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# Central interleukin-10 attenuated lipopolysaccharide-induced changes in core temperature and hypothalamic glutamate, hydroxyl radicals and prostaglandin-$E_2$

Cheng-Hsing Kao [a,b,1], Wu-Tein Huang [c,1], Mao-Tsun Lin [d,e], Wen-Shiann Wu [f,*]

[a] Department of Surgery, Chi Mei Medical Center, Tainan 710, Taiwan
[b] Center for General Education, Southern Taiwan University, Tainan 710, Taiwan
[c] Department of Recreation and Health Care Management, Chia-Nan University of Pharmacy and Science, Tainan 710, Taiwan
[d] Department of Medical Research, Chi Mei Medical Center, Tainan 710, Taiwan
[e] Graduate Institute of Disease Prevention and Control, Taipei Medical University, Taipei 112, Taiwan
[f] Department of Cardiology, Chi Mei Medical Center, Tainan 710, Taiwan

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ABSTRACT

It has been documented that intravenous lipopolysaccharide (LPS) in rabbits causes fever accompanied by increased levels of extracellular glutamate, hydroxyl radicals, and prostaglandin $E_2$ ($PGE_2$) in the hypothalamus and circulating tumor necrosis factor-alpha (TNF-α). This investigation was to determine whether central interleukin-10 (IL-10) exerted its antipyresis by reducing changes in circulating TNF-α and extracellular glutamate, hydroxyl radicals and $PGE_2$ in the hypothalamus. The microdialysis probes were stereotaxically and chronically implanted into the preoptic anterior hypothalamus of rabbit brain for determinating extracellular glutamate, hydroxyl radicals, and $PGE_2$ in situ. It was found that systemically injected LPS (2 μg/kg, intravenously) increased the levels of core temperature, and extracellular glutamate, hydroxyl radicals, and $PGE_2$ in the hypothalamus accompanied by increased plasma levels of TNF-α. Pretreatment with IL-10 (10–100 ng, intracerebroventricularly) 1 h before intravenous LPS significantly reduced the LPS-induced changes in extracellular glutamate, hydroxyl radicals, and $PGE_2$ in the hypothalamus and fever, but not the increased levels of TNF-α in rabbits. These findings suggested that directly injected IL-10 into the lateral cerebral ventricle 1 h before intravenous LPS exerted its antipyresis by inhibiting the changes in extracellular glutamate, hydroxyl radicals and $PGE_2$ in the hypothalamus during LPS fever in rabbits.



## 1. Introduction

Interleukin-10 (IL-10), an endogenous anti-inflammatory cytokine, inhibited: many actions of lipopolysaccharide (LPS) (Berg et al., 1995; Moore et al., 2001; Oberholzer et al., 2002; Strle et al., 2001; Ward et al., 2001); the production of tumor necrosis factor-alpha (TNF-α), IL-1, and IL-6, while upregulating the expression of IL-1 receptor antagonists (Howard and O'Garra, 1992; Jenkins et al., 1994). Moreover, IL-10-knockout mice had an increased likehood of inflammatory bowel disease (Rennick et al., 1997), higher mortality rates after sepsis (Berg et al., 1995), and an exacerbated and prolonged fever in response to systemically administered LPS (Leon et al., 1999). Central IL-10 has attenuated the febrile response to central LPS in rats (Ledeboer et al., 2002). However, peripheral LPS-induced fever was central IL-10-unaffected (Ledeboer et al., 2002) in rats.

It was repeatedly documented that TNF-α could be detected in either the circulating stream (Kluger ,1991) or the hypothalamus (Breder et al., 1994; Gatti and Bartfai, 1993; Roth et al., 1993; Watanabe and Hayakawa, 2003) after intravenous injection of a fever-inducing dose of LPS in rodents. Both LPS and TNF-α may have released glutamate, hydroxyl radicals, and prostaglandin $E_2$ ($PGE_2$) in the hypothalamus to induce fever (Rothwell, 1988; Huang et al., 2006, 2008; Tsai et al., 2006; Kao et al., 2007a,b). It is not known whether central IL-10 attenuates the peripheral LPS-induced fever via inhibiting the changes in circulating TNF-α and/or extracellular glutamate, hydroxyl radicals and prostaglandin $E_2$ $PGE_2$ in the hypothalamus in rabbits.

To answer the question, in the present study, we determined the changes in body core temperature (Tco), circulating levels of TNF-α, and extracellular glutamate, hydroxyl radicals, and $PGE_2$ in the hypothalamus during systemic administration of LPS in rabbit with or without prior central administration of IL-10.

## 2. Methods

### 2.1. Experimental animals

Adult male New Zealand white rabbits, aged between 4.8 and 5.6 years old and weighing between 2.1 and 3.4 kg at the study start

* Corresponding author. Tel./fax: +886 6 2517850.
*E-mail address:* 891201@mail.chimei.org.tw (W.-S. Wu).
[1] These authors contributed equally to this work.

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were unanesthetized and restrained in rabbit stocks in the pyrogen test. Between experiments the animals were housed individually at ambient temperature of 22 ± 1 °C with a 12-h light-dark cycle, with the lights switched on at 06:00. Animal chow and water were allowed *ad libitum*. Experiments were between 09:00 and 19:00, with each animal manipulated at an interval of not less than 7 days. Throughout the experiment, core temperature was measured every 5 min with a copper constantan thermocouple inserted into the rectum and connected to a thermometer (HR 1300, Yokogawa, Tokyo, Japan). The core temperature of each animal was allowed to stabilize for at least 90 min before any injections. Only animals whose core temperature was stable and in the range of 38.2 to 39.1 °C were utilized to determine the effect of drug application. All the animals were acquired from the animal center of Chi Mei Medical Center (Tainan, Taiwan, ROC). The animal protocol accounted here was approved by the Animal Care Committee of Chi Mei Medical Center.

### 2.2. Surgical techniques

An intracerebroventricular or intracerebral probe guide cannula was implanted into each animal under general anesthesia (sodium pentobarbital, 30 mg/kg, i.v.). Standard aseptic techniques were employed; so were the stereotaxic atlas and coordinates of Sawyer et al. (1954). The cannula was located in the lateral cerebral ventricle (P:4 mm, R: 3 mm, and V: 5 mm) or left organum vasculosum laminae terminalis (OVLT; A: 4.5 mm; L: 0 mm; V: 14 mm). The animals were placed in the stereotaxic apparatus, and the frontal and parietal bones were exposed by a midline incision into the scalp. After appropriately located craniotomy was trephined, two self tapping screws were inserted into the frontal or parietal bones and the cannula inserted to the depth through the craniotomy hole was anchored with dental acrylic cement to the calvarium surface, which had been scraped clean of periosteum. The reflected muscles and skin were replaced around the acrylic mound containing the cannula and screws and were sutured with chromic gut (000). Postoperatively, the guide cannula was plugged with a stylet, and animals were returned to their cages for a minimal recovery period of 1 week.

### 2.3. Drugs

All drug solutions prepared in pyrogen-free glassware that was heated for 5 h at 180 °C before use were prepared in pyrogen-free saline and passed through 0.22-μm Millipore bacteria filters. The LPS in this study, which was derived from *Escherichia coli* serotype 026:B6 (Sigma-Aldrich, Chemical Co., St Louis, MO, USA), was dissolved in sterile saline.

LPS (2 μg/kg) and human interleukin-10 (IL-10) (R&D, Minneapolis, MN, USA; 10–100 ng/rabbit) were intravenous (i.v.) and intracerebroventricular (i.c.v.), respectively. Drugs used for present study including TNF-α, glutamate, dihydroxybenzoic acid (DHBA), salicylic acid, monochloracetic acid, and $PGE_2$ were all purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA).

### 2.4. Experimental groups

Preexperimentally, the indwelling stylet of the guide cannula was replaced by a CMA-12 microdialysis probe purchased from CMA/Microdialysis (ROS-Lagsvägen, Stockholm, Sweden) so that the membrane tip protruding exactly 1.5 mm beyond the guide tube was analyzed. The microdialysis probes and perfusion procedures (1.2 μl/min) in this study were described previously (Huang et al., 2006, 2008).

In Experiment 1, an i.c.v. dose of IL-10 (10 ng, 50 ng, or 100 ng in 10 μl) or normal saline (10 μl) was randomly administered into rabbits (n = 16) 1 h before an i.v. dose of LPS (2 μg/kg) and their effects on core temperature (Tco), serum levels of TNF-α, and OVLT levels of both glutamate and DHBA were assessed.

In Experiment 2, an i.c.v. dose of IL-10 (10 ng, 50 ng, or 100 ng in 10 μl) or normal saline (10 μl) was randomly administered into rabbits (n = 16) 1 h before an i.v. dose of LPS (2 μg/kg) and their effects on OVLT levels of $PGE_2$ were assessed.

### 2.5. Microdialysis for detection of extracellular glutamate, DHBA, and $PGE_2$

For measuring extracellular levels of glutamate in OVLT of rabbit brain, the dialysis system was connected to the microdialysis pump and perfused with artificial cerebrospinal fluid at a flow rate of 1.2 μl/min. The dialysates were collected every 20 min in a CMA 140 fraction collector. Aliquots of dialysates (2 μl) were injected into a CMA600 Microdialysis Analyzer for measuring glutamate which was glutamate oxidase-oxidized enzymatically. The hydrogen peroxide formed reacted with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine and 4-amino-antipyrine, which was peroxidase-catalyzed and yielded the red-violet colored quinonediimine. The formation rate was measured photometrically at 546 nm and was proportional to the glutamate.

For measuring extracellular levels of hydroxyl radicals in the OVLT, a probe guide cannula was planted there. The morning before an experiment, after a microdialysis probe was lowered into the OVLT, the OVLT was perfused with artificial cerebrospinal fluid (149 mM $NaCl_2$; 2.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $Cl_2$, 0.125 mM ascorbic acid, and 5.4 mM D-glucose, pH7.2–7.4) containing 10 mM salicylic acid by a high pressure pump (CMA/Microdialysis; ROS-Lagsvägen, Stockhorm, Sweden) at a flow of 1.2 μl/min (Huang et al., 2006). The dialysis probe was a CMA-12 Elite Microdialysis Probe (Solna, Sweden). The concentrations of hydroxyl radicals were measured by a modified procedure based on the hydroxylation of sodium salicylate by hydroxyl radicals, leading to the production of 2,3-DHBA and 2,5-DHBA (Yang and Lin, 2002). After 2 h of stabilization the dialysates from the OVLT were collected at 10-min intervals. An Alltima reverse-phase C18 column (Bioanalytical Systems, Lafayette, Ind., USA, 150 × 1 mm inside diameter, particle size 5 mm) was used to separate the DHBAs, and the mobile phase consisted of a mixture of 0.1 M chloroacetic acid, 26.87 nmol/L disodium EDTA, 688.76 nmol/L sodium octyl sulfate, and 10% acetonitrile (PH3.0). The retention time of 2,3-DHBA and 2,5-DHBA were 8.1 and 6.0 min.

Dialysis samples from the OVLT were collected into a microdialysis vial at 60 min interval for 8 h, and they were stored at −80 °C until analyzed within 7 days. Immunoreactive $PGE_2$ concentrations in dialysates were commercially available enzyme immunoassay kits (Cayman Chemical Co., Ann Arbor, MI, USA)-determined. Triplicate aliquots of 50 μl samples were added to each well of the plate and each sample was assayed, at a minimum, at two dilutions. The quantitation limit for $PGE_2$ was 20 pg/ml.

### 2.6. Determination of TNF-α in rabbit serum

For measuring serum TNF-α, 5 ml of blood was withdrawn from the marginal ear vein of each rabbit. The blood samples were centrifuged at 1400 × *g* for 15 min at 4 °C. The serum was collected in polyethylene tubes and stored at −70 °C until the cytokine assay. The amounts of TNF-α in the serum were double-antibody sandwich ELISA (R&D systems, Minneapolis, MN)-determined according to the manufacturer. Recombinant TNF-α (5 to 300 pg/ml) represented the standards for calibration, and the detection limit of all assays was 15 pg/ml.

### 2.7. Data presentation

At the end of an experiment, the animals were sacrificed with an overdose of anesthetics and decapitated with a guillotine. Their brains were removed and stored in 10% phosphate-buffered formalin for

histologically verifying the placement of the dialysis probe tips. Only experiments in which the OVLT localization of the microdialysis probes was confirmed histologically were included in the results. The operation success-rate based on histology was about 92%.

### 2.8. Statistical analysis

Temperature response was assessed as changes from pre-injection values (Δ°C) and the fever index (FI), which was the area under the curve produced in the 5-h period after the injection of LPS, in terms of degrees centigrade per 5 h, was calculated (Huang et al., 2006). The 2,3-DHBA level of samples was expressed as a percent of the mean baseline. The results were expressed as the mean ± standard errors of the mean (S.E.M.) for n experiments. Two way analysis of variance (ANOVA) for repeated measurements (in the same animals) was for the factorial experiment, whereas Dunnett's test was for post hoc multiple comparisons among means. A $P$ value less than 0.05 was considered significantly different.

## 3. Results

### 3.1. LPS-increased Tco, the hypothalamic levels of glutamate, hydroxyl radicals and $PGE_2$ and plasma levels of TNF-α

Intravenous administration of a dose (2 μg/kg) of LPS caused a biphasic febrile response, in Tco maximal at 80 min and 200 min post-injection (Fig. 1). Each Tco elevation was accompanied by a distinct wave of hypothalamic levels of glutamate, 2,3-DHBA (Fig. 1) and $PGE_2$ (Fig. 2), and plasma levels of TNF-α (Fig. 3). If the temperature response was assessed as changes in terms of fever index, intravenous LPS (0.5–10 μg/kg) produced dose-dependent fever in rabbits (Tsai et al., 2006). Therefore, in the following experiment, an intravenous dose of 2 μg/kg of LPS was chosen as a standard. In addition, in Fig. 1,

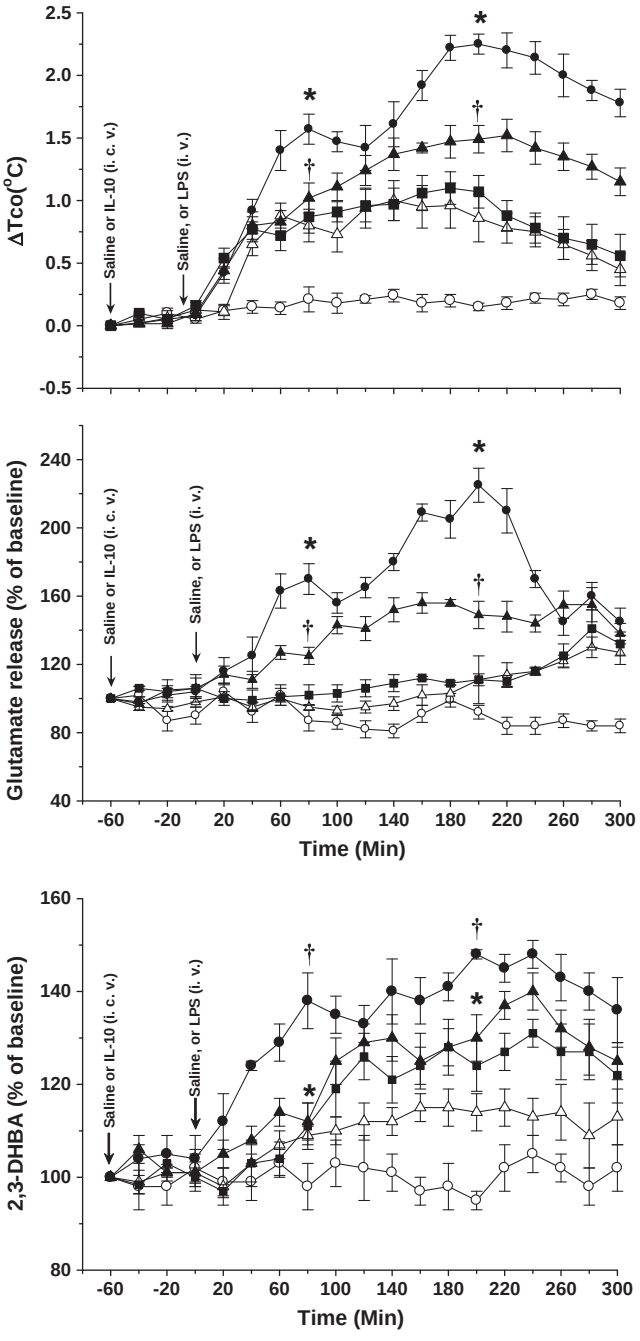


**Fig. 1.** Mean ± S.E.M. changes in core temperature (Tco), extracellular glutamate, and 2,3-DHBA generation in the hypothalamus with intracerebroventricular (i.c.v.) saline plus intravenous (i.v.) saline (○) (n = 8), i.c.v. saline plus i.v. LPS (2 μg/kg) (●) (n = 8), i.c.v. IL-10 (10 ng/rabbit) plus i.v. LPS (2 μg/kg) (▲) (n = 8), i.c.v. IL-10 (50 ng/rabbit) plus i.v. LPS (2 μg/kg) (■) (n = 8), and i.c.v. IL-10 (100 ng/rabbit) plus i.v. LPS (2 μg/kg) (△) (n = 8). IL-10 or vehicle solution was injected 60 min before LPS administration. *$P$<0.05, compared with the group (of saline plus saline); †$P$<0.05, compared with the group (of saline plus LPS). The baseline Tco, glutamate, and 2,3-DHBA were 39.1 °C, 2.4 μM, and 228 ng/ml, respectively.

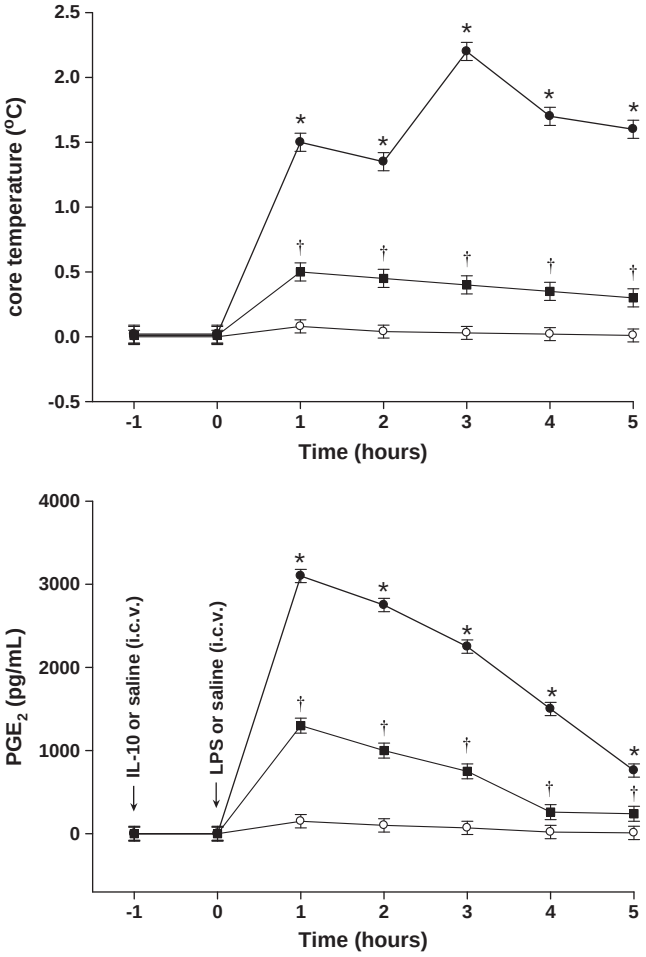


**Fig. 2.** Mean ± S.E.M. changes in core temperature (Tco) and PGE2 release in the hypothalamus with intracerebroventricular (i.c.v.) saline plus i.v. saline (○) (n = 8), i.c.v. saline plus i.v. LPS (2 μg/kg) (●) (n = 8), and i.c.v. IL-10 (50 ng/rabbit) plus i.v. LPS (2 μg/kg) (■) (n = 8). IL-10 or vehicle was injected 60 min before LPS administration. *$P$<0.05, compared with the group (of saline + saline); †$P$<0.05, compared with the group (of saline + LPS).

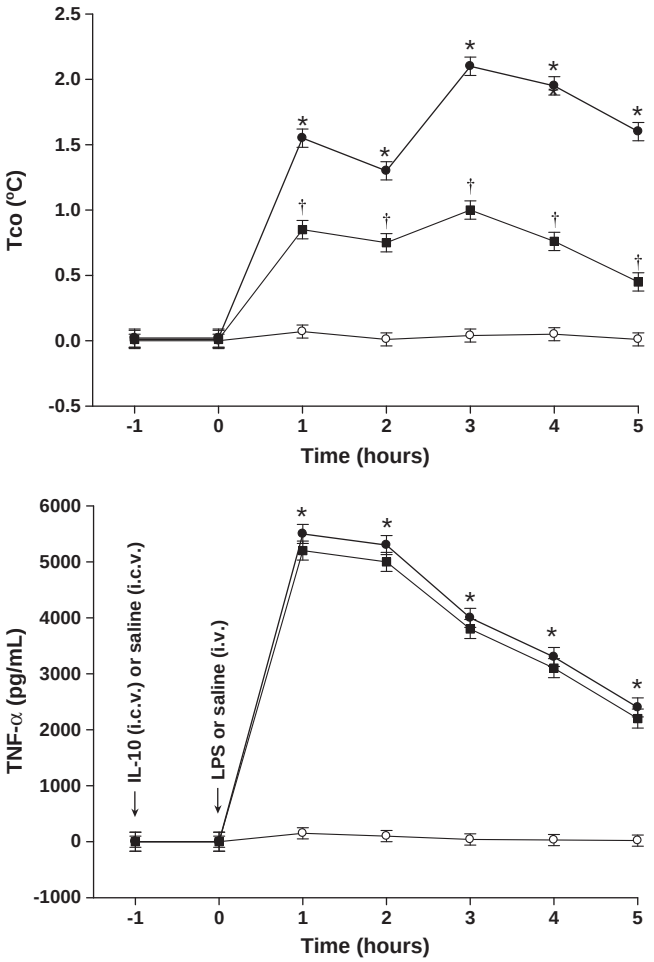


**Fig. 3.** Mean ± S.E.M. changes in core temperature (Tco) and serum levels of TNF-α with i.c.v. saline plus i.v. saline (○) (n = 8), i.c.v. saline plus i.v. LPS (2 μg/kg) (●) (n = 8), and i.c.v. IL-10 (50 ng/rabbit) plus i.v. LPS (2 μg/kg) (■) (n = 8). IL-10 or vehicle was injected 60 min before LPS administration. *$P$<0.05, compared with the group (of saline + saline); †$P$<0.05, compared with the group (of saline + LPS).

the presence or the absence of salicylic acid in artificial cerebrospinal fluid did not affect LPS fever in rabbits.

### *3.2. IL-10 preconditioning reduced the increased levels of Tco, and hypothalamic levels of glutamate, hydroxyl radicals and $PGE_2$ following LPS injection*

Both Tables 1 and 2 summarized the effects of intracerebroventricular IL-10 (10–100 ng) 1 h before the LPS injection on the peak Tco elevation, fever index, and hypothalamic glutamate and hydroxyl radical elevations in response to LPS in rabbits. It could be seen from the tables that the temperature elevations of both early and late phase (Table 2), the fever index (Table 1) as well as the elevations of hypothalamic levels of glutamate and hydroxyl radicals induced by LPS (2 μg/kg, i.v.) were dose-dependently reduced by pretreatment with IL-10 (10–100 ng, i.c.v.) 1 h before the LPS injection. The time course changes of Tco and hypothalamic levels of glutamate and 2,3-DHBA induced by IL-10 1 h before the LPS injection were in Fig. 1.

**Table 1**
Effects of intracerebroventricular IL-10 1 h before the LPS injection on the febrile response to intravenous LPS in rabbits.

| Treatments | Fever index (FI, Δ°C h) |
|---|---|
| Saline + Saline | FI = 0.90 ± 0.06 |
| Saline + LPS (2 μg/kg) | FI = 8.23 ± 0.12[a] |
| IL-10 (10 ng) + LPS (2 μg/kg) | FI = 5.82 ± 0.13[b] |
| IL-10 (50 ng) + LPS (2 μg/kg) | FI = 4.19 ± 0.08[b] |
| IL-10 (100 ng) + LPS (2 μg/kg) | FI = 3.68 ± 0.05[b] |

The values were means ± S.E.M. of 6 rabbits per group. FI represented fever index for 5 h experimental observation.
[a] Significantly different from the corresponding control value ( in the vehicle plus vehicle group) ($P$<0.05; two way analysis of variance followed by Dunnett's test).
[b] Significantly different from the corresponding control value (in the vehicle plus LPS group) ($P$<0.05; two way analysis of variance followed by Dunnett's test).

**Table 2**
Effects of intracerebroventricular IL-10 1 h before the LPS injection on peak Tco, extracellular glutamate and hydroxyl radical elevations in the hypothalamus in response to LPS in rabbits.

| Treatments | Response | |
|---|---|---|
| | Early phase | Late phase |
| | Peak Tco | (Δ°C) |
| Saline + Saline | 0.18 ± 0.05 | 0.21 ± 0.05 |
| Saline + LPS(2 μg/kg) | 1.48 ± 0.10[a] | 2.18 ± 0.11[a] |
| IL-10 (10 ng) + LPS (2 μg/kg) | 1.02 ± 0.12[b] | 1.49 ± 0.11[b] |
| IL-10 (50 ng) + LPS (2 μg/kg) | 0.87 ± 0.13[b] | 1.07 ± 0.13[b] |
| IL-10 (100 ng) + LPS (2 μg/kg) | 0.80 ± 0.14[b] | 0.86 ± 0.19[b] |
| | % of mean basal levels of glutamate | |
| Saline + Saline | 87 ± 6 | 92 ± 4 |
| Saline + LPS(2 μg/kg) | 170 ± 9[a] | 225 ± 10[a] |
| IL-10 (10 ng) + LPS (2 μg/kg) | 125 ± 5[b] | 149 ± 8[b] |
| IL-10 (50 ng) + LPS (2 μg/kg) | 102 ± 6[b] | 121 ± 4[b] |
| IL-10 (100 ng) + LPS (2 μg/kg) | 95 ± 2[b] | 101 ± 6[b] |
| | % of mean basal levels of hydroxyl radical | |
| Saline + Saline | 97 ± 4 | 96 ± 2 |
| Saline + LPS(2 μg/kg) | 136 ± 5[a] | 152 ± 13[a] |
| IL-10 (10 ng) + LPS (2 μg/kg) | 118 ± 4[b] | 130 ± 5[b] |
| IL-10 (50 ng) + LPS (2 μg/kg) | 111 ± 5[b] | 124 ± 5[b] |
| IL-10 (100 ng) + LPS (2 μg/kg) | 108 ± 2[b] | 114 ± 4[b] |

The values were means ± S.E.M. of 6 rabbits per group. Rabbits injected with LPS (2 μg/kg) produced a biphasic fever which peaked at 80 min (early phase) and 200 min (late phase) after LPS injection. The baseline Tco, glutamate, and hydroxyl radical were found to be 39.1 °C, 2.4 μM, and 228 ng/ml, respectively.
[a] Significantly different from the corresponding control value (in the vehicle plus vehicle group) ($P$<0.05; two way analysis of variance followed by Dunnett's test).
[b] Significantly different from the corresponding control value (in the vehicle plus LPS group) ($P$<0.05; two way analysis of variance followed by Dunnett's test).

Fig. 2 identified the effects of intracerebroventricular IL-10 (50 ng) 1 h before the LPS (2 μg/kg, i.v.) injection on the plasma TNF-α and Tco elevations in response to LPS in rabbits; thence, the LPS-increased Tco, but not TNF-α in plasma, was IL-10 preconditioning-reduced significantly.

Fig. 3 depicted the effects of intracerebroventricular IL-10 (50 ng) 1 h before the LPS (2 μg/kg, i.v.) injection on the hypothalamic $PGE_2$ and Tco elevations in response to LPS in rabbits; thus, the LPS-elevated hypothalamic $PGE_2$ and Tco elevations were IL-10 preconditioning-reduced significantly.

An appropriate control injection of IL-10 or normal saline insignificantly changed Tco, extracellular glutamate, 2,3-DHBA, and $PGE_2$ in the hypothalamus, or plasma levels of TNF-α.

## 4. Discussion

In our previous results (Huang et al., 2006, 2008), an intravenous dose of 2 μg/kg of LPS raised a biphasic core body temperature, maxima at 80–90 min and 180–210 min post-injection. The early phase of the fever was associated with remarkable elevations of both TNF-α and IL-1β in the serum, while the late phase of the fever was remarkable rise-related in serum levels of IL-6. It seemed that the early and the late phases of fever were associated, respectively, with the increased serum levels of TNF-α plus IL-1β and IL-6. The remarkable rise of IL-6 might be both IL-1β and TNF-α (Pedersen et al., 2001)-caused. Intravenous LPS or central injection of TNF-α, IL-1β, or IL-6 caused fever in rabbits accompanied by remarkable rise of hypothalamic glutamate, hydroxyl radicals, and $PGE_2$ (Niu et al.,

2009). In the current studies, direct injection of IL-10 into the cerebroventricular system affected no serum levels of TNF-α but significantly reduced the peripheral LPS-increased hypothalamic levels of glutamate, hydroxyl radicals and $PGE_2$ and fever in rabbits. Our results suggested that central IL-10 attenuated LPS-induced fever via reducing extracellular glutamate, hydroxyl radicals and $PGE_2$ in the hypothalamus in rabbits. The contention was in part several previous results-supported. For example, it was shown that IL-10-knockout mice had an increased fever in response to systemic injection of LPS (Leon et al., 1999). In order to test the hypothesis that endogenous IL-10 limited the LPS-induced fever, Cartmell et al. (2003) injected the LPS into a subcutaneous air pouch in rats and observed that the LPS-induced fever was significantly reduced by simultaneous administration of rat recombinant IL-10. The inhibition of fever by IL-10 was accompanied by a significant reduction of IL-6 and IL-1 receptor antagonist in the circulation. Conversely, neutralization of IL-10 in the subcutaneous air pouch increased the duration of LPS-evoked fever and the serum levels of TNF-α, IL-1β and IL-6. In fact, our results obtained from rabbits were in part consistent with the rat data of Hollis et al. (2010) showing that the peripheral LPS-induced hypophagia, reduction in motor activity, and reduction in respiratory exchange ratio could be central IL-10-attenuated. In

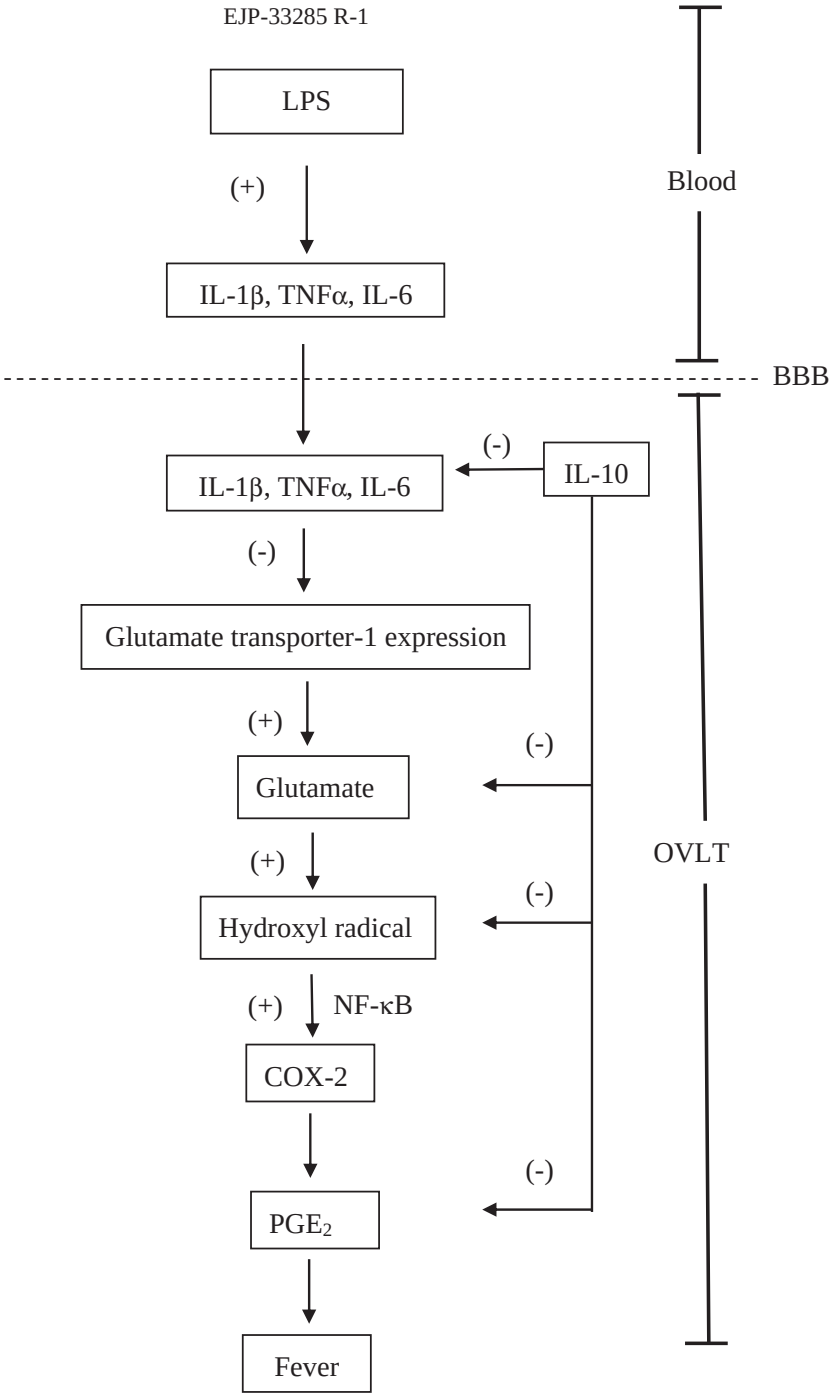


**Fig. 4.** The proposed scheme of the interacting sequence from the beginning of exposure to an LPS injection to fever occurrence in rabbits. (−) and (+) indicated "inhibiting" and "stimulating", respectively. LPS, lipopolysaccharide; IL-10, interleukin-10; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; COX-2, cyclooxygenese-2; $PGE_2$, prostaglandin E-2; BBB, blood-brain-barrier; OVLT, organum vasculosum laminae terminalis.

contrast, Ledeboer et al. (2002) reported central IL-10 attenuated the febrile response to central LPS (60 ng/rat, intracerebroventricularly) but not to peripheral LPS (10 μg/kg, intravenously) in rats. The reasons for the discrepancy in the data between these two groups of data are not apparent now.

As reviewed by Kluger (1991), LPS activated blood monocytes and/or hepatic macrophages (Kuffer cells) and released TNF-α, IL-1β, IL-6, and others. Blood-borne pro-inflammatory cytokines could activate meningeal macrophages, cerebral endothelial cells, and perivascular microglial cells, thereby locally producing $PGE_2$ and IL-6, which were implicated in fever (Cao et al., 1999; Elmquist et al., 1997; Van Dam et al., 1996). Circulating cytokines could also act on cells in the organum vasculosum of the lamina terminalis and the area postrema of the anterior hypothalamus (Blatteis, 1992; Saper and Breder, 1994). Additionally, peripherally produced cytokines could activate vagal afferent nerves that innervated the nucleus of the solitary tract in the brain stem, from which catecholaminergic projections led to the hypothalamus (Blatteis and Sehie, 1997; Ericsson et al., 1994; Simons et al., 1998). As evidenced, TNF-α, IL-1β, and IL-6 mRNA and protein were expressed in microglial cells and/or neurons in the brain after peripheral LPS (Breder et al., 1994; Buttini and Boddeke, 1995; Gatti and Bartfai, 1993; Laye et al., 1994; Tilders et al., 1994; Vallieres and Rivest, 1997; van Dam et al., 1992). Furthermore, blocking the action of these brain-derived cytokines by central administration of neutralizing antibodies or receptor antagonist of these cytokines attenuated the febrile response to peripheral LPS (Klir et al., 1994; Luheshi et al., 1996; Rothwell et al., 1991). TNF-α, IL-1β, and IL-6 induced a rapid onset of fever within minutes and required cyclooxygenase-2 and synthesis of $PGE_2$ in the hypothalamus (Dinarello, 2004). In rabbits intravenous LPS increased the circulating levels of both TNF-α (Tsai et al., 2006) and IL-6 (Niu et al., 2009), and core temperature. Systemic administration of LPS or central injection of TNF-α or IL-6 increased hypothalamic levels of glutamate, hydroxyl radicals, and $PGE_2$ and core temperature in rabbits (Tsai et al., 2006; Niu et al., 2009).

The most striking finding of this study was that central IL-10 inhibited the intravenous LPS-increased extracellular glutamate, hydroxyl radicals, and $PGE_2$ in the hypothalamus. In Fig. 4, our results indicated that central IL-10 could cause antipyresis by inhibiting the glutamate-hydroxyl radicals-$PGE_2$ pathways in the hypothalamus (Tsai et al., 2006; Huang et al., 2006; Kao et al., 2007a,b; Niu et al., 2009). However, peripheral LPS-increased circulating levels of TNF-α were not central IL-10-affected. The above-mentioned hypothesis could be in part several previous findings-supported. For example, IL-10 inhibited the LPS-stimulated macrophage production of inflammatory cytokines such as IL-1, IL-6, and TNF-α (Fiorentino et al., 1991). Glial cells-produced pyrogenic cytokines could be inhibited by IL-10 (Ledeboer et al., 2002) which inhibited systemic LPS administration- induced fever in rats and mice (Leon et al., 1999; Nava et al., 1997). IL-10 knockout mice showed exacerbated and prolonged fever (Leon et al., 1999) and elevated TNF-α and IL-6 in the brain in response to LPS. The LPS-overproduced free radicals in macrophages could be inhibited by IL-10 (Dokka et al., 2001) which promoted survival of cerebellar granule cells exposed to toxic concentrations of glutamate (Bachis et al., 2001).

It should be mentioned that, in the present results, it is not convinced that glutamate is mediating the hyperthermic effects of LPS and that IL-10 is blocking LPS-induced hyperthermia by reducing extracellular glutamate. For example, it is not known whether the increase in extracellular hypothalamic glutamate is specific to LPS-induced hyperthermia or is a nonspecific response that accompanies any hyperthermia-producing substance. In other words, it remains unclear whether hyperthermia does increase hypothalamic glutamate independent of the specific drug that raises body temperature. Further experiments are warranted to be conducted.

According to the findings of Ledeboer et al. (2002), central administration of rat recombinant IL-10 (600 ng $h^{-1}$ per rat i.c.v.) throughout the experiment attenuated the febrile response to central LPS in rats. However, in this study, the febrile responses to peripheral LPS in rabbits were attenuated by central administration of human recombinant IL-10 (10–100 ng in one bonus injection per rabbit i.c.v.). Thus, it seemed that the LPS-induced febrile responses in rabbits were quite susceptible to human recombinant IL-10. In addition, why IL-10 was administered exactly 1 h earlier before LPS administration was experimentation-based.

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