Interleukin-1 receptor antagonist as a modulator of gender differences in the febrile response to lipopolysaccharide in rats

H. Ashdown,1 S. Poole,2 P. Boksa,1 and G. N. Luu申shi1

1McGill University, Department of Psychiatry, Douglas Hospital Research Centre, Montreal, Quebec, Canada; 2National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire, United Kingdom

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Ashdown H, Poole S, Boksa P, Luu申shi GN. Interleukin-1 receptor antagonist as a modulator of gender differences in the febrile response to lipopolysaccharide in rats. Am J Physiol Regul Integr Comp Physiol 292: R1667–R1674, 2007. First published November 30, 2006; doi:10.1152/ajpregu.00274.2006.—Febrile responses to bacterial pathogens are attenuated near term of pregnancy in several mammalian species. It is unknown, however, whether this reflects a fundamental physiological adaptation of female rats or whether it is specific to pregnancy. The aims of this study therefore were 1) to determine whether febrile responses to the bacterial endotoxin lipopolysaccharide (LPS) are attenuated in female vs. male rats and, if so, to identify possible mechanisms involved in modulating this and 2) to assess whether plasma concentrations of the anti-inflammatory cytokine, interleukin-1 receptor antagonist (IL-1ra), an important regulator of fever, are dependent on the physiological state of the female and could therefore be involved in modulating febrile responses. We found febrile responses were attenuated in cycling female vs. male rats and also in near-term pregnant dams vs. cycling females after intraperitoneal injection of LPS (0.05 mg/kg). Plasma levels of IL-1ra were significantly greater in female rats after injection of LPS, particularly during pregnancy, than in males. This was accompanied by attenuated levels of hypothalamic IL-1β and cyclooxygenase-2 mRNA, two key mediators of the febrile response, in female rats. Furthermore, increasing plasma levels of IL-1ra in male rats by intraperitoneal administration of the recombinant antagonist attenuated hypothalamic mRNA levels of these mediators after LPS. These data suggest that there is a fundamental difference in febrile response to LPS between the genders that is likely regulated by IL-1ra. This may be an important mechanism that protects the developing fetus from potentially deleterious consequences of maternal fever during pregnancy.

FEVER IS A COMPLEX PHYSIOLOGICAL response mounted by the host to facilitate the resolution of infection after exposure to invading viral or bacterial pathogens. This central nervous system orchestrated response involves the production and action of prostaglandins (PGE2) on hypothalamic thermosensitive neurons, a process that is initiated through the action of systemic pyrogenic mediators of the cytokine family (16, 59). These key inflammatory proteins are readily induced after exposure to exogenous pyrogens, such as the bacterial product lipopolysaccharide (LPS), and increase in the periphery to reflect the rise in body temperature (16, 51). The proinflammatory cytokine IL-1β is a major peripheral mediator of LPS-induced fever, which is known to trigger increases in body temperature via cyclooxygenase-2 (COX-2)-dependent production of PGE2 in the brain (35). Studies in experimental animals have demonstrated that recombinant IL-1β, administered either systemically or directly into the brain, induces fever (2, 17) and that neutralization of endogenous IL-1β attenuates fever (26, 32, 36). The majority of these neutralization studies were conducted with a recombinant form of the naturally occurring IL-1 receptor antagonist (IL-1ra) (37, 52, 64), which inhibits the action of IL-1 by competing for the IL-1 receptor (16). Like IL-1, this endogenous inhibitor is induced in the periphery after LPS challenge, and its levels increase in the circulation of febrile animals in parallel with IL-1β (3). We have previously shown that increases in circulating levels of this antagonist act to “dampen” the pyrogenic effects of IL-1 in vivo (37). Furthermore, we demonstrated that neutralization of endogenous IL-1ra in vivo, using a specific antiserum, results in significantly higher fever responses to LPS in rats (12). The ability of IL-1ra to limit the pyrogenic effects of endogenous IL-1 during infection may be especially important in situations where increases in body temperature could be particularly detrimental, such as to the developing fetus during gestation (4, 18, 30, 42).

It is now well documented that febrile responses to infectious agents are significantly attenuated in several mammalian species near term of pregnancy (14, 19, 25, 41, 43, 69). Although it is tempting to speculate that this may in part be due to the actions of endogenous IL-1ra, little evidence exists to support this to date. Recent studies have demonstrated that the attenuated febrile response to LPS observed in rats near term correlated with reduced induction of COX-2 protein in the hypothalamus (22, 46). Given that peripheral IL-1 regulates brain expression of COX-2 (31, 35) and that the endogenous antagonist IL-1ra regulates IL-1 activity (12, 37), it is plausible that IL-1ra may indeed have an important role in attenuating brain levels of COX-2, and subsequently the magnitude of the fever response during pregnancy. However, although this would seem feasible, there have been contradictory reports in the literature regarding the importance of this anti-inflammatory cytokine after exposure of pregnant rats to LPS. One study reported increased expression of IL-1ra in pregnant vs. cycling female rats (20), whereas another observed comparable plasma levels of this cytokine in dams at different stages of pregnancy vs. lactating dams (47). In addition, it is unclear whether the attenuation of febrile responses observed near term is a physiological adaptation that is specific to pregnancy or whether differences also exist in the febrile responses to LPS in female vs. male rats. For example, one study found that febrile responses to IL-1β (given intraperitoneally) were comparable...
in male and female rats (45), whereas another demonstrated that febrile responses were significantly greater in male vs. cycling female rats after intravenous injection of LPS (49).

Given our earlier findings suggesting that the endogenous inhibitor IL-1ra is acting to regulate IL-1-mediated fever (12) and the critical role played by IL-1 in fever in general (16), we hypothesized that increased levels of circulating IL-1ra in pregnant vs. cycling females could be responsible for the observed suppression of the LPS-induced fever in late gestation. We also speculated that the responses observed during pregnancy may in fact be indicative of a more fundamental difference in the febrile response to LPS between males and females that could also be regulated by IL-1ra. The aim of the present investigation, therefore, was to assess whether gender influences LPS-induced fever in rats and to delineate whether IL-1ra could have a role in regulating fever responses to LPS in female rats during pregnancy.

MATERIALS AND METHODS

Animals. Randomly cycling females, pregnant dams on day 18 of gestation, and male Sprague-Dawley rats (Charles River, Montreal, Canada), with an average body weight of 243.67 ± 7.06, and 290.6 ± 5.6 g, respectively, were used in the experiments included in this study. The animals were housed individually in a controlled environment at an ambient temperature of 21 ± 2°C and a 12:12-h light-dark cycle (lights on from 0800 to 2000). Food and water were provided ad libitum. All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee.

In all experiments, animals received a single intraperitoneal injection of LPS (0.05 mg/kg; Escherichia coli 0111:B4, lot 42K4120; Sigma) or saline alone (1 ml/kg) at time 0 and human recombinant IL-1ra (1 mg/kg; National Institute for Biological Standards and Control, Hertfordshire, UK) or saline (1 ml/kg) at time 0 and 1 h where indicated.

Measurement of body temperature using remote biotelemetry. Changes in core body temperature were monitored after injection of LPS or saline with the use of remote radiobiotlemetry, as described previously (21). Temperature-sensitive transmitters (DataSciences) were implanted in the body cavity of anesthetized rats (50 mg/kg ketamine, 5 mg/kg xylazine, and 0.5 mg/kg acepromazine; 1 ml/g body wt), and the animals were allowed to recover for at least 5 days before experimentation. Transmitter output frequency (in Hz) was monitored at 10-min intervals by receiver boards placed below the cage of each animal. This information was relayed to a computer, and frequency measurements were converted to Celsius with the use of Dataquest software (DataScience). Injections were administered between 1000 and 1200, during the light phase of the normal light-dark cycle, and changes in core body temperature were monitored continuously for 8 h after treatment.

Body temperature data are presented as either 1) the net deviation from the mean baseline temperature at 0 h [i.e., change in body temperature (°C)] after saline or LPS injection in studies involving male and female rats because of gender-related differences in basal temperatures or 2) core body temperature after injection of saline or LPS in studies involving pregnant and cycling female rats because their basal body temperatures did not differ significantly.

Measurement of brain IL-1β and COX-2 mRNA levels using RT-PCR. To investigate central responses to LPS, changes in hypothalamic levels of IL-1β and COX-2 mRNA were assessed 3 h (corresponds to the first phase of the fever response) after injection of animals with LPS or saline alone, and treatment with human recombinant IL-1ra or saline where indicated (n = 5 per treatment group).

Animals were deeply anesthetized and then perfused intracardially with diethyl pyrocarbonate-treated saline. Brains were removed, and the hypothalami were dissected out and stored at −80°C until use. Brain tissue was homogenized in Trizol (Invitrogen, Burlington, Ontario) to extract total RNA. One microgram of total RNA was incubated with random primers (5 µM; Applied Bioscience) for 10 min at 65°C, and then cDNA synthesis was performed by adding DTT (10 µM; Invitrogen), murine myeloleukemia virus reverse transcriptase (200 U; Invitrogen), dNTPs (1 mM; Sigma), and first-strand buffer (Invitrogen) and incubating for 1 h at 37°C followed by 5 min at 90°C to inactivate the enzyme. PCR amplification of cDNA was performed in a GeneAmp PCR system 9700 thermocycler (Applied Biosystems) using ReadyMix RedTaq PCR reaction mix with 1.5 mM MgCl2 (Sigma) and 6 pmol of gene-specific primer sets for IL-1β (NM_031512; forward: 5'-CCCCAGCACCCTTCCTTCTCTTCATCTT-3', reverse: 5'-CAGGTTGGTGTGCGCTTTCTTC-3'), COX-2 (NM_017232; forward: 5'-TGATAGGACAGATCAAGA-3', reverse: 5'-ATGTAAGGGCCCTTCAACT-3'), and β-actin (NM_031144; forward: 5'-GCCGTTCTCCCCCTCATCGT-3', reverse: 5'-TACGACCCAGGCGATACAGGGACAAC-3'). The following cycling parameters were used: 1) denaturation for 5 min at 95°C, 2) amplification for 30 s at 95°C, 30 s at 60°C (β-actin and IL-1β) or 57°C (COX-2), followed by 1 min at 72°C for 20, 32, and 36 cycles (β-actin, IL-1β and COX-2, respectively); and 3) extension for 10 min at 72°C. PCR products were separated by electrophoresis on 2% agarose gel and visualized with ethidium bromide staining.

The amount of PCR product (band density) was quantified by GeneTool image analysis software (Syngene, Frederick, MD). To compare the expression level of gene X between animals in different treatment groups, the band density for gene X was normalized against the band density of β-actin in the same sample [relative density = (gene X/β-actin mRNA × 100)]. The linear phase of template amplification was determined in a pilot experiment by performing RT-PCR on a sample from each treatment group for an increasing number of cycles (30–50), the amount of PCR product (log scale) was plotted against cycle number, and a cycle number within the exponential phase of amplification selected and used for all subsequent PCRs.

Measurement of plasma levels of IL-1ra by ELISA. To compare circulating levels of IL-1ra in male and female rats, trunk blood samples were collected 3 h after injection of LPS or saline. However, because of the different temperature profiles observed in pregnant vs. cycling dams, plasma samples were collected 4 h after treatment to compare plasma concentrations of IL-1ra in pregnant and nonpregnant females. Animals were deeply anesthetized, and trunk blood was collected by cardiac puncture into sterile tubes containing pyrogen-free heparin (10 U/ml). The blood samples were then centrifuged (5,300 g, 10 min at 4°C), and plasma was collected and stored at −80°C. IL-1ra concentrations were measured in plasma using a two-site, rat-specific ELISA (National Institute for Biological Standards and Control) as previously described (57). All samples and standards were assayed in duplicate. Intra- and interassay coefficients of variability were <15%, with the detection limit of 62.5 pg/ml for all assays.

Statistical analysis. In the fever studies involving male and female rats, the area under the curve (AUC) was calculated on the change in body temperature data from individual animals, using zero as the baseline. In fever studies involving cycling and pregnant female rats, AUC was calculated on the core body temperature of individual animals with the use of mean body temperature of each group at time 0 as the baseline. AUC, PCR, and ELISA data were analyzed by two-way ANOVA and Tukey’s honestly significant difference post hoc test. When statistical comparisons were made between two groups, an unpaired t-test was used. Probabilities of P < 0.05 were considered significant.
RESULTS

Febrile responses to LPS are gender dependent. In this study, the mean basal body temperature of male rats (36.85 ± 0.05°C) was observed to be significantly lower (P < 0.001) than the mean basal body temperature of randomly cycling female rats (37.37 ± 0.12°C) at the time of injection (time 0). Therefore, temperature data are presented as the change in body temperature relative to the baseline at time 0 for each treatment group. All rats displayed a transient increase in body temperature, peaking 30 min after injection of LPS or saline (due to handling stress); however, the body temperature of all animals returned to baseline over the next 2 h (Fig. 1A). Statistical comparison of AUC indicated that injection of LPS (0.05 mg/kg ip) resulted in a significant increase in core body temperature, 2–8 h after treatment, in both male and female rats vs. saline alone (Fig. 1B; P < 0.01 male, saline vs. LPS and female, saline vs. LPS). The febrile response was biphasic in both sexes, commencing 2 h after injection and peaking first at 3 h and then again at 5 h after LPS (Fig. 1A). LPS induced a significantly greater change in body temperature in male vs. female rats 2–8 h after the injection of LPS (Fig. 1B; P < 0.01 LPS, male vs. female).

To investigate whether gender differences in the febrile response to LPS were due to differences in central mechanisms regulating fever, expression of IL-1β and COX-2 mRNA was assessed in the hypothalami of male and female rats 3 h after treatment with LPS or saline (n = 5 per group). In male rats, LPS induced a significant increase in hypothalamic IL-1β mRNA levels vs. saline alone (∼4-fold) (Fig. 2A; P < 0.01 male, saline vs. LPS). In female rats, LPS induced an approximately twofold increase in hypothalamic levels of IL-1β mRNA, although this did not reach statistical significance (Fig. 2A; P = 0.265). LPS induced significantly more IL-1β mRNA in the hypothalami of males than in the hypothalami of female rats (Fig. 2A; P < 0.01 LPS, male vs. female). Basal levels of IL-1β mRNA were comparable in male and female rats treated with saline alone (Fig. 2A). In addition, LPS induced a significant increase in hypothalamic levels of COX-2 mRNA in both male and female rats (Fig. 2B; P < 0.01 male, saline vs. LPS and female, saline vs. LPS). This effect was significantly greater in male than in female rats (P < 0.01 LPS, male vs. female), although basal levels of COX-2 mRNA were significantly higher in male rats treated with saline alone than in females (Fig. 2B; P < 0.01 saline, male vs. female).

Plasma levels of the anti-inflammatory cytokine IL-1ra were assessed 3 h after treatment with LPS (n = 5 per treatment group) to delineate whether plasma levels of this cytokine could account for the attenuated febrile responses (Fig. 1) and reduced hypothalamic IL-1β and COX-2 (Fig. 2, A and B) observed in female rats. LPS induced a significant increase in plasma IL-1ra concentrations in both male (17,037.5 ± 2,323 vs. 2,065.5 ± 412.5 pg/ml) and female rats (24,728 ± 2,454.5 vs. 1,661.3 ± 223.7 pg/ml) vs. saline alone, respectively (Fig. 2C; P < 0.01 male and female, saline vs. LPS). This effect was significantly greater in female than in male rats (Fig. 2C; P < 0.01 LPS, female vs. male).

Febrile responses to LPS are attenuated in pregnancy. The mean basal body temperatures of randomly cycling female rats and dams on day 18 of pregnancy were 37.23 ± 0.12°C and 37.03 ± 0.08°C, respectively, at the time of injection (time 0; n = 5/group). Randomly cycling female rats displayed a stress-dependent, transient increase in body temperature in the 30 min immediately after injection of either LPS or saline alone, although their body temperatures returned to baseline within 2 h (Fig. 3A). Pregnant dams treated with saline also displayed this transient increase in body temperature, whereas dams treated with LPS displayed a pronounced hypothermic response, 2 h after treatment (Fig. 3A). Analysis of the AUC demonstrated that injection of LPS (0.05 mg/kg ip) induced a significant increase in core body temperature in both cycling females and day 18 pregnant dams, 2–8 h after treatment, vs. that observed with saline alone (Fig. 3B; P < 0.01 cycling female, saline vs. LPS and P < 0.05 day 18 pregnant dam, saline vs. LPS). The LPS-dependent change in body temperature was significantly greater in cycling vs. day 18 pregnant females 2–8 h after injection of LPS (Fig. 3B; P < 0.05 LPS, cycling females vs. day 18 pregnant dams). The response to saline was comparable in cycling and pregnant females with no significant deviation from basal evident in either group.

Fig. 1. Febrile responses to lipopolysaccharide (LPS) are gender dependent. A: injection of LPS (0.05 mg/kg; closed symbols) induced an increase in core body temperature in male and female rats vs. that shown with saline alone (1 ml/kg; open symbols). Statistical analysis was performed on area under the curve (AUC) data. B: LPS-dependent change in body temperature was significantly greater in male than in female rats 2–8 h after treatment. **P < 0.01 male, saline vs. LPS; +++P < 0.01 female, saline vs. LPS; ###P < 0.01 LPS, male vs. female.

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To assess whether plasma levels of the anti-inflammatory cytokine IL-1ra could account for the attenuated febrile responses observed in day 18 pregnant female rats, plasma levels of this cytokine were assessed 4 h after treatment with LPS (n = 5 per treatment group). LPS increased plasma levels of IL-1ra in both cycling (1,191.8 ± 691.5 vs. 20,755.3 ± 6,050.5 pg/ml) and day 18 pregnant dams (954.5 ± 345.3 vs. 46,810.2 ± 4,982.6 pg/ml; Fig. 3C; P < 0.01 and P < 0.01, respectively) vs. saline alone; however, this effect was significantly greater in pregnant vs. cycling females (Fig. 3C; P < 0.01 LPS, day 18 vs. cycling females).

IL-1ra treatment of male rats attenuates LPS induction of hypothalamic IL-1β and COX-2 mRNA. To test the hypothesis that increasing plasma levels of IL-1ra could modulate central

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**Fig. 2. Gender differences in central and peripheral responses to LPS.**

A: LPS (0.05 mg/kg) induced a significant increase in IL-1β mRNA in the hypothalamus of male rats vs. that shown with saline alone (1 ml/kg), and this effect was significantly greater in male than in female rats. **P < 0.01 male, saline vs. LPS; ##P < 0.01 LPS, male vs. female.**

B: LPS induced significant increases in hypothalamic levels of cyclooxygenase-2 (COX-2) mRNA in male and female rats, although the effect was significantly greater in male rats. **P < 0.01 male, saline vs. LPS; ++P < 0.01 female, saline vs. LPS; ##P < 0.01 LPS, male vs. female; !P < 0.01 saline, male vs. female.**

C: Plasma levels of IL-1 receptor antagonist (IL-1ra) were significantly greater in female than in male rats 3 h after LPS challenge. **P < 0.01 male, saline vs. LPS and female, saline vs. LPS; ##P < 0.01 male, LPS vs. female LPS.

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**Fig. 3. Attenuated febrile responses near term of pregnancy correlate with increased plasma IL-1ra.**

A: Injection of LPS (0.05 mg/kg; closed symbols) induced an increase in core body temperature in both cycling and pregnant female rats vs. that shown with saline alone (1 ml/kg; open symbols). Statistical analysis was performed on AUC data. B: LPS-dependent change in body temperature was significantly greater in cycling vs. day 18 (E18) pregnant rats 2–8 h after treatment. **P < 0.01 cycling, saline vs. LPS; *P < 0.05 E18, saline vs. LPS; #*P < 0.05 E18, LPS vs. cycling.**

C: Plasma levels of IL-1ra were significantly greater in female than in male rats 3 h after LPS challenge. **P < 0.01 male, saline vs. LPS and female, saline vs. LPS; ##P < 0.01 male, LPS vs. female LPS.**
the attenuation of the hypothalamic mRNA expression of these two mediators after LPS challenge. Collectively, these data suggest that the anti-inflammatory cytokine IL-1ra may regulate gender differences in the febrile response to LPS via its effects on the IL-1β-dependent induction of hypothalamic COX-2, and this may be of particular importance during pregnancy.

Our observations that febrile responses were significantly attenuated in free-moving, cycling female rats, vs. male rats (Fig. 1B), are in agreement with previous reports (49, 65). However, we have expanded on these findings by demonstrating that hypothalamic levels of IL-1β and COX-2 mRNA are also significantly attenuated in female rats, compared with males, after injection of LPS (Fig. 2). The correlation between mRNA levels of IL-1β and COX-2 observed in the hypothalamus of male rats (Fig. 2, A and B) was highly significant (P = 0.001, r² = 0.7359), indicating a linear relationship between IL-1β and COX-2, as observed in previous studies (11, 50). However, no correlation was observed between these two mRNA species in the hypothalami of female rats (P = 0.2510, r² = 0.1607), indicating that, in females, hypothalamic levels of COX-2 are modulated by factors other than, or in conjunction with, IL-1β. Given that the anti-inflammatory molecule IL-1ra has a key role in the regulation of IL-1β activity (3), we hypothesized that differences in plasma levels of IL-1ra between male and female rats could mediate the gender differences that we observed in hypothalamic COX-2 expression and fever after exposure to LPS. Our observation that plasma levels of IL-1ra were significantly higher (Fig. 2), whereas hypothalamic COX-2 mRNA levels and febrile responses were significantly lower (Figs. 2 and 1, respectively), in female than in male rats after intraperitoneal injection of LPS (3 h) supports the notion that plasma IL-1ra could mediate gender differences in febrile response to LPS via modulation of IL-1β-dependent COX-2 expression. This is further supported by our experiment showing that LPS-induced IL-1β and COX-2 mRNA expression was attenuated in male rats after treatment with recombinant IL-1ra (Fig. 4). However, these data do not preclude that other cytokines, such as IL-6, may also influence gender differences in the febrile response. Our group (13) has previously demonstrated that this circulating mediator is a critical component of the febrile response and recently showed (60) that it is a potent activator of brain COX-2. The role of IL-6 in gender differences in the febrile response, however, remains to be determined.

Basal levels of hypothalamic COX-2 mRNA were significantly greater in male than in female rats (Fig. 2B; P < 0.01); interestingly, however, the baseline body temperature was significantly lower in male vs. female rats (36.85 ± 0.05 vs. 37.37 ± 0.12°C; P < 0.001). Boisse et al. (7) previously observed a similar dissociation between basal COX-2 levels and basal temperature in adult male rats exposed to LPS as neonates vs. controls. Why the enhanced basal COX-2 that we observed in male rats does not result in enhanced basal body temperature is unclear, although this suggests that there may be male or female differences in 1) COX-2 activity and synthesis of PGE₂, 2) PGE₂ activity or PGE₂ receptor expression, affinity, or activity, 3) PGE₂ catabolism or clearance, or 4) cellular distribution of COX-2. This discrepancy could also be due to the higher basal levels of corticosterone observed in female rats vs. their male counterparts (5, 15, 33, 44, 53). Corticosteroids interfere with the activation of NF-κB (1, 40), a key transcrip-

**Discussion**

In this study, we demonstrate that febrile responses to LPS are attenuated in randomly cycling female rats compared with their male counterparts, as well as in near-term pregnant dams vs. nonpregnant female rats. The attenuated febrile response observed in cycling female rats was accompanied by significant increases in plasma levels of IL-1ra and attenuated levels of hypothalamic IL-1β and COX-2 mRNA after injection of LPS. Furthermore, increasing plasma levels of IL-1ra in male rats by administration of the recombinant antagonist also attenuated the hypothalamic mRNA expression of these two mediators after LPS challenge. Collectively, these data suggest that the anti-inflammatory cytokine IL-1ra may regulate gender differences in the febrile response to LPS via its effects on the IL-1β-dependent induction of hypothalamic COX-2, and this may be of particular importance during pregnancy.

Responses to LPS challenge, male rats injected with LPS or saline (time 0) were treated (0 and 1 h; n = 5 per treatment group) with human recombinant IL-1ra (1 mg/kg ip) or saline alone (1 ml/kg ip), and hypothalamic expression of IL-1β and COX-2 mRNA was assessed 3 h after the initial injection. LPS induced a significant induction of hypothalamic levels of IL-1β and COX-2 mRNA (Fig. 4; P < 0.01 saline, saline vs. LPS), and this effect was significantly attenuated in animals cotreated with IL-1ra (++P < 0.01 IL-1ra, saline vs. LPS); however, this was significantly attenuated vs. animals cotreated with saline alone (###P < 0.01 IL-1ra-LPS vs. saline-LPS).

**Fig. 4.** Increasing plasma levels of IL-1ra attenuates central expression of IL-1β and COX-2 mRNA in male rats. Injection of LPS (0.05 mg/kg) induced a significant increase in IL-1β (A) and COX-2 (B) mRNA in the hypothalamus of male rats (**P < 0.01 saline, saline vs. LPS). LPS challenge also induced a significant increase in IL-1β and COX-2 mRNA in animals cotreated with IL-1ra (++P < 0.01 IL-1ra, saline vs. LPS); however, this was significantly attenuated vs. animals cotreated with saline alone (###P < 0.01 IL-1ra-LPS vs. saline-LPS).
tion factor in the expression of COX-2 (58, 68). Thus the higher basal levels of this glucocorticoid in female rats may result in lower basal levels of COX-2 vs. that shown in male rats. Alternatively, the differences in basal levels of COX-2 mRNA between male and female rats (Fig. 2B; P < 0.01) could be due to the influence of sex hormones. Indeed, treatment of cultured human decidual cells in vitro with progesterone attenuated basal expression of COX-2 protein (22, 46), and basal levels of hypothalamic COX-2 protein were found to vary with the stage in gestation in pregnant rats (46), indicating that sex hormones can influence basal levels of this enzyme.

As expected, febrile responses to LPS were significantly attenuated in pregnant rats, on day 18 of pregnancy, compared with nonpregnant, randomly cycling females (Fig. 3). This is in agreement with previous studies that reported attenuated febrile responses near term of pregnancy in rats after intravenous (41) and intraperitoneal (19) injection of LPS. In pregnant rats, attenuated febrile responses near term are also associated with reduced expression of hypothalamic COX-2 protein (22, 46). Mouihate et al. (46) reported that COX-2 expression was reduced in pregnant rat dams on day 22 of pregnancy vs. day 15 pregnant dams, and lactating females 5 days after parturition, 3 h after LPS injection (46). Subsequently, the same group reported that the changes in COX-2 protein expression observed near term are not modulated at the transcription factor level (47), suggesting that it is most likely a mechanism upstream of COX-2, perhaps at the level of IL-1β activity. Having observed significant increases in plasma levels of the anti-inflammatory cytokine IL-1ra in female vs. male rats after LPS, we hypothesized that the physiological state of the female (i.e., pregnant vs. nonpregnant) may also influence plasma levels of IL-1ra. Indeed, we observed that circulating IL-1ra concentrations were not only greater in female than in male rats (Fig. 2C) but were also significantly elevated in pregnant (day 18) vs. cycling female rats (Fig. 3C) after exposure to LPS (4 h). Our data agree with another recent study that observed significantly higher plasma levels of IL-1ra in pregnant rats at day 20 of pregnancy, vs. nonpregnant cycling rats, 4 h after intraperitoneal injection of LPS (20). However, our data appear to be somewhat contradictory to another study that demonstrated similar plasma levels of IL-1ra in rats near term of pregnancy (day 22) vs. day 15 pregnant or lactating rats 2 h after injection of LPS (day 22 pregnant rats showed attenuated febrile responses to LPS, whereas day 15 pregnant and lactating rats showed LPS-induced febrile responses that were similar to those observed in cycling females) (47). The difference between their findings and our findings is most likely due to the earlier time point after LPS administration examined (2 h compared with 4 h in our study) and/or to the different control animals used. Mouihate et al. (47) selected day 15 pregnant dams and lactating females as controls, rather than cycling females, based on their previous work indicating that febrile responses to LPS are modulated by ovarian hormones throughout the estrous cycle (48). Although this is valid, we were aiming to investigate whether the physiological state of the female (i.e., pregnant vs. nonpregnant) affects plasma levels of IL-1ra (having shown that gender modulates plasma levels of this cytokine). Therefore, nonpregnant cycling females were the most appropriate control for our study. Interestingly, increased plasma levels of IL-1ra have also been observed in human studies in healthy women vs. men and also in pregnant vs. nonpregnant women (9, 38, 54).

The underlying reason for the increased IL-1ra levels observed after LPS in the cycling female rats in our study remains undetermined; however, one possibility is that this could be linked to the higher amounts of white adipose tissue found in females, particularly during pregnancy (6, 39, 62, 66). Recently, this tissue was shown to be a target of inflammatory stimuli, such as LPS, and an important source of IL-1ra (24). In this study (24), the authors suggested that increased circulating levels of the anti-inflammatory cytokine IL-1ra may be involved in obesity through actions on the hypothalamus. Our demonstration that IL-1ra injected systemically after LPS challenge reduces hypothalamic levels of both IL-1β and COX-2 mRNA (Fig. 4) and fever (37) in male rats is in agreement with this and supports the notion that circulating IL-1ra can modulate hypothalamic activity during infection.

Other than the difference in white adipose tissue levels, another possibility is that IL-1ra concentrations could be regulated by sex hormones. Although there is no direct evidence for this, there is considerable evidence demonstrating that circulating sex hormones do modulate immune responses (28, 29, 61), and estrogens and progesterone have been found to influence IL-1 release from monocytes (55). Indeed, cytokine release from immune cells was found to vary in premenopausal women, depending on the stage in the menstrual cycle (10, 27, 55, 63). Furthermore, a recent study demonstrated that treatment of orchiectomized male rats with testosterone increased, whereas estrogen decreased, the induction of COX-2 protein in cerebral vessels after challenge with LPS (56). In a different study, sex hormones were shown to modulate febrile responses and COX-2 protein expression at different stages of the estrous cycle and therefore possibly during pregnancy. In this study, LPS-induced fever and the accompanying hypothalamic COX-2 protein expression were shown to be attenuated in ovariectomized female rats treated with estrogen and progesterone vs. ovariectomized controls (48). Circulating adrenal steroids (i.e., glucocorticoids), such as corticosterone, could also potentially regulate the differential plasma levels of IL-1ra that we observed between the genders in this study. Plasma corticosterone levels are reportedly higher in female rats than in males rats (15, 33), particularly in females at near term of pregnancy (8, 67), and this hormone is known to induce the synthesis of IL-1ra (34).

In conclusion, we have found attenuated febrile responses to LPS in cycling female vs. male rats, as well as in pregnant dams near term of pregnancy (day 18), suggesting that gender, as well as pregnancy, influences fever responses to LPS. Our data suggest that this effect may be due to the modulation of brain COX-2 by the anti-inflammatory cytokine IL-1ra, thus providing evidence for an endogenous regulatory mechanism that may protect the developing fetus from the deleterious effects of maternal fever during gestation.

GRANTS

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