**Escherichia coli** lipopolysaccharides produce serotype-specific hypothermic response in biotelemetered rats

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Akarsu ES, Mamuk S. *Escherichia coli* lipopolysaccharides produce serotype-specific hypothermic response in biotelemetered rats. *Am J Physiol Regul Integr Comp Physiol* 292: R1846–R1850, 2007. First published February 1, 2007; doi:10.1152/ajpregu.00786.2006.—We investigated whether LPS-induced hypothermia develops in a serotype-specific manner in biotelemetered conscious rats. Two different *Escherichia coli* serotypes of LPSs were injected at a dose of 250 μg/kg ip. *E. coli* O55:B5 LPS elicited an initial hypothermia and subsequent fever, but *E. coli* O111:B4 LPS caused more potent monophasic hypothermia. Serum tumor necrosis factor (TNF-α) levels were dramatically elevated at the initial phase of the hypothermia induced by both LPSs. This elevation tended to subside at the nadir of *E. coli* O55:B5 LPS-induced response but progressively increased at the nadir of *E. coli* O111:B4 LPS hypothermia. Serum IL-10 levels were moderately elevated at the initial phase of the hypothermia and persisted at the same level at the nadir of each LPS-induced response. No change was observed at the serum IL-18 levels. A selective cyclooxygenase (COX)-1 enzyme inhibitor, valeryl salicylate (20 mg/kg sc), abolished the hypothermia without any effect on the elevated cytokine levels. Another COX-1-selective inhibitor, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560; 1 mg/kg sc) inhibited hypothermic responses as well. Meanwhile, cytokine levels were also reduced by SC-560 treatment. These findings suggest that LPS-induced hypothermia may have serotype-specific characteristics in rats. *E. coli* O111:B4 LPS has more potent hypothermic activity than *E. coli* O55:B5 LPS; that may presumably be related to its higher or sustained capability to release antipyretic cytokines, such as TNF-α. COX-1 enzyme may be involved in the generation of the hypothermia, regardless of the type of LPS administered.

acute-phase response; fever; endotoxin; antipyretic cytokines; cyclooxygenase-1 selective inhibitors

LPS is a component of the outer membrane of gram-negative bacteria. Systemic LPS injection to experimental animals elicits a multisystem response which includes immune, endocrine, behavioral, and metabolic components known as the acute-phase response (13, 17). This is a nonspecific defense reaction of the organism to noxious stimuli such as LPS-induced immunological challenge. As a thermoregulatory symptom of the acute-phase response, LPS commonly causes fever in laboratory animals such as rabbits or guinea pigs. But, rats and mice respond to LPS by hypothermia with or without fever (15, 16).

Several reasons have been put forward to explain this species-specific thermoregulatory response in those rodents, the most crucial one being the dose of LPS.

It has been reported that systemic injection of a relatively high dose of LPS (such as 500 μg/kg or higher) increases the possibility for development of a hypothermic response, rather than fever, at the ambient temperatures below 30°C in rats (3, 24). Intravenous route of administration seems to result in more pronounced hypothermia (26). Thus, it has been assumed that the hypothermia could be a secondary response that is related to the cardiovascular toxicity, such as hypotension or a shocklike state leading to passive heat loss through skin vasculature due to a high dose of LPS (8, 25). Meanwhile, several experimental data have shown that hypothermia does not always occur during LPS-induced hypotensive response (9, 18), or hypothermia can be seen without any decrease in blood pressure (30). Furthermore, hypothermia may also develop by injecting lower LPS doses (such as 5.0–12.5 μg/kg) that do not cause systemic toxicity in rats (12, 19, 22, 31). It has been demonstrated that reduced thermogenesis and cold-seeking behavior may account for the mediation of LPS-induced hypothermia, implicating that this hypothermia may be a regulated adaptive strategy (like fever) against immunological challenge (2, 3). It appears that hypothermia and fever may develop independently, and the pattern of the response (i.e., fever, hypothermia, or both) is determined by the intensity of the signals leading to each response in rats (6, 24).

The other critical parameter that determines the pattern of the thermoregulatory response is the serotype of the bacteria from which the LPS was isolated. We previously showed that the pattern of the LPS-induced thermoregulatory changes may have serotype-specific characteristics, and some LPSs, such as *Escherichia coli* O111:B4 LPS, have a higher hypothermic efficiency, whereas *E. coli* O55:B5 LPS is a better pyrogen (4). Presumably, related to this observation, the pharmacological characteristics of the fever have also varied depending on the LPS serotype administered. For example, 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonyamide (SC-58236), a cyclooxygenase (COX)-2 selective inhibitor, completely inhibits the fever induced by *E. coli* O55:B5 LPS, but the same drug is not effective for the initial phase of fever induced by *E. coli* O111:B4 LPS, suggesting a possible diversity of the activated signaling pathways depending on the serotype of LPS (1, 5). These findings may give us critical information not only for the methodological validation of LPS-induced thermoregulatory changes but also for an explanation of possible mechanisms of these responses from a pathophysiological point of view.

Systemic LPS administration activates the immune system and leads to release of endogenous proinflammatory cytokines. In a concerted and interdependent sequence, the circulating proinflammatory cytokines stimulate the synthesis of other inflammatory or anti-inflammatory cytokines. Some of those
may affect the body temperature. IL-1β and IL-6 have been suggested as endogenous pyrogenic cytokines, whereas IL-10 and IL-18 may have a function as an antipyretic to limit the magnitude of fever (10, 15, 19). On the other hand, tumor necrosis factor (TNF-α) has pyrogenic, antipyretic, and hypothermic actions (20). Thus, it may be proposed that differential regulation of the cytokine release may determine the pattern of the LPS-induced body temperature changes.

Because LPS-induced hypothermia may be regarded as a thermoregulatory symptom of LPS-induced acute-phase response, we decided to evaluate whether LPS-induced hypothermia has serotype-specific characteristics. Thus, we compared the body temperature changes induced by two different E. coli serotypes of LPSs, which are commonly used ones for experimental purposes, at a dose (250 μg/kg ip) that preferentially produces hypothermia in rats (4). We chose biotelemetry technology for body temperature recording to eliminate stress-induced changes due to body temperature measurement. Alterations in serum levels of putative endogenous antipyretic cytokines such as TNF-α, IL-10, and IL-18 at the initial phase and at the nadir of LPS-induced hypothermic responses have been evaluated together with the effects of COX-1-selective inhibitors, valeryl salicylate and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560), on the hypothermia and related cytokine elevations.

MATERIALS AND METHODS

Animals. Adult male Wistar albino rats weighing 220–270 g were used. They were fed standard rat chow and water ad libitum and housed in an air-conditioned room at an ambient temperature 21 ± 1°C under a 12:12-h light-dark cycle (lights on at 7:00 AM). Rats were housed one per cage in macrolon cages. Each rat was used only once for experiments.

Body temperature measurement and surgical procedure. The abdominal temperature (T_ab) was recorded biotelemetrically (Mini-Mitter, Bend, OR). At least 1 wk before the day of experiment, a temperature transmitter (model VM-FH 3000), the output signal of which is frequency modulated by its own temperature, was implanted into the peritoneal cavity of each rat under general anesthesia (ketamine, 80 mg/kg ip) that preferentially produces hypothermia in rats (4). We chose biotelemetry technology for body temperature recording to eliminate stress-induced changes due to body temperature measurement. Alterations in serum levels of putative endogenous antipyretic cytokines such as TNF-α, IL-10, and IL-18 at the initial phase and at the nadir of LPS-induced hypothermic responses have been evaluated together with the effects of COX-1-selective inhibitors, valeryl salicylate and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560), on the hypothermia and related cytokine elevations.

Cytokine assay. Separate groups of rats (not used for T_ab recording experiments) were used. Blood samples were taken by intracardiac puncture under light diethyl ether anesthesia. The samples were kept at 4°C for 1 h, centrifuged (10,000 rpm, 5 min), and then the sera were separated and stored at −70°C. Cytokine levels were determined by using ELISA. Rat-specific ELISA kits for TNF-α, IL-10, and IL-18 were purchased from Biosource (Camarillo, CA). The detection limits of the assay were 4, 5, and 4 pg/ml for TNF-α, IL-10, and IL-18, respectively.

Compounds and drugs. Phenol-extracted LPSs of E. coli serotype O55:B5 (lot 56H4096, Sigma) and serotype O111:B4 (lot 76H4099, Sigma) were used. LPSs were aliquoted as a stock solution of 2 mg/ml under aseptic conditions and kept at −20°C until use. LPS was further diluted with sterile apyrogenic saline solution. COX-1-selective inhibitors valeryl salicylate (VS; 2-valeroyloxybenzoic acid) and SC-560 (both from Cayman Chemical, Ann Arbor MI) were suspended in 0.9% benzyl alcohol solution in physiological saline (vehicle) and injected at a dose of 20 mg/kg VS or 1 mg/kg SC-560 subcutaneously.

Experimental protocols. All experiments were carried out at an ambient temperature of 25 ± 1°C. The experiments were started at 9:00 AM. Before any treatment, rats were allowed to settle down and attain a steady T_ab. The average T_ab for 30 min preceding the treatment was calculated as the baseline T_ab. LPS (250 μg/kg ip) and/or drug injections were made between 11:00 AM and 12:00 PM. Drug and LPS injections were made at the same time point. During injections, rats were gently handled for 10–15 s. The body temperature data were evaluated as T_ab changes for every 5 min for 10 h after injection and expressed as a difference from the baseline value of each rat (∆T).

Blood samples of LPS, or LPS+drug-injected biotelemetered rats (other than those used for T_ab recordings), were taken at the initial phase or at the nadir of the hypothermia. Control samples were collected at the intervals corresponding chronologically to the initial phase (60–80 min after treatment) or to the nadir (100–110 min after injection) of the hypothermic response. Cytokine assay was carried out for three respective cytokines in the same serum sample.

The local ethical committee for Animal Care and Use of Ankara University School of Medicine approved all protocols.

Statistical analysis. ∆T values were expressed as means ± SE. Multiple comparisons of the T_ab curves were made by one-way ANOVA with a Newman-Keuls post hoc test. Significance was noted when P < 0.05. Cytokine values were analyzed by nonparametric statistical tests (Kruskal-Wallis and Mann-Whitney U-tests). The α value was corrected by using Bonferroni procedure for multiple comparisons.

RESULTS

Control values. The T_ab of vehicle (sc) + saline (ip)-injected rats was taken as control. The average baseline T_ab of the control group was 37.4 ± 0.2°C. The basal T_ab of the other treatment groups were not statistically different than the baseline T_ab of the control. There was no change in the T_ab of control rats after vehicle + saline injection for about 360 min. Then, the T_ab increased due to circadian rhythm (~1°C until the end of the observation period (600 min). In some of the experimental group, such as the saline + VS-treated group, T_ab of the rats was substantially increased in the first 30 min after injection. This transient increase in T_ab was regarded as stress response induced by handling and injection procedure.

Effects of LPSs on T_ab and serum antipyretic cytokine levels.

The injection of E. coli O55:B5 LPS (250 μg/kg ip) produced dual T_ab changes [F (2,357); 20.64; P < 0.0001 vs. vehicle+saline-injected group]. The T_ab began to fall 40 min after injection and reached its nadir in 45 min (∆T: −1.4 ± 0.24°C). The T_ab returned to baseline values 130 min after injection. Then, a pyrogenic response was observed about 300 min after LPS administration, and the rats remained febrile for 2 h (∆T: 1.4 ± 0.11°C), (Fig. 1). There was a dramatic elevation in serum TNF-α levels at the initial phase of the hypothermia (range: 330–50,686 pg/ml). This elevation tended to subside at the nadir; but the level was significantly higher than the control (range: 192–21,740 pg/ml; Table 1, treatment B). Serum IL-10 levels were also elevated at the initial phase of the hypothermia and at the nadir (range: 50–454 pg/ml and range: 23–474 pg/ml, respectively; Table 1, treatment B). There was no difference in either time marks. Serum IL-18 levels did not change in either phase of the response (Table 1, treatment B).

E. coli O111:B4 LPS (250 μg/kg ip) elicited a monophasic hypothermic response. T_ab decreased 40 min after injection and reached a significantly lower nadir (∆T: −2.1 ± 0.2°C; P <
Fig. 1. Changes on the $T_{ab}$ following intraperitoneal injection of 250 $\mu$g/kg E. coli O55:B5 or E. coli O111:B4 serotype LPS. The basal $T_{ab}$ values for LPS-injected groups were 37.6 ± 0.1°C and 37.5 ± 0.1°C, respectively. All injections were made at the time 0, indicated by the arrow. Each point represents the mean ± SE of the specified observations. *$P < 0.05$ compared with vehicle (+veh) + saline (sal)-injected group. The significantly different points of each curve were indicated as follows: ---, E. coli O55:B5 LPS group; - - -, E. coli O111:B4 LPS group.

Fig. 2. The effects of COX-1-selective inhibitor treatments [20 mg/kg sc valeryl salicylate (VS) or 1 mg/kg sc 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560)] on E. coli O55:B5 LPS-induced $T_{ab}$ changes. The basal $T_{ab}$ values for LPS + SC-560-, LPS + VS-, and saline + SC-560-injected groups were 37.5 ± 0.15°C, 37.4 ± 0.12°C and 37.7 ± 0.24°C, respectively. All injections were made at the time 0, indicated by the arrow. Each point represents the mean ± SE of the specified observations. For graphical clarity, the error bars of the saline + SC-560 treatment group were omitted. *$P < 0.05$ compared with vehicle + saline-injected group in Fig. 1. The significantly different points of each curve were indicated as follows: ---, LPS + VS group; - - -, LPS + SC-560 group.

Table 1. Changes in serum cytokine levels at the initial phase or at the nadir of the hypothermic responses of two different Escherichia coli serotypes LPSs and the effects of COX-1 selective inhibitors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNF-α, median, pg/ml</th>
<th>IL-10, median, pg/ml</th>
<th>IL-18, median, pg/ml</th>
</tr>
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The number of samples that are above detection limits are indicated in brackets. For statistical analysis, cytokine values after saline treatment were pooled as a single control group. ND, not determined. *$P < 0.05$ compared to vehicle + saline; †$P < 0.05$ compared to E. coli O55:B5 LPS (nadir); $\ddagger P < 0.05$ compared to initial phase; $\ddagger P < 0.05$ compared to E. coli O111:B4 LPS (initial).
LPS-INDUCED HYPOTHERMIA IN RATS

Our data show that LPS-induced hypothermia may have serotype-specific characteristics in rats. It appears that *E. coli* O111:B4 LPS is more capable of stimulating TNF-α release, which may result in a more potent hypothermic response. Meanwhile, COX-1 selective inhibitors effectively suppress the response regardless of the LPS administered.

It has been hypothesized that systemically injected LPS initially interacts with the peripheral immune-competent cells (such as macrophages), and subsequently, the release of various proinflammatory cytokines occurs. Those cytokines trigger multiple biological signaling mechanisms in a pleiotropic manner. It has been proposed that TNF-α, a proinflammatory antipyretic/pyretic cytokine, is predominantly responsible for the development of LPS-induced hypothermia (16, 20). Our data generally support this proposition, because serum TNF-α values were significantly elevated at the initial phase of the LPS-induced response, and the magnitude of the hypothermia was related to the progressively sustained TNF-α elevation. Furthermore, decrease of serum TNF-α levels also abolished the hypothermia.

However, recent experimental data suggest that TNF-α may not be the sole antipyretic cytokine that is responsible for the mediation of LPS-induced hypothermia, largely based on the findings that TNF-α does not fully mimic the thermoregulatory response of LPS in rats, and LPS-induced hypothermia can be observed in the absence of TNF-α release (26, 29). In this connection, we evaluated the potential contribution of another proinflammatory antipyretic cytokine, namely IL-18, for the mediation of the LPS-induced hypothermic response (10). Our results showed that IL-18 values do not change during the hypothermic response induced by either LPS, implying that IL-18 does not lend itself to a role for the development of LPS-induced hypothermia in rats.

It has been demonstrated that systemic LPS administration also causes the production of antiinflammatory cytokines such as IL-10 in rats (7). LPS-induced hypothermia and TNF-α elevation can be alleviated by systemic infusion of IL-10 (19). Thus, it may be hypothesized that the serotype-specific character of the LPS-induced hypothermia may be related to the variability of the IL-10 expression, depending on the LPS used. Meanwhile, our data indicated that serum IL-10 elevation does not change depending on either the magnitude of the hypothermia or the increased TNF-α levels. Thus, these findings do not support our hypothesis. Nevertheless, a precaution should be warranted, because it has recently been demonstrated that the serum levels measured by ELISA immunoassay may not accurately reflect serum IL-10 bioactivity in mice (14).

We have previously reported that LPS-induced hypothermia is a more sensitive parameter than fever for the suppressive effect of COX-1 selective inhibitors in rats (5). Thus, we evaluated the effectiveness of various COX-1 selective inhibitors, which abolish COX-1-dependent prostaglandin synthesis in vivo, against the hypothermic response induced by two different LPSs (11, 27). We found that VS and SC-560 are potent inhibitors of the hypothermia that may implicate COX-1 isoenzyme in being predominantly responsible for the generation of the response. Meanwhile, SC-560 has a different pharmacological activity profile than VS, having also the inhibitory activity on the elevated cytokine levels. Similar observations have previously been reported for various COX inhibitors, such as sodium salicylate. Inhibition of TNF-α elevation is presumably mediated through inhibition of certain transcription factors rather than COX-dependent prostaglandin synthesis blockade (28). Thus, the pharmacological activity differences between VS and SC-560 may be explained by COX-independent mechanisms that may be operational for SC-560.

Interestingly, when the *E. coli* O111:B4 LPS-induced hypothermia was inhibited by VS treatment, a pyrogenic response appeared. It is possible that the fever may have been masked by the potent hypothermic response, as we observed a dual response pattern (an initial hypothermia and subsequent fever) due to *E. coli* O55:B5 LPS. Thus, this observation may further support the concept that hypothermic and pyrogenic mechanisms are independently activated during LPS-induced acute phase reaction in rats (6).

Our data suggest that LPSs may have different levels of efficiency to release proinflammatory cytokines such as TNF-α in rats. LPS activates a plasma membrane receptor identified as Toll-like receptor (TLR)-4 that is present on macrophages and endothelial cells. TLR-4 activation triggers the biosynthesis of proinflammatory cytokines such as TNF-α. LPS is composed of a complex glycolipid consisting of lipid A, a core oligosaccharide and peripheral O-antigenic chains. The structure of lipid A is conserved among gram-negative bacteria, and this region is recognized by TLR-4. Thus, lipid A is thought to be responsible for all of the biological activities of LPS (23). Meanwhile, other parts of the LPS have unique sugar composition that determines the serological specificity and the mode of mechanisms for host-microbe interactions (21). It appears that these structural differences may also determine the efficiency of LPSs to activate their specific receptors.

In conclusion, LPS-induced hypothermia may have serotype-specific characteristics in rats. It seems that *E. coli* O111:B4 LPS is a more potent one to stimulate the production of endogenous antipyretic cytokines, such as TNF-α, by which...
it elicits a more potent hypothermia. Meanwhile, COX-1 isoenzyme may predominantly be involved in the generation of the hypothermia regardless of the LPS used.

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GRANTS

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