0013-7227/03/$15.00/0
Printed in U.S.A.

Endocrinology 144(6):2454–2460

doi: 10.1210/en.2002-0093

Neuroimmune Response to Endogenous and Exogenous Pyrogens Is Differently Modulated by Sex Steroids

A. MOUIHATE AND Q. J. PITTMAN

Neuroscience Research Group, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Alberta, Canada T2N 4N1

The objective of this study was to explore whether and how ovarian hormones interact with the febrile response to pyrogens. Estrogen and progesterone treatment of ovariectomized rats was associated with a reduction in lipopolysaccharide (LPS)-induced fever, compared with ovariectomized controls. LPS-fever reduction was accompanied by reduced levels of the inducible cyclooxygenase-2 (COX-2) protein expression in the hypothalamus as well as reduced plasma levels of IL-1β. The amount of LPS-induced IL-6 in the plasma was not affected by ovarian hormone replacement. In contrast, hypothalamic COX-2 expression in response to intraperitoneal injection of IL-1β was potentiated by the ovarian hormone replacement. IL-1β induced a moderate increase in plasma levels of IL-6 that was suppressed by ovarian hormone replacement. These data suggest that ovarian hormone replacement attenuated the proinflammatory response to LPS by suppressing the LPS-induced IL-1β production and COX-2 expression in the hypothalamus. The markedly different action of ovarian hormones on IL-1β and LPS effects suggests that this sex hormone modulation of the immune response is a function of the nature of infection and provides further evidence that LPS actions are different from those of IL-1β. (*Endocrinology* 144: 2454–2460, 2003)

FEVER IS CONSIDERED a hallmark of immune system activation. It consists of an actively regulated increase of core body temperature triggered on contact with pathogens (1). When exogenous pathogens (*e.g.* bacteria, virus) enter the host body, they activate synthesis of a multitude of endogenous proinflammatory cytokines (*e.g.* IL-1β, IL-6, TNFα). Peripherally produced cytokines signal to the fever responsive region of the hypothalamus via either a humoral or a neuronal pathway (2). The proinflammatory cytokines stimulate the central production of the inducible enzyme cyclooxygenase (COX)-2 (3–5) and subsequently the production of prostaglandins of the E series (3, 6). These prostaglandins activate neurons in the preoptic area of the hypothalamus (7–10), which engage heat conservation and heat production to elevate body temperature.

COX-2 is the major form of brain COX (11) and is induced almost solely in the endothelium of brain capillaries (4, 5) and perivascular cells (12) by proinflammatory cytokines, growth factors, and Gram negative bacteria-derived lipopolysaccharide (LPS) (11, 13, 14). The febrile response is closely related to the neoexpression of inducible COX-2 (3, 4, 15).

There is increasing evidence that immune responses are modulated by circulating sex hormones (16–22). However, our knowledge about the effects of sex steroids on fever development is scarce (23–26). We have previously shown IL-1β-induced fever amplitude and duration were elevated during the proestrus phase (in which ovarian hormone levels are high), compared with IL-1β fever at diestrus (in which ovarian hormones are their lowest levels). Furthermore, ovarian hormone replacement to ovariectomized rats enhanced IL-1β fever (26). The mechanisms through which ovarian hormones affect the febrile response are unclear.

Abbreviations: COX, Cyclooxygenase; LPS, lipopolysaccharide; NFκb, nuclear transcription factor κB; OVX, both ovaries removed.

In this study, we explored the effect of ovarian hormones on fever induced by Gram-negative bacteria-derived LPS. Bearing in mind that LPS-induced fever is partially mediated through IL-1β, we also investigated the effect of ovarian hormones on the neuroimmune effects of IL-1β. We asked the following questions: 1) Do estrogen and progesterone affect LPS-induced fever? 2) Can the ovarian hormones affect the expression of COX-2 in the hypothalamus after exposure to either endogenous (IL-1β) or exogenous pyrogens (LPS)? 3) Can the ovarian hormones modulate the circulating levels of the two major endogenous pyrogenic cytokines (IL-1β and IL-6) after exposure to either endogenous or exogenous pyrogens?

Materials and Methods

Female Sprague Dawley rats bred in the University of Calgary vivarium were kept in temperature-controlled quarters under a 12-h light, 12-h dark cycle (lights on 0700 h). They were individually housed, and pellet chow and water were accessible *ad libitum*. All experimental protocols were approved by the University of Calgary Animal Care Committee and carried out in accordance with the Canadian Council of Animal Care guidelines.

Ovariectomy, hormonal supplementation, and temperature measurement

Under sodium pentobarbital anesthesia (60 mg/kg ip), rats had both ovaries removed (OVX), and each rat had a precalibrated, battery-driven temperature transmitter (Mini-Mitter, Sun River, OR) implanted into the abdominal cavity. They were then returned to the vivarium to recover for 10 d after which they received a series of hormone injections to mimic hormonal changes that occur during proestrus and diestrus stages (27). On the morning of d 11 (0800 h) all rats received sc injection of a low dose (1 μg/kg) of estradiol-benzoate (1,3,5,10-estratrien-3,17β-diol3-benzoate, Steraloids, Inc., Wilton, NH) dissolved in peanut oil. On the morning of the next day (d 12 after ovariectomy), they received a larger dose of estradiol-benzoate (sc, 50 μg/kg). After 3.5–4 h (during which basal body temperatures were recorded), rats were given either an sc injection

of progesterone (4-pregnene-30,20-dione, 5 mg/kg, Sigma, St. Louis, MO) (E+Pr group) or peanut oil vehicle alone (E group). This hormone treatment regimen was followed immediately by ip injection of LPS (*Escherichia coli* serotype 026:B6, Sigma). Relatively low (50 μg/kg) and high (200 μg/kg) doses of LPS were used because the inducible COX-2 expression in brain vascular cell types is dependent on the strength of the immune challenge (12) and there is evidence that different fever profiles that arise from different doses of LPS may have different underlying mechanisms (28, 29). Core temperatures were monitored every 5 min for 6 h after LPS injection using a telemetry system (DataQuest II, Data Sciences Inc., St. Paul, MN). In subsequent independent experiments, OVX rats that received hormone replacement as described above were injected with either LPS or IL-1β (human IL-1beta, 10^8 U/mg, Immunex Corp., Seattle, WA) to study protein expression (COX-2) and cytokine release (IL-1β and IL-6). Circulating IL-1β levels in IL-1β-injected animals were also measured because IL-1β can stimulate its own release (30). Consequently, these rats were not implanted with temperature transmitters.

Protein extraction

Two and a half hours after ip injection of either LPS or IL-1β, which corresponds to the first phase of fever response (26) and LPS fever data in this study, all animals were deeply anesthetized with sodium pentobarbital (60 mg/kg ip) and transcardially perfused with PBS (NaCl, 137 mM; KCl, 2.7 mM; Na_2HPO_4, 10 mM; KH_2PO_4, 1.8 mM) to remove the blood. A basal diencephalon region that consists of the preoptic area and hypothalamus was quickly dissected and put in lysis buffer [3-(*N*-morpholino)propanesulfonic acid, 20 mM; $Mg(C_2H_3O_2)_2$, 4.5 mM; KCl, 150 mM; 1% of the detergent Triton X-100] supplemented with protease inhibitor tablets (Roche Diagnostics GmbH, Mannheim, Germany; catalog no. D68298) and a mixture of protease and phosphatase inhibitors (okadaic acid, catalog no. 495604: 1 μM; Microcystin-LR, catalog no. 475815: 1 μM, both purchased from Calbiochem, La Jolla, CA; sodium vanadate, catalog no. S-6508, Sigma: 1 mM; phenylmethylsulfonyl fluoride, catalog no. 837091, Roche Molecular Biochemicals Corp., Indianapolis, IN: 500 μM). After mechanical dissociation of the brain tissue, protein levels were assayed using a bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL; reagent A, catalog no. 23223 and reagent B, catalog no. 23224). The proteins were then put in a sample buffer composed of 125 mM Tris-HCl (T-1503, Sigma), 3% sodium dodecyl sulfate (Bio-Rad Laboratories, Inc., Richmond, CA; catalog no.161-0301), 20% glycerol (Sigma, G-5516), Bromophenol Blue (Sigma, B-5525), 20% β-mercaptoethanol (BDH Ltd., Poole, UK; catalog no. 44143) boiled for 5 min and stored at −20 C until Western blot analysis.

Western blot analysis

Protein extracts from each individual rat (30 μg/well) were separated on 10% SDS-PAGE using constant current of 30 mA/gel of 1.2 mm of thickness. Molecular weight markers (Bio-Rad Laboratories, Inc., catalog no. 161-0372) were used in each individual gel. At the end of separation, the proteins were transferred onto nitrocellulose membrane for 2 h under a constant current (1.2 mA/cm^2 of gel surface) using a transfer buffer containing 20% methanol, 50 mM Trizma base (Sigma), 40 mM glycine (Sigma), and 0.04% SDS (Bio-Rad Laboratories, Inc.). Membranes were then incubated overnight at 4 C with 10% fat-free milk in Tris-buffered saline containing Tween 20 composed of 20 mM Trizma-base (Sigma), 0.15 M sodium chloride (Fisher Scientific Co., Fairlawn, NJ), and 0.1% polyoxyethylene-sorbitan monolaurate (Sigma). A well characterized rabbit COX-2 antibody (Cayman Chemical Co., Ann Arbor, MI; catalog no. 160126) was used at 1:2000 (31, 32). The specificity of the COX-2 antibody was established by preabsorbing COX-2 antibody with the COX-2 antigen (Cayman Chemical Co., catalog no. 360106) for 1 h at room temperature. This preabsorption results in the disappearance of the COX-2 band. After 2 h of incubation with COX-2 antibody, the membrane was washed with Tris-buffered saline containing Tween 20 and incubated with goat antirabbit IgG conjugated with horseradish peroxidase (1:4000) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; catalog no. sc-2004) for 1 h at room temperature. A chemiluminescence substrate was applied to the membrane (ECL kit, Amersham, Little Chalfont, Buckinghamshire, UK), and protein bands were visualized using X-Omat film (Eastman Kodak Co., Rochester, NY). We also measured levels of actin, which served as housekeeping protein. After the COX-2 band was detected, the membrane was stripped with mercaptoethanol (BDH Ltd.) and reblotted with rabbit antiactin antibody (Sigma, catalog no. A2066) at 1:10,000 dilution.

Measurement of cytokine levels

OVX rats that received either complete hormonal replacement (E+Pr) or estrogen alone (E) were injected ip with either LPS (200 μg/kg) or IL-1β (1 μg/kg). Two and a half hours after the immune challenge, rats were anesthetized with sodium pentobarbital (60 mg/kg ip), trunk blood samples were taken, and plasma was extracted. Plasma levels of IL-1β and IL-6 were assayed using a specific rat ELISA kit (catalog no. KRC0012 and KRC0062, respectively; Biosource International, Camarillo, CA). All plasma samples were measured in the same assay. The minimum detectable concentration is less than 3 pg/ml for IL-1β assay and less than 8 pg/ml for IL-6 assay.

Data analysis

The average of body temperature recorded during 1 h immediately before LPS injection was used as baseline. Temperature readings at 10-min intervals (average of two consecutive 5-min readings) were calculated as net deviation from the mean baseline temperature. Proteins were quantified using a densitometer to give COX-2/actin ratios. These COX-2/actin ratios were normalized to the COX-2/actin values in rats treated with estrogen only (E group). All data are represented as means and SEM and were analyzed by two-way ANOVA followed by Student-Newman-Keuls *post hoc* pairwise comparisons. Following identification of significant differences between experimental groups, a two-tailed *t* test was used to reveal significance at key points. Significance was accepted at the 0.05 level.

Results

Ovarian hormone effects on fever response to LPS (50 μg/kg ip)

In the first experiment, OVX rats that were treated with estrogen/progesterone (E+Pr) or estrogen alone (E) received an ip injection of a febrile dose of LPS (50 μg/kg), and the fever response was monitored for 6 h after the injection. Basal core temperature was similar in both the E+Pr (37.81 ± 0.29 C, n = 8) and E groups (37.64 ± 0.20 C, n = 6). Immediately after LPS injection, all rats displayed a stress-induced hyperthermia because of handling (Fig. 1). The peak of the stress-induced hyperthermia occurred about 30 min after LPS injection. After this first peak, body temperature of the E group returned to baseline, whereas the E+Pr group stayed stable for about an hour. LPS-induced fever consisted of early- and late-phase peaks that occur at about 2.5 h and 4.5 h after the injection of LPS. Both groups reached an identical fever magnitude during the early phase of fever response. However, sequential estrogen and progesterone injections reduced significantly the febrile response to LPS during the late-phase peak (Fig. 1).

Ovarian hormone effects on fever response to LPS (200 μg/kg)

In this experiment, a second group of female rats were ovariectomized, received ovarian hormone replacement as described above, and were given a higher dose of LPS (200 μg/kg ip). Both E+Pr and E groups had similar basal core temperatures of 37.38 ± 0.11 C (n = 10) and 37.59 ± 0.13 C (n = 8), respectively. Both groups raised their body temperature to a peak at 30 min because of handling (Fig. 2A). The febrile response to LPS at both the early and late phases was

significantly reduced in the E+Pr group. Fever index, which integrates the increase in body temperature over the 6 h after LPS injection (200 μg/kg), similarly showed that E+Pr treatment group showed a significant attenuation in fever response, compared with the E group (Fig. 2B).

Ovarian hormone effects on COX-2 expression in the hypothalamus in response to LPS

A third group of female rats were ovariectomized and separated into E+Pr (n = 7) or E (n = 6) groups. Two and a half hours after ip injection of LPS (200 μg/kg), hypothalami were removed and proteins were extracted and individually separated by electrophoresis as described in *Materials and Methods*. Densitometric analysis showed that COX-2 expression after LPS injection was significantly reduced in the E+Pr group, compared with the E group (Fig. 3).

Ovarian hormone effects on COX-2 expression in the hypothalamus in response to IL-β

In this experiment, a different group of female rats was ovariectomized and received either estrogen/progesterone (E+PrP, n = 5) or estrogen only (E, n = 4). All these rats received IL-1β (1 μg/kg ip). In contrast to what had been seen with LPS, COX-2 expression in the hypothalamus of the E+Pr group was higher, compared with the E group (Fig. 4, A and B).

Ovarian hormone effects on plasma levels of cytokines in response to LPS or IL-1β

An independent group of rats was ovariectomized and received hormonal treatment as described above. Each of the hormonal treatment groups was injected with either LPS (200 μg/kg) or IL-1β (1 μg/kg). LPS-induced IL-1β levels in the plasma of the E+Pr group (n = 6, Fig. 5A) were suppressed, compared with the E group (n = 6). IL-1β was undetected in the plasma of rats that received ip injection of IL-1β (E+Pr group n = 6, E group n = 6; Fig. 5A). Plasma levels of IL-6 induced by LPS are more than 30-fold higher than levels of IL-6 induced by IL-1β (Fig. 5B). Ovarian hormone treatment did not affect the plasma level of IL-6 induced by LPS. As shown in Fig. 5B, the small effect of IL-1β on plasma IL-6 observed in the E group (n = 6) was suppressed in E+Pr group (n = 6).

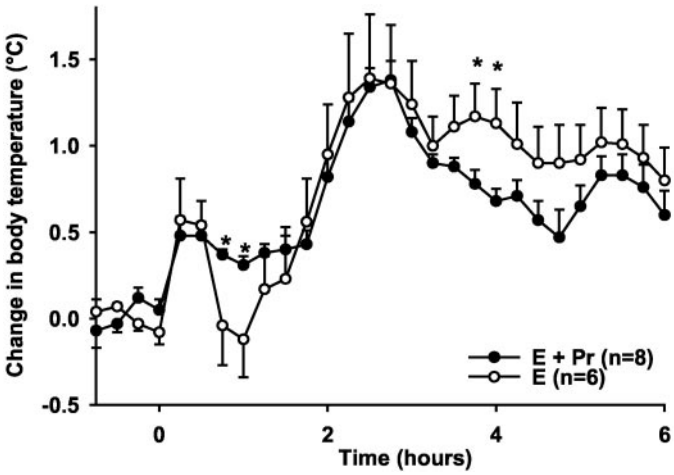

FIG. 1. Ovarian hormones effect on LPS (50 μg/kg)-induced fever. OVX animals received sc injection of either estrogen/progesterone (E+Pr) or estrogen only (E); subsequently they were ip injected with LPS (50 μg/kg) at time zero. Fever response was recorded for 6 h post LPS injection. The second phase of LPS fever was reduced in OVX rats injected with estrogen/progesterone *(filled circles)*, compared with OVX rats injected with estrogen alone *(open circles)*. The number (n) indicates the number of animals per group, *, $P < 0.05$.

Discussion

Sex hormone replacement in ovariectomized rats attenuated LPS-induced fever and reduced COX-2 expression in the hypothalamus. The ovarian hormones also suppressed plasma levels of LPS-induced IL-1β but not that of LPS-induced IL-6. In contrast, endogenous pyrogen injection (IL-1β) resulted in enhanced fever (26) and enhanced COX-2 expression in the hypothalami of rats that received ovarian

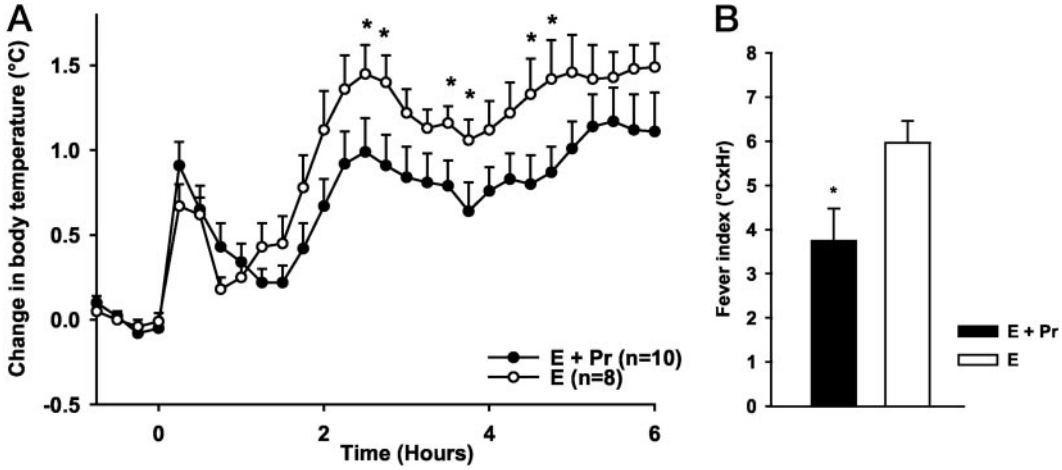

FIG. 2. Ovarian hormone effects of LPS (200 μg/kg)-induced fever. OVX animals received sc injection of either estrogen/progesterone (E+Pr) or estrogen alone (E); subsequently they were ip injected with LPS (200 μg/kg) at time zero. Fever response was recorded for 6 h post LPS injection. Both early and late phases of LPS fever were reduced in OVX rats injected with estrogen/progesterone *(filled circles)*, compared with OVX rats injected with estrogen alone *(open circles)*. The number (n) indicates the number of animals per group. *, $P < 0.05$.

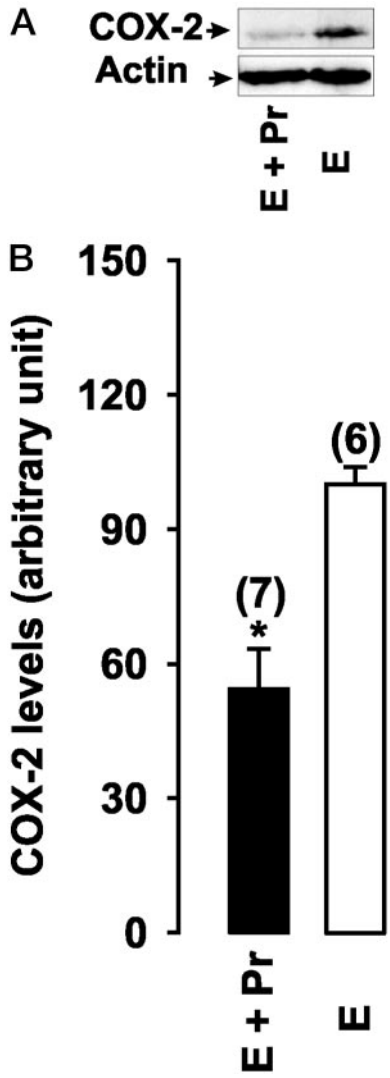

FIG. 3. Estrogen-progesterone effects on LPS-induced COX-2 protein expression. Intraperitoneal injection of endotoxin (LPS: 200 μg/kg) induced COX-2 expression in the hypothalamic region of female rat. OVX rats that received sc injections of estrogen/progesterone (E+Pr) showed reduced LPS-induced COX-2 protein expression in the hypothalamus, compared with OVX rats that received estrogen alone (E). Arbitrary unit values of OD analysis of COX-2/actin ratios, normalized to the COX-2/actin ratios in rats that received oil instead of progesterone, are shown in Fig. 3B. The *number in parentheses* indicates the number of animals per group. *, $P < 0.05$.

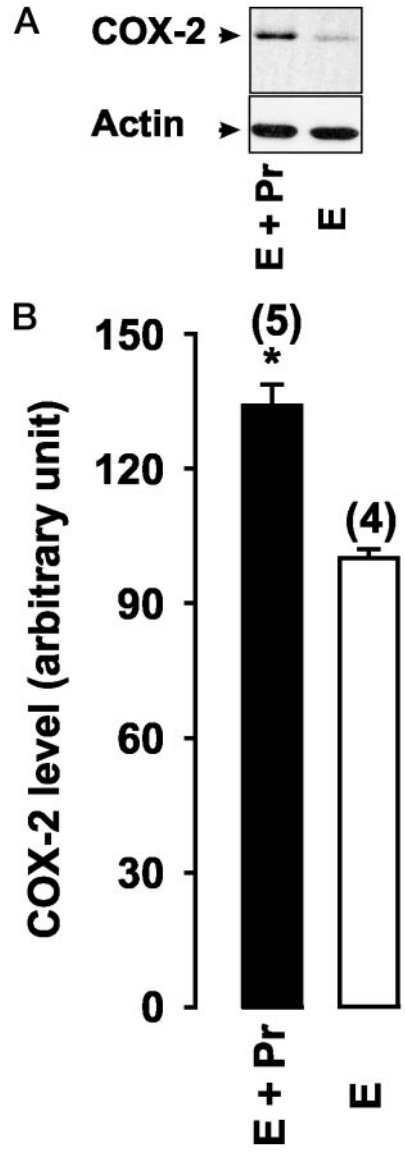

FIG. 4. Estrogen-progesterone effects on IL-1β-induced COX-2 protein expression. OVX rats that received sc injections of estrogen/progesterone (E+Pr) showed enhanced IL-1β (1 μg/kg)-induced COX-2 protein expression in the hypothalamus, compared with OVX rats that received estrogen alone (E) (A). The *number in parentheses* indicates the number of animals per group. *, $P < 0.05$.

hormone replacement. These data strongly suggest that the reduction in the febrile response to LPS is due to a suppressive effect of these ovarian hormones on LPS-induced release of endogenous pyrogens (such as IL-1β) from peripheral macrophages. The data presented in this study suggest that ovarian hormones may affect an upstream component of the LPS-activated pathway that leads to COX-2 expression. Indeed, circulating levels of IL-1β induced by LPS were suppressed by hormonal replacement. This reduced IL-1β level in the plasma may account, in part, for the attenuated LPS-induced fever. Interestingly, LPS induced similar high circulating levels of IL-6 in the presence or absence of complete hormone replacement, thus suggesting that IL-6 is not the ovarian hormone target for attenuating LPS fever. Our data also indicate that IL-6 production does not seem to be dependent on the circulating levels of IL-1β. Despite the general view of the mandatory role of IL-1β in LPS-induced IL-6 production, LPS can stimulate IL-6 production in the absence of the IL-1β gene (33). Similarly, depletion of macrophages does not affect LPS-induced IL-6 but blunts plasma level of IL-1β induced by LPS (34, 35). Our data do not, however, rule out a role for an interaction between IL-1β and IL-6 because IL-1β has been shown to sensitize brain tissues to IL-6 effects (36). Thus, the decrease in IL-1β is sufficient to lower fever.

IL-6 is regarded as the ultimate mediator of LPS fever because mice with disrupted IL-6 gene lack febrile response to both IL-1β and LPS (37). Our observation that LPS fever

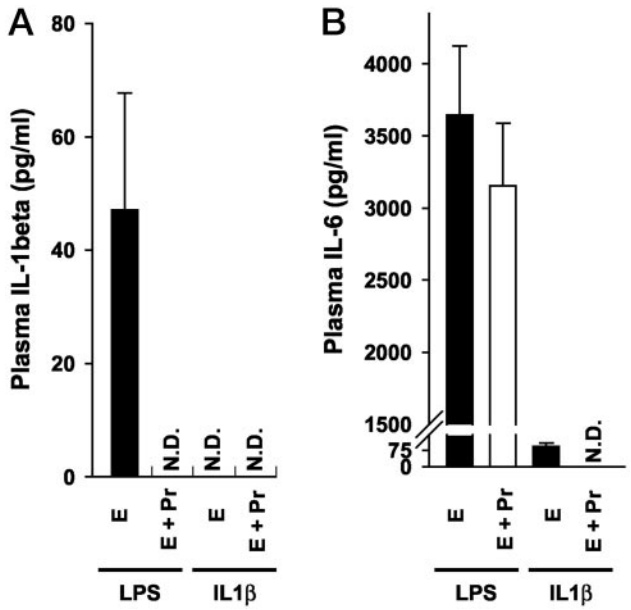

FIG. 5. Estrogen-progesterone effects on LPS- or IL-1β-induced cytokines production. A, OVX rats that received sc injection of estrogen/progesterone (E+Pr, n = 6) showed a complete suppression of plasma levels of IL-1β (A) induced by LPS (200 μg/kg), compared with OVX rats that did receive estrogen alone (E, n = 6). No detectable circulating levels of IL-1β were observed in IL-1β (1 μg/kg) ip-injected rats in both E+Pr (n = 6) and E (n = 6) groups. B, LPS-induced release of IL-6 in the plasma was similarly high in both rats that received estrogen/progesterone (n = 6) and in rats that received estrogen alone (n = 6). IL-1β induced a small increase in plasma levels of IL-6 in rats that received estrogen alone (n = 6). Hormonal replacement inhibited IL-1β-induced IL-6 production (n = 6).

is attenuated but not totally suppressed by ovarian hormone replacement despite equal circulating levels of IL-6 favor the idea that the synergistic effect of IL-1β and IL-6 causes a more enhanced fever. Indeed, IL-1β enhances IL-6 receptor gene expression but not IL-6 gene itself in rat brains (36). We chose a time during the early phase of fever in which IL-6 protein and mRNA levels are maximally induced (36) to measure circulating cytokine levels (38), but the possibility cannot be ruled out that at some later phase of the fever, levels would be different. However, if this were the case, it would be difficult to explain how fever would be altered earlier than this. A final possibility that we cannot discount is that estrogen/progesterone regimen may have a direct effect on neuronal activity in addition to that on cytokines.

It is noteworthy that the plasma concentrations of IL-1β and IL-6 are in agreement with previous studies (39, 40). These data are also in agreement with previous findings in which estrogen and progesterone were shown to exert an inhibitory effect on IL-1β mRNA synthesis (41) and IL-1β release but not IL-6 secretion by human peripheral blood mononuclear cells (42). These results are also in line with studies in women. Indeed, in premenopausal women, the levels of endotoxin-induced cytokines released from immune cells vary with the menstrual cycle. Namely, the luteal phase, characterized by a high circulating levels of progesterone is associated with a reduced cytokine release from monocytes exposed to LPS (43, 44). Whether ovarian hormones also affect antiinflammatory cytokines is not clear. Although elevated levels of IL-10 were correlated with sex steroid levels in peritoneal fluid of patients with ovarian hyperstimulation syndrome (45), estrogen inhibits endotoxin-induced IL-1ra in postmenopausal women (46) and did not affect LPS-induced IL-10 production by macrophages (47). We are not aware of any published work that explored the combined effect of estrogen and progesterone on antiinflammatory cytokines during immune system activation.

It was curious to observe that the attenuated fever response induced by ovarian hormone replacement was more pronounced when rats received relatively higher doses of LPS. Besides its pyrogenic effect, LPS activates the hypothalamo-pituitary-adrenal axis (48) that contributes to the production of antipyretic glucocorticoids (49). It is possible that this negative feedback is potentiated by ovarian hormone replacement when female rats are exposed to higher doses of endotoxins (50, 51).

In addition to their effects on circulating cytokines, ovarian hormones can exert their modulatory effects at the transcriptional level of the immune-activated *cox-2* gene. The *cox-2* gene activation and COX-2 protein induction mainly are due to an activation of upstream proinflammatory signaling molecules, namely the dissociation between the inhibitory κB (Iκb) and nuclear transcription factor κB (NFκb) and subsequently the translocation of NFκb from the cytoplasm to activate transcription of *cox-2* gene (52). There is evidence that ovarian hormones reduce LPS-induced COX-2 via an inhibition of NFκb activation (53–56).

It is generally accepted that IL-1β is an important component of the LPS-activated cascade of cytokines involved in inflammatory responses (57). However, we were surprised to find that sex hormone replacement in OVX rats attenuated LPS fever (Figs. 1 and 2), whereas it potentiated IL-1β-induced fever (26). Similarly, LPS-induced COX-2 expression in the hypothalamus was reduced, whereas IL-1β-induced COX-2 was enhanced in estrogen/progesterone-treated rats (Figs. 3 and 4). These differential effects of LPS and IL-1β can be explained by the suppressive effect of ovarian hormones on LPS-induced plasma IL-1β (Fig. 5A). The reduction of LPS fever in estrogen/progesterone-treated animals can thus be brought about, at least in part, by reduced LPS-induced IL-1β. However, it is also possible that ovarian hormones may modulate either the binding properties or expression levels of IL-1β and IL-6 in the hypothalamus (58). In addition, a direct effect of ovarian hormones on LPS receptors cannot be discounted (59, 60).

The reported thermogenic property of ovarian hormones, namely that of progesterone (61, 62), seems to contrast with both our observed ovarian hormone-induced attenuated LPS fever and the similar baseline temperatures in both groups of rats. This apparent discrepancy can be explained by differences in the experimental design we and others had used. In the above-mentioned reports, the thermogenic effect of ovarian hormones in rats is observed only when ovarian hormones were injected daily for at least 14 d. The current results are similar to those we reported previously in which ovarian hormone replacement did not affect nonfebrile temperatures (26). In addition to its action through an IL-1β-dependent pathway, LPS can induce an inflammatory response through an IL-1β-independent pathway (63). For

instance, systemic injection of LPS induces COX-2 mRNA expression in endothelial cells of the blood vessels in the brain of transgenic mice that lack IL-1β (64). The possibility that ovarian hormones may target a component of an IL-1β-independent pathway cannot be ruled out.

The reduction in the magnitude of fever and COX-2 induction brought about by estrogen/progesterone treatment raises questions as to the relevance of these findings to the human condition. Very few studies had investigated the occurrence or lack thereof of fever at various stages of the menstrual cycle or after menopause in women (65–67). However, this could be of interest, given the fact that fever is thought to have a survival value, *i.e.* it is of benefit in fighting infections (1). Similarly, one might ask whether hormonal replacement therapy after menopause may have potential deleterious effects on the body's ability to respond to Gram-negative infection (68).

These data underlie the complexity of the interaction between the female gonadal axis and the immune system activation and call for more studies to understand the differential effects of sex hormones with regard to the nature and route of infection.

Acknowledgments

We appreciate the comments of Dr. Stephen Kent.

Received December 2, 2002. Accepted February 28, 2003.

Address all correspondence and requests for reprints to: Abdeslam Mouihate, Ph.D., Neuroscience Research Group, Department of Physiology and Biophysics, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1. E-mail: mouihate@ucalgary.ca.

This work was supported by the Canadian Institutes of Health Research. Q.J.P. is an Alberta Heritage Foundation for Medical Research Medical Scientist.

References

1. **Kluger MJ** 1991 Fever: role of pyrogens and cryogens. Physiol Rev 71:93–127
2. **Dantzer R, Konsman JP, Bluthe RM, Kelley KW** 2000 Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? Auton Neurosci 85:60–65
3. **Cao CY, Matsumura K, Yamagata K, Watanabe Y** 1997 Involvement of cyclooxygenase-2 in LPS-induced fever and regulation of its mRNA by LPS in the rat brain. Am J Physiol 272:R1712–R1725
4. **Matsumura K, Cao C, Ozaki M, Morii H, Nakadate K, Watanabe Y** 1998 Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever: light and electron microscopic immunocytochemical studies. J Neurosci 18:6279–6289
5. **Lacroix S, Rivest S** 1998 Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. J Neurochem 70:452–466
6. **Komaki G, Arimura A, Koves K** 1992 Effect of intravenous injection of IL-1 beta on PGE2 levels in several brain areas as determined by microdialysis. Am J Physiol 262:E246–E251
7. **Lipton JM, Welch JP, Clark WG** 1973 Changes in body temperature produced by injecting prostaglandin E1, EGTA and bacterial endotoxins into the PO-AH region and the medulla oblongata of the rat. Experientia 29:806–808
8. **Williams JW, Rudy TA, Yaksh TL, Viswanathan CT** 1977 An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. Brain Res 120:251–262
9. **Stitt JT** 1991 Differential sensitivity in the sites of fever production by prostaglandin E1 within the hypothalamus of the rat. J Physiol 432:99–110
10. **Scammell TE, Elmquist JK, Griffin JD, Saper CB** 1996 Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. J Neurosci 16:6246–6254
11. **Feng L, Sun W, Xia Y, Tang WW, Chanmugam P, Soyoola E, Wilson CB, Hwang D** 1993 Cloning two isoforms of rat cyclooxygenase: differential regulation of their expression. Arch Biochem Biophys 307:361–368
12. **Schiltz JC, Sawchenko PE** 2002 Distinct brain vascular cell types manifest inducible cyclooxygenase expression as a function of the strength and nature of immune insults. J Neurosci 22:5606–5618
13. **O'Banion MK** 1999 Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. Crit Rev Neurobiol 13:45–82
14. **Hinz B, Brune K** 2002 Cyclooxygenase-2–10 years later. J Pharmacol Exp Ther 300:367–375
15. **Matsumura K, Cao C, Watanabe Y** 1997 Possible role of cyclooxygenase-2 in the brain vasculature in febrile response. Ann NY Acad Sci 813:302–306
16. **Krzych U, Strausser HR, Bressler JP, Goldstein AL** 1981 Effects of sex hormones on some T and B cell functions as evidenced by differential immune expression between male and female mice and cyclic pattern of immune responsiveness during the estrous cycle. Prog Clin Biol Res 70:145–150
17. **Weinstein Y, Ran S, Segal S** 1984 Sex-associated differences in the regulation of immune responses controlled by the MHC of the mouse. J Immunol 132:656–661
18. **Myers MJ, Petersen BH** 1985 Estradiol induced alterations of the immune system: I. Enhancement of IgM production. Int J Immunopharmacol 7:207–213
19. **Erbach GT, Bahr JM** 1988 Effect of chronic or cyclic exposure to estradiol on the humoral immune response and the thymus. Immunopharmacology 16:45–51
20. **Gallichan WS, Rosenthal KL** 1996 Effects of the estrous cycle on local humoral immune responses and protection of intranasally immunized female mice against herpes simplex virus type 2 infection in the genital tract. Virology 224:487–497
21. **Van Vollenhoven RF, McGuire JL** 1994 Estrogen, progesterone, and testosterone: can they be used to treat autoimmune diseases? Cleve Clin J Med 61:276–284
22. **Schuurs AH, Verheul HA** 1990 Effects of gender and sex steroids on the immune response. J Steroid Biochem 35:157–172
23. **Pittman QJ, Malkinson TJ, Kasting NW, Veale WL** 1988 Enhanced fever following castration: possible involvement of brain arginine vasopressin. Am J Physiol 254:R513–R517
24. **Martin SM, Malkinson TJ, Veale WL, Pittman QJ** 1996 Prostaglandin fever in rats throughout the estrous cycle late pregnancy and post parturition. J Neuroendocrinol 8:145–151
25. **Heath HL, Blagburn BL, Elsasser TH, Pugh DG, Sanders LG, Sartin EA, Steele B, Sartin JL** 1997 Hormonal modulation of the physiologic responses of calves infected with Eimeria bovis. Am J Vet Res 58:891–896
26. **Mouihate A, Chen X, Pittman QJ** 1998 Interleukin-1β fever in rats: gender difference and estrous cycle influence. Am J Physiol 275:R1450–R1454
27. **Boling JL, Blandau RJ** 1939 The estrogen-progesterone induction of mating responses in the spayed female rats. Endocrinology 25:359–364
28. **Romanovsky AA, Kulchitsky VA, Akulich NV, Koulchitsky SV, Simons CT, Sessler DI, Gourine VN** 1997 The two phases of biphasic fever—two different strategies for fighting infection? Ann NY Acad Sci 813:485–490
29. **Romanovsky AA, Kulchitsky VA, Simons CT, Sugimoto N** 1998 Methodology of fever research: why are polyphasic fevers often thought to be biphasic? Am J Physiol 275:R332–R338
30. **Dinarello CA, Ikejima T, Warner SJ, Orencole SF, Lonnemann G, Cannon JG, Libby P** 1987 Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits *in vivo* and in human mononuclear cells *in vitro.* J Immunol 139:1902–1910
31. **Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF** 1993 Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron 11:371–386
32. **Mouihate A, Clerget-Froidevaux MS, Nakamura K, Negishi M, Wallace JL, Pittman QJ** 2002 Suppression of fever at near term is associated with reduced COX-2 protein expression in rat hypothalamus. Am J Physiol 283:R800–R805
33. **Alheim K, Chai Z, Fantuzzi G, Hasanvan H, Malinowsky D, Di Santo E, Ghezzi P, Dinarello CA, Bartfai T** 1997 Hyperresponsive febrile reactions to interleukin (IL) 1α and IL-1β, and altered brain cytokine mRNA and serum cytokine levels, in IL-1β-deficient mice. Proc Natl Acad Sci USA 94:2681–2686
34. **Derijk R, van Rooijen N, Tilders FJ, Besedovsky HO, Del Rey A, Berkenbosch F** 1991 Selective depletion of macrophages prevents pituitary-adrenal activation in response to subpyrogenic, but not to pyrogenic, doses of bacterial endotoxin in rats. Endocrinology 129:330–338
35. **Derijk RH, Strijbos PJ, van Rooijen N, Rothwell NJ, Berkenbosch F** 1993 Fever and thermogenesis in response to bacterial endotoxin involve macrophage-dependent mechanisms in rats. Am J Physiol 265:R1179–R1183
36. **Vallieres L, Rivest S** 1997 Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1β. J Neurochem 69:1668–1683
37. **Chai Z, Gatti S, Toniatti C, Poli V, Bartfai T** 1996 Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1β: a study on IL-6-deficient mice. J Exp Med 183:311–316
38. **Cartmell T, Poole S, Turnbull AV, Rothwell NJ, Luheshi GN** 2000 Circulating interleukin-6 mediates the febrile response to localised inflammation in rats. J Physiol 526(Part 3):653–661
39. **Rees GS, Ball C, Ward HL, Gee CK, Tarrant G, Mistry Y, Poole S, Bristow AF** 1999 Rat interleukin 6: expression in recombinant *Escherichia coli,* purification and development of a novel ELISA. Cytokine 11:95–103
40. **Johnson JD, O'Connor KA, Deak T, Stark M, Watkins LR, Maier SF** 2002

Prior stressor exposure sensitizes LPS-induced cytokine production. Brain Behav Immun 16:461–476
41. **Polan ML, Loukides J, Nelson P, Carding S, Diamond M, Walsh A, Bottomly K** 1989 Progesterone and estradiol modulate interleukin-1β messenger ribonucleic acid levels in cultured human peripheral monocytes. J Clin Endocrinol Metab 69:1200–1206
42. **Li ZG, Danis VA, Brooks PM** 1993 Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells *in vitro.* Clin Exp Rheumatol 11:157–162
43. **Schwarz E, Schafer C, Bode JC, Bode C** 2000 Influence of the menstrual cycle on the LPS-induced cytokine response of monocytes. Cytokine 12:413–416
44. **Konecna L, Yan MS, Miller LE, Scholmerich J, Falk W, Straub RH** 2000 Modulation of IL-6 production during the menstrual cycle *in vivo* and *in vitro.* Brain Behav Immun 14:49–61
45. **Manolopoulos K, Lang U, Gips H, Braems GA** 2001 Elevated interleukin-10 and sex steroid levels in peritoneal fluid of patients with ovarian hyperstimulation syndrome. Eur J Obstet Gynecol Reprod Biol 99:226–231
46. **Puder JJ, Freda PU, Goland RS, Wardlaw SL** 2001 Estrogen modulates the hypothalamic-pituitary-adrenal and inflammatory cytokine responses to endotoxin in women. J Clin Endocrinol Metab 86:2403–2408
47. **Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD** 1997 Estradiol down-regulates LPS-induced cytokine production and NFκB activation in murine macrophages. Am J Reprod Immunol 38:46–54
48. **Lenczowski MJ, Van Dam AM, Poole S, Larrick JW, Tilders FJ** 1997 Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS. Am J Physiol 273:R1870–R1877
49. **Morrow LE, McClellan JL, Klir JJ, Kluger MJ** 1996 The CNS site of glucocorticoid negative feedback during LPS- and psychological stress-induced fevers. Am J Physiol 271:R732–R737
50. **Shanks N, McCormick CM, Meaney MJ** 1994 Sex differences in hypothalamic-pituitary-adrenal responding to endotoxin challenge in the neonate: reversal by gonadectomy. Brain Res Dev Brain Res 79:260–266
51. **Frederic F, Oliver C, Wollman E, Delhaye-Bouchaud N, Mariani J** 1993 IL-1 and LPS induce a sexually dimorphic response of the hypothalamo-pituitary-adrenal axis in several mouse strains. Eur Cytokine Netw 4:321–329
52. **Rivest S** 1999 What is the cellular source of prostaglandins in the brain in response to systemic inflammation? Facts and controversies. Mol Psychiatry 4:500–507
53. **Sharma RV, Gurjar MV, Bhalla RC** 2001 Selected contribution: estrogen receptor-α gene transfer inhibits proliferation and NF-κB activation in VSM cells from female rats. J Appl Physiol 91:2400–2406
54. **Evans MJ, Eckert A, Lai K, Adelman SJ, Harnish DC** 2001 Reciprocal antagonism between estrogen receptor and NF-κB activity *in vivo.* Circ Res 89:823–830
55. **Van der Burg B, van der Saag PT** 1996 Nuclear factor-κ-B/steroid hormone receptor interactions as a functional basis of anti-inflammatory action of steroids in reproductive organs. Mol Hum Reprod 2:433–438
56. **McKay LI, Cidlowski JA** 1999 Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signaling pathways. Endocr Rev 20:435–459
57. **Kakucska I, Qi Y, Clark BD, Lechan RM** 1993 Endotoxin-induced corticotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is mediated centrally by interleukin-1. Endocrinology 133:815–821
58. **Watanobe H, Yoneda M** 2003 A mechanism underlying the sexually dimorphic ACTH response to lipopolysaccharide in rats: sex steroid modulation of cytokine binding sites in the hypothalamus. J Physiol 547:221–232
59. **Laflamme N, Rivest S** 2001 Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. FASEB J 15:155–163
60. **Nguyen MD, Julien JP, Rivest S** 2002 Innate immunity: the missing link in neuroprotection and neurodegeneration? Nat Rev Neurosci 3:216–227
61. **Freeman ME, Crissman Jr JK, Louw GN, Butcher RL, Inskeep EK** 1970 Thermogenic action of progesterone in the rat. Endocrinology 86:717–720
62. **Marrone BL, Gentry RT, Wade GN** 1976 Gonadal hormones and body temperature in rats: effects of estrous cycles, castration and steroid replacement. Physiol Behav 17:419–425
63. **Dunn AJ** 2000 Effects of the IL-1 receptor antagonist on the IL-1- and endotoxin-induced activation of the HPA axis and cerebral biogenic amines in mice. Neuroimmunomodulation 7:36–45
64. **Laflamme N, Lacroix S, Rivest S** 1999 An essential role of interleukin-1β in mediating NF-κB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. J Neurosci 19:10923–10930
65. **Brooks EM, Morgan AL, Pierzga JM, Wladkowski SL, O'Gorman JT, Derr JA, Kenney WL** 1997 Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women. J Appl Physiol 83:477–484
66. **Brooks-Asplund EM, Cannon JG, Kenney WL** 2000 Influence of hormone replacement therapy and aspirin on temperature regulation in postmenopausal women. Am J Physiol 279:R839–R848
67. **Tankersley CG, Nicholas WC, Deaver DR, Mikita D, Kenney WL** 1992 Estrogen replacement in middle-aged women: thermoregulatory responses to exercise in the heat. J Appl Physiol 73:1238–1245
68. **Pfeilschifter J, Koditz R, Pfohl M, Schatz H** 2002 Changes in proinflammatory cytokine activity after menopause. Endocr Rev 23:90–119