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

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Running head: IL-1ra, gender and fever
Abstract

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 versus 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 versus male rats, and also in near term pregnant dams versus cycling females, following intraperitoneal (ip) injection of LPS (0.05 mg kg\(^{-1}\)). Plasma levels of IL-1ra were significantly greater in female rats after injection of LPS, particularly during pregnancy, versus males. This was accompanied by attenuated levels of hypothalamic IL-1\(\beta\) and COX-2 mRNA, two key mediators of the febrile response, in female rats. Furthermore, increasing plasma levels of IL-1ra in male rats by administration (ip) of the recombinant antagonist attenuated hypothalamic mRNA levels of these mediators following LPS. These data suggest 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. **KEY WORDS**: Fever, pregnancy, COX-2, IL-1β, cytokines
Introduction

Fever is a complex physiological response mounted by the host to facilitate the resolution of infection following exposure to invading viral or bacterial pathogens. This CNS orchestrated response involves the production and action of prostaglandins (PGE₂) on hypothalamic thermo-sensitive 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 following 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 pro-inflammatory cytokine interleukin (IL)-1β is a major peripheral mediator of LPS-induced fever, which is known to trigger increases in body temperature via cyclooxygenase (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 using a recombinant form of the naturally occurring IL-1 receptor antagonist (IL-1ra) (37; 53; 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 following 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 acts 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). Whilst 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 following exposure of pregnant rats to LPS. One study reported increased expression of IL-1ra in pregnant versus cycling female rats (20), whereas another observed comparable plasma levels of this cytokine in dams at different stages of pregnancy versus 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 versus male rats. For example, one study found febrile responses to IL-1β (ip) were comparable in male and female rats (45), whilst another demonstrated that febrile responses were significantly greater in male versus cycling female rats following intravenous (iv) 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 versus 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 current 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 $243.67 \pm 2.1$, $300.36 \pm 7.06$ and $290.6 \pm 5.6$ 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 $21 \pm 2 \, ^{\circ}C$ and a 12 h light/dark cycle (lights on from 08:00 to 20:00 h). 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 (ip) injection of LPS (0.05 mg kg$^{-1}$; *E. Coli* 0111:B4, Lot 42k4120, Sigma, Canada) or saline alone (1 ml kg$^{-1}$) at $t=0$, and human recombinant IL-1ra (1 mg kg$^{-1}$; NIBSC, UK), or saline (1 ml kg$^{-1}$), at $t=0$ and 1 h where indicated.

Measurement of body temperature using remote biotelemetry

Changes in core body temperature were monitored following injection of LPS, or saline, using remote radio-biotelemetry as described previously (21). Temperature sensitive transmitters (DataSciences, USA) were implanted in the body cavity of anaesthetized rats (50 mg kg$^{-1}$ ketamine, 5 mg kg$^{-1}$ xylazine and 0.5 mg kg$^{-1}$ acetaprazine; 1 µl g$^{-1}$ body weight) and the animals were allowed to recover for at least 5 days prior to experimentation. Transmitter output frequency
(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 converted to °C using Dataquest software (DataScience, USA). Injections were administered between 10 am and 12 am, during the light phase of the normal light-dark cycle, and changes in core body temperature monitored continuously for 8 h post treatment. Body temperature data are presented as either, (i) the net deviation from the mean baseline temperature at 0 h [ie. change in body temperature (°C)] following injection of saline or LPS in studies involving male and female rats because of gender related differences in basal temperatures or, (ii) core body temperatures following injection of saline or LPS in studies involving pregnant and cycling female rats since 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 anaesthetized and then perfused intracardially with diethyl pyrocarbonate-treated saline. Brains were removed and the hypothalami dissected out and stored at - 80 °C until use. Brain tissue was homogenized in TRIzol (Invitrogen, Burlington, Ontario) in order to extract total RNA. One µg of total RNA was incubated with random primers (5 µM; Applied
Bioscience, Canada) for 10 min at 65 °C and then cDNA synthesis was performed by adding dithiothreitol (10 µM; Invitrogen, Canada), murine myeloleukemia virus reverse transcriptase (MMLV-RT, 200 units; Invitrogen, Canada), dNTPs (1 mM; Sigma, Canada) and first strand buffer (Invitrogen), and incubating for 1 h at 37 °C followed by 5 min at 90 °C in order to inactivate the enzyme. PCR amplification of cDNA was performed in a Gene Amp 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’-CCCAAGCACCTTCTTTTCATCTT-3’, reverse: 5’-CAGGGTGGGTGTGCCGTCTTTC-3’), COX-2 (NM_017232; forward: 5’-TGATAGGAGAGAGACGATCAAG-3’, reverse: 5’-ATGGTAGAGGCTTTCAACT-3’), and β-actin (NM_031144; forward: 5’-GCCGTCTTCCCCCTCCATCGTG-3’, reverse: 5’-TACGACCAGAGGCATACAGGGACAAC-3’). The following cycling parameters were used: 1) denaturation, 5 min at 95 °C; 2) amplification, 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); 3) extension, 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 using GeneTool image analysis software (Syngene, Frederick, MD). In order 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 x 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 using ELISA**

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

**Statistical analysis**

In the fever studies involving male and female rats, 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 using mean body temperature of each group at t=0 h as the baseline. AUC, PCR and ELISA data were analyzed using a two way ANOVA and Tukey's HSD 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 ⁰C) was observed to be significantly lower (*** P<0.001) than the mean basal body temperature of randomly cycling female rats (37.37 ± 0.12 ⁰C) at the time of injection (t=0 h). Therefore, temperature data are presented as the change in body temperature relative to the baseline at t=0 h for each treatment group. All rats displayed a transient increase in body temperature, peaking 30 min after injection of LPS/saline (due to handling stress), however, the body temperature of all animals returned to baseline over the next 2 h (Figure 1A). Statistical comparison of the area under the curve (AUC) indicated that injection of LPS (0.05 mg kg⁻¹, ip) resulted in a significant increase in core body temperature, 2-8 h after treatment, in both male and female rats versus saline alone (Figure 1B; ** P<0.01 male saline vs LPS; ++ P<0.01 female saline vs LPS). The febrile response was biphasic in both sexes, commencing 2 h after injection and peaking first 3 h, and then again 5 h, after LPS (Figure 1A). LPS induced a significantly greater change in body temperature in male versus female rats, 2-8 h after the injection of LPS (Figure 1B; ## P<0.01 LPS male vs female).

In order 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/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
versus saline alone (approximately 4 fold) (Figure 2A; ** P<0.01 male saline vs LPS). In female rats, LPS induced an approximately 2 fold increase in hypothalamic levels of IL-1β mRNA although this did not reach statistical significance (Figure 2A, P=0.265). LPS induced significantly more IL-1β mRNA in the hypothalami of male versus female rats (Figure 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 (Figure 2A). In addition, LPS induced a significant increase in hypothalamic levels of COX-2 mRNA in both male and females (Figure 2B; ** P<0.01 male saline vs LPS; ++ P<0.01 female saline vs LPS). This effect was significantly greater in males than 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 versus females (Figure 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) in order to delineate if plasma levels of this cytokine could account for the attenuated febrile responses (Figure 1), and reduced hypothalamic IL-1β and COX-2 (Figure 2A and 2B), observed in female rats. LPS induced a significant increase in plasma IL-1ra concentrations in both male (17 037.5 ± 2323 versus 2 065.5 ± 412.5 pg ml⁻¹) and female rats (24 728 ± 2454.5 versus 1 661.3 ± 223.7 pg ml⁻¹) versus saline alone respectively (Figure 2C; ** P< 0.01 male and female saline vs LPS). This effect was significantly greater in female versus male rats (Figure 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 (t=0 h; 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 temperature returned to baseline within 2 h (Figure 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 (Figure 3A). Analysis of the AUC demonstrated that injection of LPS (0.05 mg kg⁻¹, ip) induced a significant increase in core body temperature in both cycling females and E18 pregnant dams, 2-8 h after treatment, versus saline alone (Figure 3B; ** P<0.01 cycling female saline vs LPS; * P<0.05 E18 pregnant dam saline vs LPS). The LPS-dependent change in body temperature was significantly greater in cycling versus E18 pregnant females, 2-8 h after the injection of LPS (Figure 3B; # P<0.05 LPS cycling female vs E18 pregnant dam). The response to saline was comparable in cycling and pregnant females with no significant deviation from basal evident in either group.

In order to assess if plasma levels of the anti-inflammatory cytokine IL-1ra could account for the attenuated febrile responses observed in E18 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 versus 20 755.3 ± 6 050.5 pg ml⁻¹) and E18 pregnant dams (954.5 ± 345.3 versus 46 810.2 ± 4 982.6 pg ml⁻¹; Figure 3C; ** P<0.01 and ## P<0.01 respectively) versus saline alone, however, this effect was significantly greater in pregnant versus cycling females (Figure 3C; && P<0.01 LPS E18 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 responses to LPS challenge, male rats injected with LPS or saline (t=0 h) were treated (0 and 1 h; n=5 per treatment group) with human recombinant IL-1ra (1 mg kg⁻¹, ip) or saline alone (1 ml kg⁻¹, ip), and hypothalamic expression of IL-1β and COX-2 mRNA assessed 3 h after the initial injection. LPS induced a significant induction of hypothalamic levels of IL-1β and COX-2 mRNA (Figure 4A and B; ** P<0.01 saline saline vs saline LPS), and this effect was significantly attenuated in animals co-treated with IL-1ra (Figure 4A and B, ## P<0.01 saline LPS vs IL-1ra LPS). Treatment with IL-1ra did not however affect basal levels of IL-1β or COX-2 mRNA in control animals injected with saline alone (Figure 4A and B).
Discussion

In this study we demonstrate that febrile responses to LPS are attenuated in randomly cycling female rats compared to their male counterparts, as well as in near term pregnant dams versus non-pregnant 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 following 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, versus male rats (Figure 1B), is in agreement with previous reports (49; 65). We have however expanded on these findings by demonstrating that hypothalamic levels of IL-1β and COX-2 mRNA are also significantly attenuated in female rats, compared to males, following injection of LPS (Figure 2). The correlation between mRNA levels of IL-1β and COX-2 observed in the hypothalamus of male rats (Figure 2A 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 we observed in hypothalamic COX-2 expression and fever following exposure to LPS. Our observation that plasma levels of IL-1ra were significantly higher (Figure 2), whilst hypothalamic COX-2 mRNA levels and febrile responses significantly lower (Figure 2 and 1 respectively), in female versus male rats following LPS (ip, 3h), 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 following treatment with recombinant IL-1ra (Figure 4). These data do not however preclude that other cytokines, such as IL-6, may also influence gender differences in the febrile response. We have previously demonstrated that this circulating mediator is a critical component of the febrile response (13) and recently showed that it is a potent activator of brain COX-2 (60). 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 versus female rats (Figure 2B, P<0.01), interestingly however, the baseline body temperature was significantly lower in male versus female rats (36.85 ± 0.05 versus 37.37 ± 0.12 °C, P<0.001). Boisse et al (2004) have previously
observed a similar dissociation between basal COX-2 levels and basal temperature in adult male rats exposed to LPS as neonates versus controls (7). Why the enhanced basal COX-2 that we observed in male rats does not result in enhanced basal body temperature is unclear, however, this suggests that there may be male/female differences in (i) COX-2 activity and synthesis of PGE2, (ii) PGE2 activity or PGE2 receptor expression, affinity or activity, (iii) PGE2 catabolism or clearance, or (iv) cellular distribution of COX-2. This discrepancy could also be due to the higher basal levels of corticosterone observed in female rats versus their male counterparts (5; 15; 33; 44; 52). Corticosteroids interfere with the activation of nuclear factor κB (NFκB) (1; 40) a key transcription factor in the expression of COX-2 (58; 68) thus the higher basal levels of this glucocorticoid in female rats may result in the lower basal levels of COX-2 versus male rats. Alternatively, the differences in basal levels of COX-2 mRNA between male and female rats (Figure 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 (23), 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 to non-pregnant, randomly cycling females (Figure 3). This is in agreement with previous studies that reported attenuated febrile responses near term of pregnancy in rats following
intravenous (41) and intraperitoneal injections of LPS (19). In pregnant rats, attenuated febrile responses near term are also associated with reduced expression of hypothalamic COX-2 protein (22; 46). Mouihate et al (2002) reported that COX-2 expression was reduced in pregnant rat dams on day 22 of pregnancy (E22), versus E15 pregnant dams and lactating females 5 days after parturition (L5), 3 h after LPS (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, at the level of IL-1β activity perhaps. Having observed significant increases in plasma levels of the anti-inflammatory cytokine IL-1ra in female versus male rats after LPS, we hypothesized that the physiological state of the female (ie. pregnant vs non-pregnant) may also influence plasma levels of IL-1ra. Indeed, we observed that circulating IL-1ra concentrations were not only greater in female versus male rats (Figure 2C), but were also significantly elevated in pregnant (E18) versus cycling female rats (Figure 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, versus non-pregnant cycling rats, 4 h after injection of LPS (ip) (20). Our data however appears to be somewhat contradictory to another study which demonstrated similar plasma levels of IL-1ra in rats near term of pregnancy (E22), versus E15 pregnant or lactating rats, 2 h after injection of LPS [E22 pregnant rats show attenuated febrile responses to LPS while E15 pregnant and lactating rats show LPS-induced febrile responses that are similar to those observed in cycling
females] (47). The difference between their and our findings is most likely due to the earlier time point after LPS administration examined (2 h compared to 4 h in our study) and/or to the different control animals used. Mouihate et al (2005) selected E15 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). Whilst this is valid, we were aiming to investigate whether the physiological state of the female (ie. pregnant versus non pregnant) affects plasma levels of IL-1ra (having shown that gender modulates plasma levels of this cytokine). Therefore non pregnant 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 females versus males, and also in pregnant versus non pregnant females (9; 38; 54).

The underlying reason for the increased IL-1ra levels observed following 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 (WAT) 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 the authors suggested that increased circulating levels of the anti-inflammatory cytokine IL-1ra may be involved in obesity through actions on the hypothalamus (24). Our demonstration that IL-1ra injected systemically following LPS challenge reduces hypothalamic levels of both IL-1β and COX-2 mRNA (Figure 4), as well as 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 WAT 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, versus ovariectomized controls (48). Circulating adrenal steroids (i.e. glucocorticoids), such as corticosterone, could also potentially regulate the differential plasma levels of IL-1ra we observed between the genders in this study. Plasma corticosterone levels are reportedly higher in female rats than in males rats (15; 33), particularly 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 versus male rats, as well as in pregnant dams near term of pregnancy (E18), 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.
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Figure 1. Febrile responses to LPS are gender-dependent
Injection of LPS (0.05 mg kg⁻¹, filled symbols) induced an increase in core body temperature in male and female rats, versus saline alone (1 ml kg⁻¹, open symbols) (A). Statistical analysis was performed on area under the curve (AUC) data. The LPS-dependent change in body temperature was significantly greater in male versus female rats, 2-8 h after treatment (B). ** P<0.01 male saline vs LPS; ++ P<0.01 female saline vs LPS; ## P<0.01 LPS male vs female.

Figure 2. Gender differences in central and peripheral responses to LPS
LPS (0.05 mg kg⁻¹) induced a significant increase in IL-1β mRNA in the hypothalamus of male rats versus saline alone (1 ml kg⁻¹) and this effect was significantly greater in male versus female rats. ** P<0.01 male saline vs LPS; ## P<0.01 LPS male vs female (A). LPS induced significant increases in hypothalamic levels of 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 (B). Plasma levels of IL-1ra were significantly greater in female versus male rats, 3 h after LPS challenge. ** P<0.01 male saline vs LPS and female saline vs LPS; ## LPS male vs LPS female (C).

Figure 3. Attenuated febrile responses near term of pregnancy correlate with increased plasma IL-1ra
Injection of LPS (0.05 mg kg⁻¹, filled symbols) induced an increase in core body temperature in both cycling and pregnant female rats, versus saline alone (1 ml kg⁻¹, open symbols) (A). Statistical analysis was performed on area under the curve (AUC) data. The LPS-dependent change in body temperature was significantly greater in cycling versus E18 pregnant rats, 2-8 h after treatment. ** P<0.01 cycling females saline vs LPS; * P<0.05 E18 dams saline vs LPS; # P<0.05 LPS E18 pregnant vs cycling females. (B). LPS (0.05 mg kg⁻¹) induced a significant increase in plasma IL-1ra in cycling and E18 pregnant rats versus saline alone (1 ml kg⁻¹), however, this response was significantly greater in pregnant versus cycling rats. ** P<0.01 cycling saline vs LPS; ## E18 saline vs LPS; && LPS E18 vs cycling (C).

Figure 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⁻¹) induced a significant increase in IL-1β (A) and COX-2 (B) mRNA in the hypothalamus of male rats (saline saline vs saline LPS ** P<0.01). LPS challenge also induced a significant increase in IL-1β and COX-2 mRNA in animals treated with IL-1ra (IL-1ra saline vs IL-1ra LPS ++ P<0.01), however, this was significantly attenuated versus animals treated with saline alone (## P<0.01).
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Figure 1. Febrile responses to LPS are gender-dependent
Figure 2. Gender differences in central and peripheral responses to LPS
Figure 3. Attenuated febrile responses near term of pregnancy correlate with increased plasma IL-1ra
Figure 4A

![Graph showing IL-1ra and IL-1 mRNA levels in SALINE and LPS conditions.]

Figure 4B

![Graph showing COX-2 mRNA levels in SALINE and LPS conditions.]

Figure 4. Increasing plasma levels of IL-1ra attenuates central expression of IL-1β and COX-2 mRNA in male rats.