Role of hypothalamic interleukin-1β in fever induced by cecal ligation and puncture in rats

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Gourine, Alexander V., Karin Rudolph, Johannes Tesfaigzi, and Matthew J. Kluger. Role of hypothalamic interleukin-1β in fever induced by cecal ligation and puncture in rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R754–R761, 1998.—Bacterial endotoxin induces fever by causing the release of interleukin (IL)-1β into the circulation or the brain. IL-1β is believed to mediate fever via triggering the production and/or release of IL-6 in the hypothalamus. The present study examined whether IL-1β triggers the production and/or release of IL-6 in the hypothalamus. The present study examined whether IL-1β and IL-6 in the hypothalamus of the rat are also involved in fever during bacterial sepsis caused by cecal ligation and puncture (CLP). CLP induces fever for 2 days. Polyclonal rabbit antibody against rat IL-1β (anti-IL-1β, 2 µg/µl) or control rabbit IgG (2 µg/µl) was unilaterally microinjected into the hypothalamus of rats immediately after or 24 h after CLP or sham-CLP surgery. Anti-IL-1β injected 24 h after CLP (when fever was already present) or sham-CLP surgery did not affect fever. Microinjection of anti-IL-1β into the hypothalamus immediately after surgery caused a significant decrease in body temperature during the night after CLP surgery and a 48% reduction of fever on the following day. Although blood plasma levels of IL-6 were significantly elevated 1, 5, 6, 24, and 48 h after CLP surgery, there were no differences in IL-6 concentrations in the extracellular fluid of the anterior hypothalamus (collected by push-pull perfusion). These data suggest that fever due to bacterial sepsis is initiated by IL-1β within the hypothalamus, and this febrile response, unlike endotoxin-induced fever, is not accompanied by elevation in the hypothalamic concentration of IL-6.

cytokines; endotoxin; push-pull perfusion; temperature regulation

FEVER IS A REGULATED RISE in body temperature (Tb) and one of the most common responses to infection, injury, or trauma. Administration of bacterial endotoxin lipopolysaccharide (LPS) is used widely as a laboratory model of fever. Some endogenously produced proteins are thought to be responsible for the induction of fever by altering the “set point” for Tb regulation. Interleukin (IL)-1 is thought to be an endogenous pyrogen during LPS-induced fever. Intraperitoneal, intravenous, intracerebroventricular, and intrahypothalamic injections of recombinant IL-1 cause fever in various species [for review, see Kluger (13)]. It is believed, on the basis of the observation that intravenous administration of antiserum to murine recombinant IL-1α does not affect the rise in Tb during fever and that IL-1α is not involved in LPS-induced fever (16). On the other hand, intraperitoneal injection of neutralizing antibody to IL-1β led to a significant attenuation of LPS-induced fever in rats (17, 26). Intraperitoneal (29) or intracerebroventricular (18, 22) injections of IL-1 receptor antagonist have also attenuated LPS-induced fever in rats. Klir et al. (11) found that microinjection of neutralizing antibody to IL-1β into the anterior hypothalamus of rats led to an attenuation of LPS-induced fever. The presence of IL-1 type I receptors in thermoregulatory centers has been demonstrated (34). IL-6 has also been reported to be pyrogenic when injected [for review, see Kluger (13)]. Hypothalamic concentrations of IL-6 rise during LPS-induced fever in guinea pigs (25) and rats (12). Infusion of IL-6 into the anterior hypothalamus at a concentration that simulates levels seen after injection of LPS causes a significant rise in Tb (12). Rothwell et al. (27) found that a central injection of antibody to IL-6 inhibits the febrile response to LPS in rats. The probable site of action of endogenous pyrogens is the anterior hypothalamus. IL-1β in vivo (10) and in vitro (20) and IL-6 in vitro (33) predominantly decrease the unit activity of warm-sensitive neurons and increase that of cold-sensitive neurons of the rostral hypothalamus. These data are consistent with the hypothesis that IL-1β and IL-6 act on the thermoregulatory neurons to cause a rise in Tb. Thus numerous data indicate that IL-1β and IL-6 are endogenous pyrogens during LPS-induced fever, the production of these cytokines in the brain increases after administration of exogenous pyrogens, and IL-1β and IL-6 might possess pyrogenic action via their actions on thermosensitive neurons of the hypothalamus.

There is evidence that the rise in IL-6, which is responsible for fever, is triggered in part by IL-1β. Shalaby et al. (28) demonstrated that IL-1β is a strong inducer of IL-6 production in vivo, and LeMay et al. (15) found that the rise in plasma and cerebrospinal fluid concentration of IL-6 in rats injected with LPS was attenuated when the rats were pretreated with anti-IL-1β. Klir et al. (11) showed that the intrahypothalamic administration of the antibody to IL-1β not only blocked a significant portion of the rise in Tb in response to LPS, but also completely abrogated the rise in hypothalamic IL-6. Klir and colleagues (11) concluded that IL-1β mediated LPS-induced fever via an increase in intrahypothalamic IL-6.

Although injection of LPS is a reasonable model of real infection, LPS-induced fever is not the “typical” fever. LPS is usually cleared from the circulation within 1 h after intravenous injection and induces relatively short-lasting fever. However, in most cases

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fever is induced and maintained for long periods of time by continuous pyrogenic stimulation. On the other hand, there is little clinical evidence that plasma LPS levels correlate with the development of sepsis (6), and the failure of the antiendotoxin approach in clinical trials of sepsis treatment (2a) also suggests that LPS is probably only one of several agents responsible for fever and sickness behavior during infections. These data support the hypothesis that the mechanisms of LPS-induced febrile response (laboratory model of fever) may be different from that of naturally occurring fever.

The model of cecal ligation and puncture (CLP) was used in this study. It is widely accepted by many investigators as a model of sepsis, acute infection, and bacterial peritonitis (7, 31, 32). This model involves continuous pyrogenic stimulation and simulates a real infection much better than infusion of constant amounts of LPS (simulated injection).

The purpose of the present study was to investigate whether hypothalamic IL-1β and IL-6 are involved in the development of fever in response to bacterial infection (CLP model). Because of the poor correlation between circulating and brain levels of IL-1 and fever (4, 12, 13), we did not measure the hypothalamic levels of this cytokine. Neutralizing antibody to IL-1β was injected intrahypothalamically, and the effect of this injection on the development of CLP-induced fever was studied. In addition, because IL-6 levels in the hypothalamus correlate well with fever (11, 12), we measured this cytokine in push-pull perfusate.

### MATERIALS AND METHODS

#### Animals

Specific pathogen-free male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 260–300 g were used. Rats were housed one per cage in specific pathogen-free animal quarters in a room maintained at a constant temperature of 25 ± 1°C, a temperature within the thermoneutral zone of rats, and in a 12:12-h light-dark cycle with light onset at 0600. Drinking water and laboratory rodent chow were provided ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Lovelace Respiratory Research Institute. All studies were conducted in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

#### Surgery

The animals were anesthetized with a mixture of ketamine hydrochloride (87.0 mg/kg) and xylazine hydrochloride (13.0 mg/kg) injected intramuscularly. First, a miniature battery-operated, temperature-sensitive telemetry transmitter (model VMFH; Mini-Mitter, Sunriver, OR) was implanted into the abdominal cavity for continuous monitoring of Tb. Then a 26-gauge guide injection cannula (Plastic Products, Roanoke, VA) was stereotaxically implanted into the brain, according to the atlas developed by Paxinos and Watson (24). Coordinates for injections or perfusions of the central part of the anterior hypothalamus were 1.8 mm posterior to bregma, 0.5 mm lateral to right of midline, and 8.6 mm below surface of skull, and coordinates of the lateral ventricle were 0.8 mm posterior to bregma, 1.5 mm lateral to right of midline, and 4.0 mm below the surface of the skull. Two small screws were placed into the skull, and the cannula was secured in place by dental acrylic. The guide cannula was closed with a dummy cannula that extended from the tip of the guide cannula by ±0.2 mm. Animals were allowed to recover for at least 7 days before any experiment. At the end of the experiment, rats were perfused transcardially with saline followed by 4% paraformaldehyde solution, brains were removed, and the location of the tip of cannula was histologically confirmed.

#### Tₜ Measurements

Deep Tₜ (±0.1°C) was monitored with implanted telemetry units (Mini-Mitter). Recordings were made at 5-min intervals by use of a peripheral processor (Datacol III system, Mini-Mitter) connected to an IBM PC, as described previously (15).

#### CLP

The rats were anesthetized with a mixture of 4% halothane in air. A 2-cm midline abdominal incision was made to expose the cecum. The base of the cecum was ligated with 3–0 silk just below the ileocecal valve to permit intestinal continuity. Then the antimesenteric cecal surface was punctured two times with an 18-gauge needle, the cecum was placed back into the abdominal cavity, and the incision was sutured. The wound area was then swabbed with topical antibiotics. The control sham-operated rats had their ceca exposed but not ligated and punctured.

#### Microinjections

The hypothalamus or lateral ventricle was microinjected with an internal injection cannula connected to PE-20 tubing attached to a 10-µl syringe (Hamilton, Reno, NV). The volumes of injections were 1 µl for the hypothalamus and 3 µl for the lateral ventricle.

#### Push-Pull Perfusion

The dummy cannula was unscrewed, and the internal cannula with the rest of the push-pull assembly was attached. The assembly consisted of the internal cannula inserted into the guide cannula, two PE-20 tubing lines (push and pull), and the infusion-withdrawal pump (Harvard Apparatus, South Natick, MA). A small area near the tip of the cannula was continuously bathed with artificial cerebrospinal fluid (aCSF) at a flow rate of 20 µl/min. Each perfusion lasted ~15 min. The samples of perfusate were immediately stored at -20°C. The rat could move freely in its cage during the whole experiment.

#### Animal Perfusion and Histology

Rats were anesthetized with halothane (4% in air mixture) and perfused transcardially with saline followed by phosphate-buffered 4% paraformaldehyde. The brains were removed, stored in the same fixative for 24 h, and submerged in 20% sucrose for an additional 24 h. Series of coronal sections from the hypothalamic region were cut at 10 µm. Slide-mounted sections were stored at −20°C until immunohistochemical staining was initiated.

#### Immunohistochemistry

The distribution of the rabbit IgG injected in the hypothalamus was determined by immunohistochemistry using a biotinylated goat anti-rabbit IgG. Tissue sections were sequentially incubated in 2% hydrogen peroxide in methanol for 30 s to inactivate endogenous peroxidases and blocked in 1% normal...
ROLE OF IL-1β IN FEVER DURING BACTERIAL SEPSIS

goat serum and 0.1% Triton X-100 in 100 mM Tris·HCl (pH 7.8) for 45 min. After the reaction with the biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA) in 0.5% normal goat serum and 0.1% Triton X-100 in 100 mM Tris·HCl (pH 7.8) for 1 h, tissues were incubated with an avidin-biotin complex (Vector Elite kit; 1:100 in 100 mM Tris·HCl, pH 7.8) for an additional hour at room temperature. After extensive rinsing, the distribution of IgG was visualized using the chromagen dianobenzidine (Vector DAB Staining kit, Vector Laboratories, Burlingame, CA). The dianobenzidine reaction was terminated after 10 min, and sections were counterstained with hematoxylin, dehydrated in alcohol, cleared in xylene, and permanently placed under a coverslip with Permount. Sections were observed with a Zeiss microscope, and IgG distribution was determined.

IL-6 Assay

The IL-6-dependent mouse B9 hybridoma cell line was used to determine IL-6 levels. The B9 line was kindly provided by Dr. Lucien Aarden; see Aarden et al. (1). Details of the IL-6 bioassay have been described (11, 12). The sensitivity of the bioassay was 0.2 U/ml (1 U = 0.01 ng; 1st International Standard Code 89/548, Stephen Poole, National Institute for Biological Standards and Control). Because we did not specifically neutralize the activity of the proliferative factor with IL-6 blocking antibodies, the reported values must be considered “IL-6-like” activity. Immunoactive IL-6 was also measured using a rat IL-6 ELISA kit (Biosource International, Camarillo, CA).

Materials

Anti-IL-1β. The rabbit anti-rat IL-1β antibody used for hypothalamic microinjection was obtained from Cytokine Sciences (Boston, MA). This polyclonal antibody neutralized both recombinant and natural rat IL-1β. It was dissolved in aCSF (see aCSF below) to obtain a final concentration of 2 µg in 1 µl. We confirmed the activity of these antibodies by demonstrating that injection into the hypothalamus in a dose of 2 µg blocked a significant portion of fever induced by intraperitoneal injection of LPS, data similar to those of Klir et al. (11).

IgG. Purified rabbit IgG used as a control for hypothalamic microinjection of anti-IL-1β was obtained from Sigma (St. Louis, MO). It was dissolved in aCSF to a final concentration of 2 µg in 1 µl of aCSF.

hrIL-1β. Human recombinant IL-1β (hrIL-1β; specific activity 1.66 × 109 IU/mg) was obtained from Immunex (Seattle, WA). It was dissolved in aCSF to a final concentration of 50 ng (8.3 × 103 IU) in 3 µl of aCSF.

hrIL-1ra. Human recombinant IL-1 receptor antagonist (hrIL-1ra) was obtained from Synergen (Boulder, CO). It was dissolved in aCSF to a final concentration of 100 µg in 3 µl of aCSF.

aCSF. The aCSF used for microinjections consisted of (in mM) 145.0 NaCl, 3.3 KCl, 1.3 CaCl2, and 1.0 MgCl2 dissolved in sterile pyrogen-free water.

Experimental Design

Experiment 1: Effect of intrahypothalamic injection of neutralizing antibody to IL-1β (24 h after surgical procedure) on fever caused by CLP. Tb was monitored for 2 days before and 3 days after the surgical procedure. Animals were conditioned to handling once a day for 5 days before the experiment. Each animal was anesthetized with 4% halothane for ~10 min during the CLP or sham-CLP surgery. Anti-IL-1β or control IgG was injected intrahypothalami

24 h after the surgery in a 2-µg dose. This dose has been effective in attenuating the LPS-induced fever after intrahypothalamic administration (11).

Experiment 2: Effect of intrahypothalamic injection of neutralizing antibody to IL-1β (immediately after surgical procedure) on fever caused by CLP. Tb was monitored for 2 days before and 3 days after the surgical procedure. CLP or sham surgery was performed between 0900 and 1100. Intrahypothalamic injections of anti-IL-1β or control IgG were performed immediately after surgery. Antibody was injected in the same dose of 2 µg. During surgical procedure and subsequent injections of antibodies, each animal was anesthetized with 4% halothane for ~15 min.

Experiment 3: Distribution of rabbit IgG in hypothalamus after unilateral microinjection. Eight rats received an intrahypothalamic microinjection of IgG (2 µg) between 0900 and 1000. Brains were removed for histology and immunohistochemistry 2 h (4 rats) and 24 h (4 rats) after injections. IgG was identified using the procedure described.

Experiment 4: Effect of intracerebroventricular injection of hrIL-1β on Tb in rats. To test the ability of IL-1β to induce long-lasting fever, we injected hrIL-1β into the lateral ventricle of the rats, and Tb was monitored for 48 h. The hrIL-1β was administered in a dose of 50 ng (8.3 × 104 IU). Earlier studies from this laboratory on the pyrogenic activity of this cytokine revealed that 50 ng (8.3 × 104 IU) is the lowest dose of hrIL-1β injected into the lateral ventricle of rats that elicits a febrile response (L. R. Leon, unpublished observations). The hrIL-1ra was administered into the same brain site before hrIL-1β treatment to confirm that the effect of hrIL-1β was realized through its specific receptors. The hrIL-1ra was injected in a dose of 100 µg, which is an average dose from the amounts effective in attenuating LPS-induced fevers after central administration (18, 22).

Experiment 5: Level of IL-6 activity in hypothalamus and plasma during fever caused by CLP. CLP or sham surgery was performed between 0900 and 1100. After 1.5, 6, 24, and 48 h, push-pull perfusion samples of the extracellular fluid of the hypothalamus were collected and stored at −20°C until assayed. For comparison of hypothalamic and circulatory levels of IL-6, another set of CLP and sham rats was anesthetized with 4% halothane and blood was collected by cardiac puncture at the same time points. Immediately after the collection, plasma was separated by centrifugation and stored at −20°C.

Statistical Analysis

Data are reported as means ± SE. Comparisons between experimental groups were made using one-way ANOVA followed by Fisher’s post hoc test. A value of P < 0.05 was considered significant.

RESULTS

Experiment 1: Effect of Intrahypothalamic Injection of Neutralizing Antibody to IL-1β, 24 h After Surgical Procedure, on Fever Caused by CLP

The temperature response of rats to CLP and sham surgery followed in 24 h by an intrahypothalamic administration of anti-IL-1β or control IgG is shown in Fig. 1. The day before surgery, animals showed the normal circadian rhythm of Tb, which rises during the dark period (day, −28 to −17 h; night, −16 to −5 h). Because of anesthesia, the surgical procedure itself induced a drop in Tb in all groups of rats. During the first (20–31 h) and second (44–55 h) days after surgery,
CLP rats developed fever and maintained significantly (P < 0.05) higher T_b (38°C) than sham-operated rats (Fig. 1). The handling (day before surgery) as well as the injection procedure (day after surgery) induced short-term, stress-induced rises in T_b. Intrahypothalamic injection of anti-IL-1β 24 h after the surgical procedure did not significantly influence CLP-induced fever (Fig. 1).

Experiment 2: Effect of Intrahypothalamic Injection of Neutralizing Antibody to IL-1β Immediately After Surgical Procedure, on Fever Caused by CLP

Figure 2 shows a 2-h average T_b for the groups of animals during the whole experiment. Figure 3 shows a 12-h average T_b during the night and the day after surgery. As shown in Figs. 2 and 3, injection of the neutralizing antibody to IL-1β considerably modified the response to CLP. Intrahypothalamic administration of anti-IL-1β induced a significant decrease in T_b in CLP rats during the night after surgery (Figs. 2 and 3). Average core temperatures for the night after surgery were 37.30 ± 0.14 for CLP + anti-IL-1β versus 37.74 ± 0.12 for CLP + IgG (P < 0.05). Compared with sham rats injected intrahypothalamically with IgG, the CLP rats injected the same way with IgG developed a 0.99°C rise in T_b (average temperatures during next 12 h of daylight on day after surgery). CLP rats injected intrahypothalamically with anti-IL-1β compared with sham-operated rats injected with anti-IL-1β showed a 0.51°C rise in T_b during the same time, which represents a 48% reduction in fever. The actual average T_b on the day after surgery of the CLP rats injected with IgG was 38.46 ± 0.09, whereas the T_b of CLP rats injected with anti-IL-1β was 38.09 ± 0.08 during this time (P < 0.05). On the second day after surgery, there was no difference in the magnitude of fever in CLP rats injected with anti-IL-1β compared with CLP rats injected with IgG (Fig. 2).

Experiment 3: Distribution of Rabbit IgG in Hypothalamus After Unilateral Microinjection

To determine the site of action of anti-IL-1β in the attenuation of fever induced by CLP, we examined the distribution of the antibody in the hypothalamus 2 and 24 h after injection. An immunohistochemical study of the injected IgG distribution revealed widespread distribution of the antibody through the hypothalamic tissue 2 h after a unilateral microinjection into the central part of the anterior hypothalamus (1.8 mm posterior to bregma, 0.5 mm lateral to right of midline, and 8.6 mm below surface of skull). The area of IgG distribution was limited in the rostral direction by the medial preoptic area at the level of anterior commissure, 0.26 mm posterior to bregma (24), and in the caudal direction by the dorso medial hypothalamic nucleus, 3.30 mm posterior to bregma (24). In the mediolateral direction, IgG was distributed between the third ventricle and the lateral hypothalamic area and distribution was not exclusively limited by the wall of the third ventricle. Moderate staining was also observed on the contralateral side. However, 24 h after injection, the IgG was not

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**Figure 1.** Effect of microinjection of anti-rat interleukin (IL)-1β antibody (anti-IL-1β; 2 µg in 1 µl of artificial cerebrospinal fluid (aCSF)) or control IgG (in equivalent dose and volume) into hypothalamus on cecal ligation and puncture (CLP)-induced fever in rats (2-h averages of body temperature (T_b)). Rats were injected with anti-IL-1β or IgG 24 h after CLP or sham-CLP surgery. I, time of conditioning on day before surgery; II, time of surgical procedure; III, time of anti-IL-1β or IgG injections on day after surgery. Black horizontal bars indicate dark periods in a 12:12-h light-dark cycle. Numbers in parentheses indicate sample sizes. Data are presented as means ± SE.

**Figure 2.** Effect of microinjection of anti-rat interleukin (IL)-1β antibody (anti-IL-1β; 2 µg in 1 µl of aCSF) or control IgG (in equivalent dose and volume) into hypothalamus on CLP-induced fever in rats (2-h averages of Tb). Rats were injected intrahypothalamically with anti-IL-1β (2 µg in 1 µl of aCSF) or control IgG (in equivalent dose and volume). Numbers in parentheses indicate sample sizes. Data are presented as mean ± SE.

**Figure 3.** Twelve-hour averages of T_b in CLP- and sham-CLP-operated rats to intrahypothalamic microinjection of anti-rat IL-1β antibody (anti-IL-1β; 2 µg in 1 µl of aCSF) or control IgG (in equivalent dose and volume). Numbers in parentheses indicate sample sizes. Data are presented as means ± SE. Left columns (8–19 h) represent first dark period after surgery, and right columns (20–31 h) represent next light period after surgery. *Significant difference in values at P < 0.05.
detected, suggesting that the antibody was cleared from the hypothalamus during this time.

Experiment 4: Effect of Intracerebroventricular Injection of hrIL-1β on T[b] in Rats

Although the fever observed 24 h after CLP was attenuated by the anti-IL-1β injected shortly after surgery, anti-IL-1β may not be present at this time point, based on the histological finding. Therefore, we decided to test the ability of IL-1β to cause long-lasting fever in rats. As shown in Fig. 4, injection of hrIL-1β induced long-lasting elevation in T[b] in rats. The initial temperature response (0 h) represents a stress-induced rise in T[b] caused by the injection procedure. During the day of injection (0–8 h) and the next light period (20–31 h), rats injected with aCSF and hrIL-1β were febrile and this rise in T[b] was completely blocked by the preliminary injection of hrIL-1ra. The T[b] of the rats injected with hrIL-1ra and hrIL-1β was not different from the values the day before (control).

Experiment 5: Level of IL-6 Activity in Hypothalamus and Plasma During Fever Caused by CLP

Table 1 shows IL-6 levels in extracellular fluid of the hypothalamus and plasma 1.5, 6, 24, and 48 h after CLP or sham surgery. At all time points, IL-6 levels in plasma of CLP rats were significantly higher in comparison with sham-operated animals. The maximum level of IL-6 was reached 6 h after CLP surgery (Table 1). IL-6 levels in the hypothalamus measured by bioassay or by the immunoassay were very low (near detection limit) in both groups of animals, and there were no differences between experimental groups at all time points tested.

DISCUSSION

This study shows that fever in response to live bacterial infection is significantly attenuated by intrahypothalamic administration of neutralizing antibody to IL-1β, and this febrile response is not accompanied by an elevation in hypothalamic concentration of IL-6.

As mentioned in the introduction, there is evidence that mechanisms of LPS-induced febrile response (laboratory model of fever) may be different from that of naturally occurring fever. The lack of correlation between plasma LPS levels and the development of sepsis (6), together with the failure of the anti-endotoxin therapy in clinical trials of sepsis treatment (2a), suggests that LPS is not the only factor responsible for the elevation of core temperature and other symptoms of sickness during infections. The CLP model, used in this study, closely resembles the clinical situation of bowel perforation and mixed bacterial infection of intestinal origin and is to a large extent different from LPS-induced febrile response. LPS is usually eliminated from the circulation within 1 h after intravenous injection, whereas in the case of CLP, endotoxin is detectable in serum for at least 21 h after surgery (31). Variability of the approach is an obvious disadvantage of this model of fever. Indeed, the amount and type of bacteria and the time course of leakage into the peritoneal cavity after CLP can vary considerably among animals. However, several experiments showed that

Table 1. Interleukin-6 activity in blood plasma and extracellular fluid of hypothalamus 1.5, 6, 24, and 48 h after CLP or sham-CLP surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Time From Surgery, h</th>
<th>1.5</th>
<th>6</th>
<th>24</th>
<th>48</th>
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<tr>
<td></td>
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<tr>
<td>Plasma bioassay, IU/ml</td>
<td>Sham operated</td>
<td>92.92 ± 9.96 (6)</td>
<td>72.83 ± 35.08 (6)</td>
<td>7.52 ± 9.46 (6)</td>
<td>3.27 ± 0.21 (6)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>206.93 ± 25.73 (6)</td>
<td>297.08 ± 11.91 (6)</td>
<td>165.99 ± 19.72 (6)</td>
<td>52.70 ± 12.43 (6)</td>
</tr>
<tr>
<td>Hypothalamus bioassay, IU/ml</td>
<td>Sham operated</td>
<td>0.36 ± 0.07 (9)</td>
<td>0.35 ± 0.09 (8)</td>
<td>0.36 ± 0.09 (12)</td>
<td>0.20 ± 0.06 (8)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>0.49 ± 0.10 (13)</td>
<td>0.38 ± 0.08 (12)</td>
<td>0.37 ± 0.09 (11)</td>
<td>0.34 ± 0.11 (10)</td>
</tr>
<tr>
<td>Hypothalamus immunoassay, pg/ml</td>
<td>Sham operated</td>
<td>UD (4)</td>
<td>13.06 ± 9.32 (4)</td>
<td>0.71 ± 0.70 (4)</td>
<td>UD (4)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>19.82 ± 12.94 (5)</td>
<td>6.30 ± 6.30 (4)</td>
<td>UD (5)</td>
<td>UD (6)</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Numbers in parentheses indicate sample sizes. UD, values under detection limit of assay. *Significant difference between cecal ligation and puncture (CLP) and sham-CLP-operated rats (P < 0.05).
the febrile response of rats to CLP is very consistent (Figs. 1 and 2). Despite the inherent variability of the approach, it was found that temperature responses of individual rats to CLP were virtually identical within an experiment.

Earlier studies from this laboratory reported that LPS-induced fever was attenuated by intrahypothalamic microinjection of a neutralizing antibody to IL-1β (11). The present data provide further evidence that IL-1β is also important for the development of fever due to acute bacterial infection and that the hypothalamus is the site of its pyrogenic action. Unilateral injection into the hypothalamus of a neutralizing antibody to IL-1β (administered immediately after CLP surgery) resulted in a 48% reduction of fever. The injection of antibody to IL-1β also induced a significant decrease in Tb in CLP-operated rats the night after surgery. After the CLP-induced fever had developed (24 h after surgery), the intrahypothalamic injection of anti-IL-1β did not antagonize it. Although the fever observed on the next day after CLP was attenuated by anti-IL-1β injected shortly after surgery, histological findings indicate that anti-IL-1β may not be present at this time point, because it was found that antibody was completely cleared from the hypothalamus within 24 h. Acute central injection of hrIL-1β in its lowest pyrogenic dose (L. R. Leon, unpublished observations) induced long-lasting elevation in Tb in rats. In most studies on IL-1β pyrogenic activity, Tb was measured only during the day of injection. In this study, Tb was monitored for 48 h and it was found that fever in response to hrIL-1β was observed even on the day after injection (20–30 h postinjection). Thus fever after acute central administration of hrIL-1β was observed during the same time when fever in response to CLP was attenuated by acute injection of anti-IL-1β. It was also found that fever in response to live bacterial infection, unlike LPS-induced febrile response, was not accompanied by changes in hypothalamic levels of IL-6. Thus the present study revealed similarities, as well as differences, between LPS and live bacteria-induced fever.

These data suggest that IL-1β within the hypothalamus is probably involved in inducing, rather than maintaining, fever. 1) Anti-IL-1β attenuates the rise in Tb when injected intrahypothalamically before the development of fever; 2) anti-IL-1β does not affect Tb when the fever has already developed; 3) injected antibody is completely eliminated from the site of administration within 24 h, indicating that when the significant difference in fever is observed, anti-IL-1β is not present in the hypothalamus; 4) exogenous IL-1β can induce long-lasting (30 h) fever in rats after acute central administration; and 5) earlier studies from this laboratory did not find a rise in IL-1 in extracellular fluid from the hypothalamus during the LPS-induced fever (12), and it was suggested that IL-1 in this area quickly becomes cell associated. On the basis of these data, we speculate that initial binding of the IL-1β (during the first hours after CLP surgery) is critically important for the subsequent fever.

The origin of the hypothalamic IL-1β (i.e., from the brain or periphery) remains unclear. Banks et al. (3) found that IL-1β can cross the blood-brain barrier to enter the central nervous system by a saturable transport system. However, data of Cocceani et al. (4) indicate that the blood-brain barrier is impermeable to IL-1. There is also considerable evidence that IL-1 can be synthesized in the brain. Tringali et al. (30) generated data suggesting that IL-1β released by rat hypothalamus might be of neuronal origin. IL-1β production in the brain was found during LPS-induced fever (19) and after peripheral (9, 14) or central (5) endotoxin application. These observations suggest that IL-1β, which exerts its pyrogenic action on the hypothalamic level, might be of peripheral (by crossing blood-brain barrier) as well as brain (synthesized by neurons or glial cells) origin.

At the same time, when plasma levels of IL-6 were markedly elevated during CLP-induced fever, there were no differences in IL-6 levels in the extracellular fluid of the hypothalamus between the CLP and sham rats. The present data indicating that hypothalamic IL-6 levels did not increase during bacterial infection-induced fever do not support earlier findings from this laboratory of increased hypothalamic IL-6 levels during LPS-induced fever (12) or observations that the IL-6 measured in the push-pull perfusate from the hypothalamus during LPS fever is produced at that site in response to a local increase in IL-1β (11). We speculate that IL-1β pyrogenic action during live bacterial infection, unlike during LPS-induced fever, is not achieved via an increase in intrahypothalamic IL-6. IL-1β at the hypothalamic level may initiate fever by inducing the synthesis of prostaglandins (PGs), which are considered the ultimate mediators of the febrile response. There is evidence that supports this hypothesis. 1) IL-1 can directly induce the expression of cyclooxygenase inducible isoform COX-2 and production of PGs (21, 23), 2) hypothalamic neuronal responses to IL-1β are effectively blocked by mepracine (a phospholipase A2 inhibitor) and by sodium salicylate (a cyclooxygenase inhibitor) (10, 20). Thus it is suggested that IL-1β in the hypothalamus directly induces prolonged synthesis of PGs, which in turn decreases the activity of warm-sensitive neurons and increases the activity of cold-sensitive neurons, resulting in an increase in heat production and decrease in heat loss.

The data obtained in this study might be useful in terms of the development of anti-cytokine approaches in the treatment of sepsis. IL-1 together with tumor necrosis factor are believed to play central roles in the pathophysiological state of sepsis (simulated by CLP model). Animal studies show that hrIL-1ra is effective in the attenuation of LPS-induced fever (18, 22, 29) and in preventing morbidity and mortality of sepsis in rats (2); however, soluble IL-1ra did not protect against sepsis in clinical trials (2a). We hypothesize that anti-IL-1 therapy might be effective only during the early stages of sepsis, because we have shown that anti-IL-1β treatment was effective in attenuation of fever when it
was applied before but not after the development of sepsis. In conclusion, this study characterizes the role of hypothalamic IL-1β and IL-6 in the development of fever during acute bacterial infection in rats. The data obtained revealed similarities as well as differences between LPS and live bacteria-induced fever. The results show that fever caused by bacterial infection, like LPS-induced fever, involves IL-1β as an endogenous pyrogen and that the hypothalamus is the site of its pyrogenic action. It is speculated that IL-1β within the hypothalamus is probably involved in inducing, rather than maintaining, fever. The results also indicate that fever in response to bacterial sepsis, unlike LPS-induced fever, is not accompanied by an elevation in hypothalamic concentration of IL-6.

Perspectives

Fever is probably one of the most common adaptive responses to infection, injury, or trauma. Most models of fever involve the injection of LPS, a component of the cell wall of gram-negative bacteria. These models have shown that IL-1β at the level of the anterior hypothalamus is responsible for a portion of fever (11). In addition, the IL-1β results in a rise in hypothalamic IL-6 (11). Are all fevers identical? That is, do they all operate via the induction of the same cytokine cascade? In the present study, we measured hypothalamic concentrations of IL-6 in a model of “real” infection: CLP. We found that CLP caused a rise in circulating concentrations of IL-6 but no rise in the hypothalamic concentration of this cytokine. However, when the rats had neutralizing antibody to IL-1β microinjected into the hypothalamus, this resulted in a significant attenuation of fever. We conclude that 1) CLP-induced fevers act in part via hypothalamic IL-1β, and 2) these fevers may not require the production of IL-6.

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transcriptional control of COX-2 gene expression by IL-1β.


