group) as described previously [29,30]. Time spent in the center region (21 cm × 21 cm) of the open-field apparatus (45 cm × 45 cm × 30 cm) and the number of open-arm entries and duration in the open-arms of the elevated plus maze during 5-min sessions were counted.

2.3. Immunohistochemistry

Immunohistochemistry for c-Fos was performed as described previously [31]. Briefly, mice were i.p. injected with LPS and placed back into home cages. At 2, 6, 12 or 24 h after injection, mice were deeply anesthetized with 50 mg/kg pentobarbital, and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate-buffered saline. Whole brains were dissected and postfixed in the same fixative overnight at 4 °C. Then, brain blocks were cryoprotected in 20% sucrose in phosphate-buffered saline for 48 h at 4 °C. For c-Fos staining, coronal brain sections (20 μm) containing the BST (at 0.26 mm from the bregma), the PVN (at −0.82 mm from the bregma), the CeA (at −0.94 mm from the bregma), and the NTS (at −7.20 mm from the bregma) were prepared, and processed for immunohistochemistry with anti-c-Fos rabbit polyclonal primary antibody (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA, 1:2000 dilution) and biotin-labeled anti-rabbit IgG secondary antibody (Nichirei, Tokyo, Japan). The number of c-Fos-positive cells in a 1 mm × 1 mm region was counted manually by experienced observers blinded to the treatment (n = 3–5 per group).

2.4. Statistical analysis

All the data are expressed as means + SEM. Statistical evaluation was carried out with Statview software (SAS Institute Japan Ltd., Tokyo, Japan). The statistical significance of differences was assessed using the Student's t-test, Dunnett's test, repeated two-way ANOVA, or two-way ANOVA followed by the Tukey–Kramer test. The threshold for statistical significance was defined as $P < 0.05$. Because significant effects of age were observed in some data, we only evaluated data from 8-week-old mice in most experiments. Data from 12-week-old mice is shown only in Fig. 1F (33% of mice) and 2B (48% of mice), because two-way ANOVA revealed no significant effects of age in these data sets ($F_{1,46} = 0.22$, $P = 0.64$ in Fig. 1F and $F_{1,36} = 1.28$, $P = 0.27$ in Fig. 2B).

3. Results

3.1. Time- and dose-dependency of LPS-induced sickness behavior

The temporal expression patterns and dose–response relationships of LPS-induced sickness behavior were determined in BALB/c mice at our animal facility (Fig. 1). Five micrograms of LPS