Day–Night Variations of Behavioral and Autonomic Thermoregulatory Responses to Lipopolysaccharide in Rats

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Abstract: This study investigated the day–night differences in behavioral and autonomic thermoregulatory responses to bacterial lipopolysaccharide (LPS) in rats. Male rats were housed individually in cages with a 12:12 h light dark cycle at an ambient temperature of 24°C. The rats were placed in a box with a temperature gradient and intraperitoneally injected with LPS (10 µg/kg). The preferred ambient temperature (T_{pr}) was estimated by the location of the rats in the box, and intraperitoneal temperature (T_b) was measured by a biotelemetry system. Measurements were taken during the light and dark phases of the day. LPS produced fever in both phases. The magnitude of rise in T_b did not differ between the two periods. In the dark phase, T_{pr} significantly increased during the development of fever and decreased during the defervescence,

while it did not change throughout the febrile course during the light phase. In a separate experiment, rats were loosely restrained and placed in a direct calorimeter. Their colonic temperature (T_{col}), evaporative and nonevaporative heat loss and heat production were measured before and after intraperitoneal injections of LPS (10 µg/kg). Measurements were taken during the light and dark phases of the day. LPS induced fever in both phases. The magnitude of change in T_{col} , heat loss, and heat production due to LPS did not differ between the two periods. These results suggest that the febrile response of rats to intraperitoneal LPS is not affected by the time of day. However, it seems that during LPS-induced fever, thermoregulatory behavior is not fully activated during the light phase of the day. [Japanese Journal of Physiology, 46, 451–456, 1996]

Key words: fever, preferred ambient temperature, heat loss and production, calorimetry.

Fever is a unique event occurring in the thermoregulatory system. It has been shown that febrile response to bacterial endotoxin is modified by various physiological and pathological conditions of the host animals such as sex [1, 2], age [1, 3], body size [2, 4], hydration [5, 6], and nutrition [7, 8]. For instance, a 60-h fast attenuates bacterial lipopolysaccharide (LPS)-induced fever due to a reduction of nonshivering thermogenesis in guinea pigs [8], whereas a 24-h dehydration enhances LPS-induced fever in rats [6]. The thermoregulatory function of mammals has also been demonstrated to vary according to the time of day [9–15]. However, it is not yet clear whether there are nycthemeral variations in thermoregulatory responses to pyrogens. The first objective of this study was, therefore, to investigate this issue.

Endothermic animals use both behavioral and autonomic modes of effector response to achieve and maintain a stable core body temperature. Thus, total thermoregulatory response should be analyzed giving consideration to behavioral and autonomic components. Thermoregulatory behavior can be studied by the observation of preferred ambient temperature (T_{pr}) [10, 16–25] in various classes of vertebrates. Indeed, febrile animals prefer a higher ambient temperature to raise their core body temperature [10, 16, 22–26]. The second objective of this study was to assess the day–night differences in behavioral and autonomic thermoregulatory responses to LPS by examining the changes in T_{pr} and heat balance during LPS-induced fever.

Received on May 20, 1996; accepted on October 11, 1996

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METHODS

Animals. Male rats (Std: Wistar/ST) with weights of 320–340 g were used. All animals were housed individually in wire mesh cages maintained at an ambient temperature of 24°C and had access to food and water *ad libitum*. Lighting was controlled automatically on a 12:12 h cycle (light on 0800–2000). The animals used in this study were maintained in compliance with the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences,” published by The Physiological Society of Japan.

Preferred ambient temperature during LPS-induced fever. A temperature-gradient box was constructed from 0.78-cm-thick aluminum, with the interior dimensions of 200.0 (length) × 12.0 (width) × 12.0 (height) cm. Position sensors activated by an infrared light source (PZ-41, KEYENCE, Osaka, Japan) were located along the length of the temperature-gradient box at 10 cm intervals. The ends of the box were maintained at 18.2 and 35.3°C, which resulted in air temperatures ranging from 19.5 to 33.5°C. The gradient was established almost perfectly. AM receivers (RLA3000 and RMX10, Data Sciences, St Paul, MN, USA) were attached to the temperature-gradient box at 25 cm intervals and were interfaced to a personal computer (PC-9801VM, NEC, Tokyo, Japan).

At least 10 d before measurements, the rats were intraperitoneally anesthetized with pentobarbital sodium (50 mg/kg) and a temperature transmitter (TA10TA-F40, Data Sciences) implanted in the peritoneal cavity. The rats were then adapted to the temperature-gradient box for 4 d before the measurements.

Measurements were taken both during the light and dark phases of the day using 7 rats in each period. The rats were placed in the temperature-gradient box at 0900 (light phase) or 2100 (dark phase). Two hours later, the animals were injected with either LPS (0111:B4, Difco Labs, Detroit, MI, USA) suspended in saline at a dose of 10 µg/kg or saline alone, and then spent the following 7 h in the temperature-gradient box. Before and after LPS or saline injection, intraperitoneal temperature (T_b) was measured every minute using a biotelemetry system. The location of the rats in the temperature-gradient box was also detected every minute, and their T_{pr} was estimated by the calibration curve of the air temperature as a function of the location. Three days later, the rats were again placed in the temperature-gradient box at the same time of day as that for the first measurements. Then, the rats which had been injected with LPS were injected with the same volume of saline, and those

which had been injected with saline were injected with LPS. Each animal was given only one injection of LPS and saline.

Heat balance during LPS-induced fever.

Three days before the measurements, the rats were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg) and a polyethylene catheter (0.5 mm ID, 0.9 mm OD: Imamura, Tokyo, Japan) implanted in the intraperitoneal cavity. The catheter was filled with pyrogen-free saline, and the free end was sealed. The catheter was passed subcutaneously to a point at the midscapular level behind the neck and maintained there until the measurements were completed. Before the catheter surgery, the rats were loosely restrained in individual cylindrical wire mesh cages (7 cm diameter, 15 cm long) for 8 h every day for at least 6 d before the first surgery to habituate to the experimental condition.

Measurements were taken both during the light and dark phases of the day using 6 rats in each period. Each animal was restrained in the cylindrical wire mesh cage and put into a gradient-type direct calorimeter [27] at 1000 (light phase) or 2200 (dark phase). One hour later, they were injected with LPS at a dose of 10 µg/kg intraperitoneally through the catheter. Colonic body temperature (T_{col}) was recorded by a fine copper constantan thermocouple inserted 5 cm into the colon. Fresh dry air was introduced into the direct calorimeter at a constant rate of 1.8 l/min. A fraction of the air (100 ml/min) withdrawn from the chamber was sent into a Zirconia O₂ analyzer (LC-700E, Toray, Tokyo, Japan), and O₂ consumption was calculated from measurements of O₂ content and air flow. Metabolic heat production (M) was calculated by multiplying the O₂ consumption times the caloric equivalent for O₂ (4.823 kcal/l). Nonevaporative heat loss ($R+C+K$) was measured by the direct calorimeter, and evaporative heat loss (E) was calculated from the airflow and humidity of the air, which was monitored by a humidity sensor (HMI-11, Vaisala, Helsinki, Finland). Total heat loss (HL) was estimated as $(R+C+K)+E$. Heat storage (S) was estimated as $M-HL$. These recordings were done every minute using a personal computer (PC-9801-VX, NEC) through an intelligent A/D converter (ADC-12IB, Kanazawa Control Kiki, Kanazawa, Japan).

Data analysis. The results are presented as means ± SE. A two-factor repeated-measure analysis of variance was used for comparisons between the groups. A post hoc analysis was done using Scheffe's F test. The critically significant level was set at $p < 0.05$.

RESULTS

Preferred ambient temperature during LPS-induced fever

The initial values of T_b just before LPS or saline injection (37.80 ± 0.19 , 37.91 ± 0.18 , respectively) during the dark phase were significantly higher than those (36.75 ± 0.27 , 36.78 ± 0.25 , respectively) during the light phase. In accordance with previous reports [17, 28], the initial values of T_{pr} just before LPS or saline injection (22.3 ± 1.22 , 22.5 ± 1.14 , respectively) during the dark phase were significantly lower than those (28.6 ± 1.63 , 28.6 ± 2.85 , respectively) during the light phase.

The changes in T_b (ΔT_b) and T_{pr} (ΔT_{pr}) from the initial values after the injection of LPS during the light

and dark phases of the day are shown in Fig. 1. The intraperitoneal injections of LPS significantly increased T_b in both the light and dark phases. The changes in T_b were not affected by the time of day. T_{pr} varied significantly after the LPS injection during the dark phase, while the LPS injection had no significant effect on T_{pr} during the light phase. In the dark phase, T_{pr} increased gradually between 0.5 and 3.5 h after the LPS injection, the period corresponding to fever development. Then, T_{pr} decreased rapidly and reached a level below the initial value 5 h after the injection of LPS. It was noted that a decrease in T_{pr} preceded defervescence. The ΔT_{pr} levels during the dark phase were significantly higher than those during the light phase between 1.5 and 4 h after the administration of LPS, and significantly lower 5 h after the LPS injection. The saline injection did not affect T_b and T_{pr} in either phase of the day.

Heat balance during LPS-induced fever

The initial levels of T_{col} , HL, and M ($38.79 \pm 0.07^\circ\text{C}$, $56.78 \pm 1.78 \text{ W/m}^2$, $57.42 \pm 2.41 \text{ W/m}^2$, respectively) during the dark phase were significantly higher than those ($38.34 \pm 0.15^\circ\text{C}$, $52.24 \pm 1.38 \text{ W/m}^2$, $51.24 \pm 2.58 \text{ W/m}^2$, respectively) during the light phase. The initial values of S were not different between the dark and light phases of the day (0.64 ± 2.02 , $-1.00 \pm 1.76 \text{ W/m}^2$, respectively).

The changes in T_{col} (ΔT_{col}) from the initial values after the intraperitoneal injection of LPS during the light and dark phases of the day are illustrated in Fig. 2. T_{col} changed significantly after the LPS injection regardless of the time of day. However, there was no significant difference in ΔT_{col} between the two phases of

Fig. 1. Changes in abdominal body temperature (T_b) and preferred ambient temperature (T_{pr}) from the initial values after LPS injection during the light (○: $n=7$) and dark (●: $n=7$) phases of the day. Data are plotted every 30 min. Values are means $\pm$ SE. * Significant difference between the light and dark phases of the day.

Fig. 2. Changes in colonic body temperature (T_{col}) from the initial values after LPS injection during the light (○: $n=7$) and dark (●: $n=7$) phases of the day. Data are plotted every 30 min. Values are means $\pm$ SE.

the day. Figure 3 shows the changes in M (ΔM), total heat loss (ΔH), and S (ΔS) from the respective initial values after the LPS injection during the light and dark phases of the day. The LPS injection had significant effects on these parameters. ΔM and ΔH during the light phase of the day tended to be higher than those during the dark phase. However, the changes of heat balance (e.g., ΔS) did not differ between the light and dark phases of the day.

Fig. 3. Changes in total heat loss (HL), metabolic heat production (M), and heat storage (S) from the initial values after LPS injection during the light (○: $n=7$) and dark (●: $n=7$) phases of the day. Data are plotted every 30 min. Values are means $\pm$ SE.

DISCUSSION

This study confirmed that, in rats, intraperitoneal injections of LPS at a dose of 10 μ g/kg produced fever both during the light and dark phases of the day. The levels of core body temperature were consistently higher in the dark phase than in the light phase during the febrile course. However, the magnitude of change in core body temperature after the LPS injection did not differ according to the time of day either when the rats were allowed to move freely (Fig. 1) or when they were restrained. Thus, it seems that febrile response to intraperitoneal LPS in rats is not affected by the time of day.

Feng *et al.* [9] showed that changes in core body temperature after central injections of prostaglandin E_2 , a possible mediator of fever, were a function of the day in rats. They, however, did not show that thermoregulatory responses after peripheral injections of pyrogens depend on the time of day. Recently, Watkins *et al.* [29] observed that subdiaphragmatic vagal transection disrupts the rise in core body temperature after the intraperitoneal injection of interleukin-1. Their results suggest the importance of vagal afferents from the intraabdominal region in transducing febrile signals from the periphery to the center when pyrogens are administered into the peritoneal cavity. Thus, the mechanism of fever induction appears to be rather different between the central and intraperitoneal applications of pyrogens. The results reported here cannot be directly compared with the observations made by Feng *et al.* [9].

In this study, during the dark phase, the T_{pr} increased during the period of fever development, and decreased during defervescence (Fig. 1). As is well documented [10, 16, 22–26], these changes in T_{pr} indicate the occurrence of behavioral thermoregulation for gaining or dissipating heat to adjust core body temperature to a target level. However, such behavior did not take place here in the light phase of the day during LPS-induced fever.

The difference in behavioral thermoregulation between the light and dark phases may be interpreted simply in two ways: 1) autonomic thermoregulation was primarily induced by LPS during the light phase, while behavioral thermoregulation was rarely induced during the light phase and mainly brought about by LPS during the dark phase, or 2) thermoregulation depended exclusively on the autonomic system during the light phase, whereas it depended on the behavioral system during the dark phase. However, there is substantial evidence showing that both effector systems can be activated in rats by various thermal stimuli re-

ardless of the time of day. For instance, Szymusiak *et al.* [30] reported that behavioral thermoregulation to escape cold can be brought about during the light phase. In our experiments, autonomic thermoregulatory responses to LPS during the dark phase did not differ from those during the light phase when movement was not allowed (Figs. 2 and 3). Thus, the observations presented here indicate that the predominant effector mode of thermoregulatory response to intraperitoneal LPS may be behavioral during the dark phase and autonomic during the light phase. It seems, therefore, that the dark phase is preferable to investigate behavioral thermoregulation in rats.

In the thermal-gradient box, high levels of T_b were maintained for more than 5 h after the intraperitoneal injection of LPS (Fig. 1). This febrile course was consistent with that of the foregoing reports in freely moving rats [31–33]. When the rats were restrained in cylindrical cages, however, changes in core body temperature after LPS injections were obviously different from those in the freely moving rats, (i.e., defervescence started 3 h after the LPS injection) (Fig. 2). It has been shown that restraint modifies a febrile response to i.v. IL-1 and LPS in rats [31]. Thus, the difference in febrile responses to LPS between the two experiments might have been caused by the difference in the level of restraint. This mechanism remains to be investigated.

We are grateful to A. Yachi and K. Kitai for their technical assistance. This study was supported by the Grants-in-Aid for Scientific Research (05557005, 06404018, 05670064, and 07557186) from the Ministry of Education, Science, Sports and Culture of Japan.

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