Interleukin-1β fever in rats: gender difference and estrous cycle influence

A. MOUIHATE, X. CHEN, AND Q. J. PITTMAN
Neuroscience Research Group, Department of Physiology and Biophysics,
University of Calgary, Calgary, Alberta, Canada T2N 4N1

Mouihate, A., X. Chen, and Q. J. Pittman. Interleukin-1β fever in rats: gender difference and estrous cycle influence. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1450–R1454, 1998.—Evidence exists to support the concept of sex difference in immune system activation by pyrogenic cytokines. In this study, fever development was monitored to analyze the effect of peripheral administration of interleukin (IL)-1β (1 µg/kg) in adult male and cycling or ovariectomized female rats with or without ovarian hormonal replacement. In male and randomly cycling female rats, a similar increase in body temperature occurred after intraperitoneal IL-1β injection. Two representative stages of estrus with higher and lower levels of ovarian hormones (proestrus and diestrus, respectively) were chosen for study of the febrile response induced by IL-1β. The fever induced by IL-1β was found to be significantly higher and more prolonged in females at proestrus than at diestrus. The differential fever response seems to be mainly linked to the ovarian hormonal levels because bilaterally ovariectomized females, supplemented with sequential injections of estradiol 17β and progesterone, showed a significantly higher IL-1β fever than did ovariectomized rats receiving estradiol 17β only. These results indicate that gonadal hormones can influence fever development and raise the possibility of interaction between sex hormones and thermogenesis in females during the estrous cycle.

IL-1β; estrogen; progesterone; ovariectomy

Fever development is one of the major processes by which mammals enhance the efficiency of their immune system when they are challenged with pathogens (e.g., bacteria and viruses). The influence of gender on the amplitude and the duration of fever is still unclear. Intracerebroventricular injection of PGE2 induces a higher fever in females than in males (12), whereas intravenously infused lipopolysaccharide (LPS) induces a higher fever in male rats (25). The fever response also changes depending on the physiological states of the females. Notably, a significant attenuation of febrile responses to intravenous infusion of either LPS or interleukin (IL)-1β, or to intracerebroventricular injection of PG (PGE2 or PGE3), is observed in pregnant rats at near term (22, 23, 33). However, estrous cycle states did not affect the fever response generated by centrally injected PGE2 (23).

Peripheral immune activation, which results in cytokine production, is modulated by circulating hormones such as glucocorticoids and gonadal hormones (for review, see Ref. 31). There is evidence that females have more pronounced immune responses than males (20). Moreover, these responses change during the estrous cycle in rodents, being more active at proestrus than at diestrus (19). Ovarian hormones whose levels increase at proestrus (estrogen and progesterone) (7) modulate the immune activity of cultured rat peritoneal macrophages (8–10). Reports of investigations of a causal relationship between gonadal hormones and fever induction in females are surprisingly sparse. Nonetheless, if immune system activity is modulated by ovarian hormones, fever development to peripherally injected cytokines may also change with regard to the estrous cycle.

In this study, we used a well-established fever induction model, intraperitoneal injection of IL-1β (1 µg/kg) and measurements of body temperatures of Mini-Mitter-equipped rats, to answer the following questions: 1) does peripheral injection of IL-1β elicit fever of different magnitude or duration in female compared with male rats? 2) does IL-1β fever change during the estrous cycle? and 3) do ovarian hormones influence IL-1β fever in females?

MATERIALS AND METHODS

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

General animal preparations and surgery. Male and female rats weighing 220–250 g were anesthetized with pentobarbital sodium (50–60 mg/kg ip). A precalibrated, battery-driven temperature transmitter (Mini-Mitter, Sunriver, OR) was inserted into the abdominal cavity of each rat. After at least 1 wk of recovery, rats were transferred to an environmentally isolated and temperature-controlled (22°C) testing room and allowed to acclimatize to the environment for a day. Core temperatures were monitored using a telemetry system (DataQuest II; Data Sciences, St. Paul, MN) that automatically took a reading every 5 min. Baseline temperatures were monitored for at least 2 h, after which intraperitoneal injection of IL-1β (10³ U/mg Immunex) was given at approximately the same time of day (1200).

Estrous cycle determination. To follow the estrous stage of female rats, we monitored daily vaginal smears 1 wk after the implantation of the Mini-Mitters. At least two consecutive estrous cycles were monitored, after which females were divided into proestrus and diestrus groups, both of which received a dose of IL-1β (1 µg/kg ip).

Ovariectomy and hormonal supplementation. Under pentobarbital sodium anesthesia, rats had both ovaries removed.
(OVX) and each had a temperature transmitter implanted into the abdominal cavity. They were then returned to the vivarium to recover for 10 days. On the morning of the eleventh day (0800), all rats received subcutaneous injection of a low dose (1 µg/kg) of estradiol benzoate [(17β)-estradiol-1,3,5(10)-triene-3,17-diol-3-benzoate; Steraloids, Wilton, NH] in sesame oil. The morning of the following day, they received a larger dose of estradiol benzoate (50 µg/kg). After 3.5–4 h (during which basal body temperatures were recorded; only body temperatures of the last hour were used as baseline), each was given a subcutaneous injection of either sesame oil or progesterone (4-pregnene-30,20-dione; Sigma, St. Louis, MO) at a dose of 5 mg/kg in sesame oil to mimic the hormonal changes that occur during proestrus (5). This estrogen-progesterone regimen was followed immediately by intraperitoneal injection of IL-1β (1 µg/kg) or pyrogen-free saline. Body temperature was recorded for the following 6 h.

Data analysis. All data are represented as means ± SE. Original temperature readings of 5-min intervals were calculated as net deviation from the mean baseline temperature. Data were analyzed by one-way or two-way ANOVA followed by Student-Newman-Keuls post hoc pairwise comparisons. After identification of significant differences between experimental groups, a two-tailed t-test (where only 2 points were compared) or an ANOVA (where 3 or more values were compared) were used to reveal significance at key time points. Significance was accepted at the 0.05 level.

RESULTS

Males and randomly cycling females develop similar IL-1β fever. The first experiment was designed to test whether males and females respond differently to peripherally injected IL-1β. As presented in Fig. 1, males (basal temperature of 36.81 ± 0.06°C) and randomly cycling females (basal temperature of 36.87 ± 0.1°C) showed no significant difference in fever in response to intraperitoneal injection of 1 µg/kg of IL-1β (ANOVA; F = 2.53, P = 0.136). Because males grow faster than females, at the age when the experiments were done, males were heavier than the females (males 363.67 ± 14.53 g vs. females 284.00 ± 5.58 g, P < 0.001), and thus they received more IL-1β. We therefore also compared IL-1β fever between weight-matched males and females and again observed identical fever responses (ANOVA, F = 0.142, P > 0.05; data not shown).

Proestrus rats develop higher and longer IL-1β fever than diestrous rats. Female rats undergo an estrous cyclicity during which ovarian hormone levels change dramatically. An experiment was designed to test whether hormonal change in these females affects their febrile response to peripherally injected IL-1β. Two representative ovarian stages with higher and lower levels of ovarian hormones (proestrus and diestrus, respectively) (7) were chosen. Baseline body temperatures during proestrus (36.77 ± 0.07°C) and diestrus (36.81 ± 0.11°C) were similar (P = 0.698). Proestrus females showed a significantly higher and sustained fever response compared with that of diestrous rats (ANOVA; F = 4.994, P < 0.05) (Fig. 2).

Estrogen and progesterone supplementation to ovariectomized rats potentiate IL-1β fever. To further characterize the involvement of ovarian hormones in the differential response between female rats at proestrus and diestrus, we surgically removed the ovaries and the two major ovarian hormones, estrogen and progesterone, were supplemented. Ovariectomized rats were either sequentially injected with estrogen followed by progesterone (subcutaneous injection) or injected with estrogen followed by vehicle only. The baseline body temperatures in ovariectomized rats were not distinguishable [OVX + (estrogen, progesterone) + IL-1β, 36.75 ± 0.09°C; OVX + (estrogen, oil) + IL-1β, 36.70 ± 0.15°C; OVX + (estrogen, progesterone) + Sal, 36.75 ± 0.06°C; OVX + (estrogen, oil) + Sal, 36.64 ± 0.07°C]. Figure 3 shows that, under these conditions, OVX rats fully supplemented with estrogen and progesterone responded to intraperitoneal injection of IL-1β with a significantly greater increase in body temperature compared with the OVX rats that did not receive progesterone (ANOVA; F = 31.27, P < 0.001). Student-Newman-Keuls post hoc pairwise comparisons revealed no difference between saline-injected groups but a significant difference between both IL-1β-injected groups and

Fig. 1. Change (Δ) in body temperature of male and randomly cycling female rats after intraperitoneal injection of interleukin (IL)-1β (1 µg/kg). IL-1β was injected at time 0. Data are presented as means ± SE.

Fig. 2. Change in body temperature of proestrous and diestrous female rats after intraperitoneal injection of IL-1β. Intraperitoneal injection of IL-1β (1 µg/kg) at time 0 induced a larger and more sustained fever in proestrous females. Data are presented as means ± SE. For ANOVA results, see text. *P < 0.05, **P < 0.01.
Estrogen-progesterone involvement in the increased hormones (i.e., estradiol and progesterone) occur at modulating effects of peripherally secreted ovarian is most likely that the changes are related to the response during the estrous cycle are not yet known. It is maximized and receiving estrogen only. 

significantly higher IL-1β injected with estrogen followed by progesterone develop a fever response during diestrus. In addition, rats bilaterally ovariectomized and sequentially injected with IL-1β results in attenuated fever response in progesterone and oil-pretreated groups. Progesterone-oil regimen shows more responsive to the pyrogenic effect of peripherally endogenous pyrogen, IL-1β. This result can also be interpreted as a fever response to IL-1β was confirmed in ovariectomized rats on an estradiol-progesterone replacement regimen. Our results are consistent with a recent study in which ovarian hormone replacement was found to modulate thermoregulation in postmenopausal women.

The question therefore arises as to how ovarian hormones might affect the febrile response. Several possibilities come to mind, involving either a peripheral action of the IL-1β or the central responses to the cytokine. IL-1β has numerous actions on peripheral immune tissues, including the induction of synthesis of both further IL-1β and other cytokines (for review, see Ref. 16). Because evidence exists to support a direct interaction between sex steroids and immune system function (19) (for review, see Ref. 31), ovarian hormones may modulate these actions of IL-1β on peripheral immune tissues and thereby differentially alter levels of pyrogenic cytokines as a function of hormonal status. Another possibility is that, at different times of the estrous cycle, the access of IL-1β to its sites of action within the body varies; this could prevent it from activating responsive tissues. However, there do not appear to be any available data indicating changes in blood flow and distribution or capillary permeability throughout the estrous cycle that could account for the changes in febrile responses we have observed.

With respect to central actions of IL-1β that could be modulated by hormonal status, it is thought that IL-1β acts at circumventricular organs or brain capillaries or at peripheral afferent nerves to activate cells within the brain to elicit synthesis and release of PGs of the E series (3, 14, 18). Because we previously showed that direct injection of PG into the lateral ventricle of female rats resulted in identical fevers at all stages of the estrous cycle (23), the attenuated IL-1β fevers seen at diestrus must be at a locus before the action of PGs. There are several steps where this could occur and which might be subject to modulation by physiological changes (most likely hormonal in nature) throughout the estrous cycle. Because available evidence indicates that IL-1β fevers are mediated by PGs (14), it is possible that the brain synthesis and/or release of PGs, particularly in cerebral microvessels (3), in response to IL-1β is altered as a function of estrous status. We are unaware of any experiments addressing this possibility. Nonetheless, in a variety of peripheral tissues, PGE2 synthesis and release was greater at proestrus than at diestrus (11, 37). Furthermore, oxytocin-stimulated PGE2 release in cultured bovine endometrium is enhanced by estrogen (1). It will be important to determine if similar variations exist for brain PG production.

In addition to causing brain synthesis of PGs, IL-1β administration is associated with activation of central corticotropin-releasing hormone (CRH) pathways to cause fever (29, 30). The differential fever response during the estrous cycle may be due to a differential CRH expression (and subsequent release) in thermogenic brain areas in females as a function of their physiological states. Indeed, CRH gene is highly expressed in the hypothalamus of proestrus rat (4),

![Graph](http://ajpregu.physiology.org/)
Possibly the most remarkable finding of this series of experiments is that the enhanced fever we previously observed in females after intracerebroventricular PGE2 is not seen in response to intraperitoneal IL-1β. Thus the manner in which gonadal hormones affect peripheral and central aspects of the febrile response is different. Because IL-1β represents only one of the peripheral cytokines released during a peripheral immune challenge, it will be important to determine if there are similar sex differences in response to LPS in animals equipped with telemetry devices.

REFERENCES


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