Metyrapone restores the febrile response to *Escherichia coli* LPS in pregnant rats

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Alexander BN, Fewell JE. Metyrapone restores the febrile response to *Escherichia coli* LPS in pregnant rats. *Am J Physiol Regul Integr Comp Physiol* 300: R1588–R1595, 2011. First published April 13, 2011; doi:10.1152/ajpregu.00785.2010.—Fever, an important component of the host’s defense response to immune challenge, is absent or attenuated in rats near the term of pregnancy. The current experiments were carried out to determine the role of endogenous glucocorticoids in mediating the altered core temperature (Tc) response to exogenous pyrogen (i.e., *Escherichia coli* LPS). For the experiments, metyrapone—a glucocorticoid synthesis inhibitor—was administered to near-term pregnant rats prior to an EC100 dose of *E. coli* LPS. Administration of LPS following vehicle elicited a significant corticosterone response and resulted in a decrease in Tc (i.e., hypothermia). Prior administration of metyrapone, however, which abolished the corticosterone response and altered the pyrogenic/cryogenic cytokine response to LPS, eliminated hypothermia and restored the febrile response. Our results provide evidence that endogenous glucocorticoids play a role in mediating the altered febrile response to immune stimuli observed in rats near the term of pregnancy.

Numerous physiological changes accompany the maternal adaptation to pregnancy in rats, including reversible changes in blood hormone concentrations (e.g., corticosterone) and basal thermoregulatory control, as well as the cytokine, prostanoid, and Tc responses to immune challenge (4, 17, 19, 21, 34). For example, Fote and Fewell (21) observed that Tc responses to intraperitoneal administration of 160 μg/kg *Escherichia coli* LPS (EC100 in nonpregnant rats) are different in pregnant rats as early as day 10 of the 21-day gestational period compared with nonpregnant oophorectomized rats. In nonpregnant rats, 160 μg/kg *E. coli* LPS elicits an increase in Tc (i.e., a fever) with a latency, magnitude, and duration of 1.5 h, 1.9°C, and at least 4.5 h, respectively (21). Accompanying the febrile response are significant increases in plasma IL-1β, IL-6, TNF-α, and IL-1ra (22). In pregnant rats, however, 160 μg/kg *E. coli* LPS elicits a “regulated” decrease in Tc (i.e., hypothermia)—the magnitude and duration of which increase with increasing LPS dose and gestation—before a modest increase in Tc above baseline is recorded (21, 52); accompanying the hypothermia are significant increases in plasma TNF-α, and IL-1ra, but not IL-1β and IL-6 (22). The mechanism and consequences for the mother and fetus of this altered thermoregulatory component of the acute phase response remain largely unknown.

It has long been known that glucocorticoids have an anti-pyretic action on natural fever in man (27), and Coelho et al. (13) and Morrow et al. (38) have shown that endogenous glucocorticoids modulate bacterial pyrogen-induced fever in male rats. Furthermore, Moore and Fewell (36) have recently shown that oral administration of mifepristone (RU38486)—a progestosterone and glucocorticoid type II receptor antagonist—attenuates the transient hypothermic response following intraperitoneal administration of 160 μg/kg *E. coli* LPS in near-term pregnant rats. As basal blood concentrations of corticosterone increase from day 10 of gestation, reaching a maximum at term of gestation in rats (4, 15, 47, 55), we speculated that the altered Tc response resulted from a corticosterone-mediated feed-forward mechanism, which served to hold back pyrogenic cytokine and prostaglandin synthesis/secretion upon exposure to the immune stimulus. Despite the fact that Brunton et al. (9) have shown that systemic administration of a small dose of IL-1β (i.e., 500 ng/kg) elicits a corticosterone response in nonpregnant rats but not in pregnant rats, our results do not preclude the possibility that systemic administration of a relatively large dose of bacterial pyrogen activates the hypothalamic-pituitary-adrenal axis, resulting in the secretion of corticosterone, which acts via a negative feedback mechanism to modulate the Tc response. Indeed, we have recently found that near-term pregnant rats reveal a significant corticosterone response when exposed to a psychological stressor (i.e., a simulated open field) (47). Furthermore, given that RU38486 is a progestosterone, as well as a glucocorticoid receptor antagonist, we cannot rule out a possible role for this hormone in mediating the altered Tc response to bacterial pyrogen near the term of pregnancy in rats (36).

Accordingly, our current experiments have been carried out to further define the role of corticosterone in mediating the altered Tc response to bacterial pyrogen observed in rats near the term of pregnancy. Our approach has been first, to determine whether intraperitoneal administration of an EC100 dose of *E. coli* LPS elicits a systemic corticosterone response in near-term pregnant rats as it does in nonpregnant rats. Second, we have carried out experiments to determine whether intraperitoneal administration of metyrapone (SU-4885)—a specific glucocorticoid synthesis inhibitor—eliminates the corticosterone response elicited by an EC100 dose of *E. coli* LPS in near-term pregnant rats. Third, we have carried out experiments to test the hypothesis that administration of metyrapone and elimination of the corticosterone response restores the febrile response to an EC100 dose of *E. coli* LPS in near-term pregnant rats.

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MATERIALS AND METHODS

Experiments were carried out on 12 nonpregnant Sprague-Dawley female rats and 119 time-mated Sprague-Dawley rats (Charles River Laboratories) undergoing their first pregnancy. The rats were housed individually in Plexiglas cages lined with Aspen-Chip Laboratory Bedding (Northeastern Products) and kept in a humidity (30–40%) and temperature (25 ± 1°C), the selected ambient temperature and thermoneutral temperature of nonpregnant and pregnant rats (17)-controlled environmental chamber on a 12:12-h light-dark cycle with lights on at 0700. They were handled several times prior to an experiment to familiarize the animals with the investigator and experimental procedures. All animals had continuous access to food (Lab Diet 5001) and tap water.

All surgical and experimental procedures were carried out in accordance with ethical codes provided by the Canadian Council on Animal Care, with the approval of the Animal Care Committee of the University of Calgary.

Surgical Procedure

Five days before an experiment, each animal was placed in a cylindrical anesthesia chamber (Kent Scientific), and anesthesia was rapidly induced with halothane in oxygen. Anesthesia was then maintained via mask using an open-circuit anesthesia system delivering ~2% halothane in oxygen with a gas flow rate of 1 liter/min. A paramedian laparotomy was performed using sterile technique and a free-floating, battery-operated telemetry device (TA10TA-F20, Data Sciences International) was placed in the peritoneal cavity for measurement of Tc. In addition, a sterile catheter of silicone tubing (Dow Corning Silastic) was inserted into the peritoneal cavity for administration of the various injectates. The catheter was then tunneled subcutaneously, exteriorized at the dorsal scapular area, and sealed with a ligature. Intraperitoneal administration of injectate by this technique does not elicit stress-induced fever in rats, as normally occurs when once pierces the abdominal wall with a needle for drug administration (16). Finally, the skin was sutured to close the abdominal incision and the catheter was secured in place with a purse-string suture and tissue adhesive (Vetbond). Topical antibiotic (Topazone) and spray adhesive bandage (OpSite) were applied to all wounds.

Experiment Protocols

Experimental series I. Nonpregnant rats were studied after they were randomly allocated to one of two experimental groups: rats that received vehicle (n = 6) or 160 μg/kg E. coli LPS (n = 6). On the day prior to an experiment, each rat was removed from its cage and weighed. On the day of an experiment, following an acceptable control period defined as five, 2-min measurements of Tc that did not vary by more than ± 0.2°C, each rat was removed from its cage and trunk blood was collected following decapitation. The blood was collected on ice in centrifuge tubes containing heparin and was centrifuged for 10 min; the resulting plasma was stored at ~70°C until plasma corticosterone, IL-1β, IL-6, and TNF-α were measured.

Experimental series II. Pregnant rats (day 20 or 21 of gestation) were studied after random allocation to one of four experimental groups according to whether they received vehicle followed at 1 h by vehicle (Veh-Veh group; n = 7), vehicle followed at 1 h by E. coli LPS (Veh-LPS group; n = 7), metyrapone, followed at 1 h by vehicle (Met-Veh group; n = 8) or metyrapone followed at 1 h by E. coli LPS (Met-LPS group; n = 5). On the day prior to an experiment, each rat was removed from its cage and weighed. On the day of an experiment, following an acceptable control period defined as five, 2-min measurements of Tc that did not vary by more than ± 0.2°C, each rat was removed from its cage and given one of the pairs of aforementioned intraperitoneal injections after the injectates were warmed to body temperature. The rat was then returned to its cage, and Tc was measured at 2-min intervals for 6 h.

Conditions of Observation

All experiments were carried out during the light cycle and began at ~1100 to avoid circadian variations in basal Tc and blood corticosterone and cytokine concentrations or their response to perturbation. During an experiment, each animal was studied in her cage in the aforementioned environmental chamber. Each cage was placed over a platform antenna (PhysioTel CTR 86; RLA-1020 Data Sciences International), which received the output frequency (Hz) from the implanted telemetry device. The platform antenna was interfaced with a peripheral processor for determination of Tc.

We emphasize that 1) animals in our experiments received intraperitoneal injections of solution warmed to body temperature in a nonstressful manner to avoid stress-induced hyperthermia (8, 16); 2) our experiments were carried out in a tightly regulated neutral environmental temperature as environmental temperature has been shown to influence the Tc responses of rodents to LPS (44); and 3) the dose of E. coli LPS used in our experiments was selected from full dose-response experiments carried out in identical experimental conditions (21).

E. coli Lipopolysaccharide

E. coli LPS (serotype 0111:B4, Lot 31K4121) was purchased from Sigma-Aldrich as a lyophilized powder. The powder was reconstituted by adding sterile saline to make a stock solution, which was stored at ~4°C. On the day of an experiment, a sample of stock solution was diluted with sterile saline to make a total injected volume of 200 μL. Vehicle was sterile saline, and all injectates were followed by 200 μL of sterile saline to flush the dead space of the catheter. We have previously shown that 160 μg/kg E. coli LPS is the EC10, in that it is the smallest dose that elicits a maximal febrile response in nonpregnant rats (21).

Metyrapone

Metyrapone was purchased from Sigma-Aldrich as a powder. The powder was reconstituted by adding 40% polyethylene glycol 60% saline to make a stock solution, which was further diluted with sterile saline to make a total injected volume of 200 μL. Vehicle was 40% polyethylene glycol 60% sterile saline, and all injectates were followed by 200 μL of sterile saline to flush the dead space of the catheter. In preliminary dosing experiments testing doses of 12.5 to 100 mg/kg, we found that 50 mg/kg was the smallest dose of metyrapone that prevented an increase in plasma corticosterone fol-
lowing administration of an EC$_{100}$ dose of *E. coli* LPS without significantly altering basal Tc.

**Corticosterone**

Corticosterone concentrations were measured using a Coat-A-Count Rat Corticosterone solid-phase RIA kit (Siemens Medical Solutions Diagnostics). Sensitivity of the corticosterone RIA was 5.7 ng/ml and the intra- and inter-assay coefficients of variation averaged 8% and 10%, respectively.

**Cytokines**

Plasma IL-1β was measured using ELISA, employing a polyclonal antibody specific for rat IL-1β (Quantikine Rat IL-1β/IL-1F2, R&D Systems); sensitivity of this assay was 15 pg/ml and the intra- and inter-assay coefficients of variation averaged 6.4% and 4.9%, respectively. Plasma IL-6 was measured using ELISA, employing a monoclonal antibody specific for rat IL-6 (Quantikine Rat IL-6, R&D Systems); sensitivity of the assay was 5 pg/ml and the intra- and inter-assay coefficients of variation averaged 6.7% and 7.6%, respectively. Plasma TNF-α was also measured using ELISA employing a monoclonal antibody specific for rat TNF-α (Quantikine Rat TNF-α/ TNFSSF1A, R&D Systems); sensitivity of the assay was 5 pg/ml and the intra- and inter-assay coefficients of variation averaged 3.1% and 9.4%, respectively.

**Statistical Analysis**

Statistical analysis was carried out using ANOVA. For hormone and cytokine data, which were all normally distributed (i.e., corticosterone, IL-1β, IL-6, and TNF-α), a two-way ANOVA followed by a Student-Newman-Keuls multiple-comparison test was used to determine whether experimental group (Veh-Veh; Veh-LPS; Met-Veh; Met-LPS) or time (2 h, 3 h, 5 h) influenced the measured variables. For Tc data, which were normally distributed, we also carried out a two-factor ANOVA for repeated measures followed by a Student-Newman-Keuls multiple-comparison test to determine whether experimental group or time influenced Tc. All results are reported as means ± SD; *P* < 0.05 was considered to be of statistical significance.

**RESULTS**

In nonpregnant rats, plasma concentration of corticosterone averaged 39 ± 25 ng/ml (± SD) 2 h following intraperitoneal administration of vehicle and 646 ± 58 ng/ml 2 h following intraperitoneal administration of 160 μg/kg *E. coli* LPS (*P* < 0.05). In near-term pregnant rats, plasma concentration of corticosterone averaged 167 ± 53 ng/ml 2 h following intraperitoneal administration of vehicle-vehicle and increased to more than 700 ng/ml 2 h following vehicle-LPS (Fig. 1). Plasma concentrations of corticosterone following vehicle-LPS waned with time but remained significantly elevated above vehicle-vehicle plasma concentrations for at least 5 h. Following metyrapone, however, intraperitoneal administration of LPS did not elicit an increase in corticosterone compared with that observed following metyrapone-vehicle. Basal plasma concentrations of corticosterone varied with time-of-day and were increased at 5 h after vehicle-vehicle in near-term pregnant rats (i.e., late afternoon), as previously observed by Atkinson and Waddell (4); the late afternoon increase in basal plasma concentration of corticosterone was not observed following metyrapone-vehicle.

Basal Tc’s were similar between the four experimental groups of near-term pregnant rats and averaged 36.7 ± 0.1°C (vehicle-vehicle), 36.8 ± 0.1°C (metyrapone-vehicle), 36.8 ± 0.0°C (vehicle-LPS), and 36.8 ± 0.1°C (metyrapone-LPS). Intraperitoneal administration of LPS following vehicle resulted in a transient but significant decrease in Tc (i.e., hypothermia) with a latency, duration, and magnitude of 90 min, 70 min, and −0.8°C, respectively (Fig. 2). Conversely, a biphasic increase in Tc (i.e., fever) was observed when metyrapone preceded intraperitoneal administration of LPS. Minimal changes in Tc from baseline were observed when vehicle followed either vehicle or metyrapone.

Following intraperitoneal administration of vehicle-LPS, plasma concentrations of IL-1β increased and peaked at 3 h and remained above vehicle-vehicle at 5 h; prior administration of metyrapone did not influence the IL-1β response following LPS (Fig. 3). Also, IL-6 increased and peaked at 3 h following intraperitoneal administration of vehicle-LPS, but in contrast to IL-1β, it did not remain significantly elevated at 5 h; the increase and duration of the IL-6 response to LPS were accentuated and prolonged by prior administration of metyrapone. Following intraperitoneal administration of vehicle-LPS, plasma concentrations of TNF-α increased at 2 h and peaked at

![Fig. 1. Plasma corticosterone concentrations measured 2, 3, and 5 h following vehicle-vehicle (Veh-Veh), Veh-LPS, metyrapone-Veh (Met-Veh), and Met-LPS in near-term pregnant rats. Data are presented as means ± SD for *n* = 8 in each group except Met-Veh at 3 h, where *n* = 7 and Met-LPS at 3 h, where *n* = 5. *P* < 0.05 vs. Veh-Veh at same time point. #*P* < 0.05 vs. Veh-Veh at 2 h.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00233.2010?journalCode=ajpregu)
3 h but returned to plasma concentrations observed following vehicle-vehicle by 5 h; prior administration of metyrapone abolished the TNF-α response to LPS.

**DISCUSSION**

Our experiments provide new information about the role of endogenous glucocorticoids in modulating the febrile response observed following intraperitoneal administration of bacterial pyrogen in rats near the term of pregnancy (21, 34). Novel findings in our study were 1) that intraperitoneal administration of an EC100 dose of *E. coli* LPS (i.e., 160 μg/kg) elicited a significant increase in plasma corticosterone concentration in near-term pregnant rats as in nonpregnant rats, 2) that intraperitoneal administration of metyrapone abolished the corticosterone response to intraperitoneal administration of an EC100 dose of *E. coli* LPS in near-term pregnant rats, 3) that a biphasic increase in Tc (i.e., fever) rather than a transient decrease in Tc (i.e., hypothermia) was observed when metyrapone was administered prior to an EC100 dose of *E. coli* LPS in near-term pregnant rats, and 4) that administration of metyrapone shifted the balance of systemic cytokines from cryogenic (e.g., TNF-α) to pyrogenic (e.g., IL-6) following administration of an EC100 dose of *E. coli* LPS in near-term pregnant rats. Thus, our data provide evidence that endogenous glucocorticoids play a role in modulating the Tc response to a relatively large dose of bacterial pyrogen in rats near the term of pregnancy, at least in part, through their influence on the balance of pyrogenic and cryogenic cytokines.

In male and nonpregnant female rats, systemic administration of exogenous (e.g., LPS) or endogenous (e.g., IL-1) pyrogen elicits fever and activates the hypothalamic-pituitary-adrenal axis, resulting in the secretion of corticosterone, which acts to modulate the Tc response and to ensure survival (6, 7, 9, 13, 38). In near-term pregnant rats, however, exposure to psychogenic stimuli (e.g., a novel environment, such as a simulated open field) and physical stimuli (e.g., LPS and IL-1β) has been shown to elicit an attenuated or no glucocorticoid response compared with that observed in nonpregnant rats (9, 25, 39). For example, Brunton et al. (9) found that intravenous administration of a threshold dose of IL-1β (i.e., 500 ng/kg) elicits a prompt adrenocorticotropic hormone and corticosterone response in nonpregnant rats but not in pregnant rats. Furthermore, they provided evidence that the hyporesponsiveness of the hypothalamic-pituitary-adrenal axis is mediated through an opioid mechanism, brought about by the neurosteroid allopregnanolone, acting at the level of the paraventricular nucleus corticotrophin-releasing hormone neurons (9, 10). In our current experiments, we found that intraperitoneal administration of a relatively large dose of *E. coli* LPS (i.e., EC100) elicited significant and similar increases in plasma corticosterone in near-term pregnant and nonpregnant rats. Thus, although pregnancy may shift the dose-response relationship be-
between a physical stressor and the resulting corticosterone response in rats, a relatively large stimulus clearly engenders a significant systemic glucocorticoid response.

We have previously found that oral administration of mifepristone (RU38486)—a progesterone and glucocorticoid type II receptor antagonist—attenuates the early hypothermic response but does not alter the later Tc response following ip administration of 160 μg/kg E. coli LPS in near-term pregnant rats (36). Given the aforementioned data of Brunton et al. (9) and the fact that basal blood concentrations of corticosterone...
increase from around day 10 of gestation reaching a maximum at term of gestation in rats (4, 15, 47, 55), we speculated that the altered Tc response resulted from a corticosterone-mediated feed-forward mechanism, which served to hold back pyrogenic cytokine and prostaglandin synthesis/secretion upon exposure to the immune stimulus. Our previous results are somewhat different than what we observed in our current experiments and may have resulted from incomplete glucocorticoid type II receptor antagonism or perhaps a confounding effect of progesterone receptor antagonism. Our current experiments clearly show that halting the biosynthesis of corticosterone with metyrapone, and thus eliminating the pregnancy-related, accentuated late-afternoon increase in basal corticosterone concentration, as well as the corticosterone response following intraperitoneal administration of E. coli LPS, not only eliminates the early hypothermic response but also restores the later febrile response in near-term pregnant rats. Restoration of the febrile response to bacterial pyrogen in near-term pregnant rats, therefore, likely results from abolition of a corticosterone-mediated negative feedback mechanism, as well as a corticosterone-mediated feedforward mechanism, as previously speculated (36), both of which govern the balance of pyrogenic and cryogenic cytokines following administration of bacterial pyrogen, as well as corticosterone-mediated effects downstream to the release of cytokines, as discussed below.

Perspectives and Significance

Our experiments provide evidence that endogenous glucocorticoids play a role in modulating the cytokine and Tc response to a relatively large dose of bacterial pyrogen in rats near the term of pregnancy. Given that fever in response to infectious stimuli is adaptive and thought to be of survival value to the host (29), what are the possible consequences for the mother and fetus of this altered cytokine response and “regulated” decrease in Tc (i.e., hypothermia) rather than a “regulated” increase in Tc (i.e., fever) in response to infectious stimuli? Considering that pregnancy is a hypermetabolic and hyperdynamic state, perhaps a hypothemic response to infectious stimuli aimed at energy conservation, which defends the host’s vital systems is of greater survival value than the antimicrobial and immunostimulating effects of a febrile response. Fetal Tc is normally “clamped” 0.4°C to 0.8°C higher
than maternal Tc, (1), and in some species, such as the sheep, where the febrile response to bacterial pyrogen occurs until very late in gestation, fetal Tc increases in parallel (1, 23) or exceeds (30) the rise in maternal Tc with a resulting increase in oxygen demand secondary to the temperature coefficient of metabolism (i.e., Q10). If this occurred at a time when fetal oxygen availability was limited, such as asphyxia during birth, it could potentially exacerbate neuronal injury (11, 31) and increase perinatal morbidity and mortality. Studies in primates have shown that hyperthermia alone is associated with the development of fetal hypoxia, metabolic acidosis, and hypertension (37). It has also been suggested that some maternal bacterial and viral infections may be associated with later neurodevelopmental disorders such as schizophrenia in the offspring (41, 50). The potential role that pyrogenic vs. cryogenic cytokines and/or that the maternal Tc response play in the etiology of neurodevelopmental disorders is unknown. One might speculate, however, that the maternal hypothermic response to infectious stimuli modulates the subsequent generation of proinflammatory cytokines, which may place the fetus at risk for brain injury, particularly when coupled with the asphyxia of parturition (12, 28, 45). Lastly, Hull et al. (24) have provided evidence that newborn rabbits select ambient temperatures that mimic their intrauterine experience and regulate their Tc to a level of ~0.8°C above the maternal body-core temperature on the day prior to delivery (i.e., at a level similar to that which they experienced late in fetal life). Thus, it is also possible that an altered cytokine and maternal febrile response to infection may influence central nervous system thermoregulatory “ imprinting” for the newborn.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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