Peri-OVLT E-series prostaglandins and core temperature do not increase after intravenous IL-1β in pregnant rats

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Fewell, James E., Heather L. Eliason, and Roland N. Auer. Peri-OVLT E-series prostaglandins and core temperature do not increase after intravenous IL-1β in pregnant rats. J Appl Physiol 93: 531–536, 2002; 10.1152/japplphysiol.01036.2001.—Rats have an attenuated febrile response to endogenous pyrogen near the term of pregnancy. Given the fundamental role of E-series prostaglandins (PGEs) in mediating the febrile response to blood-borne endogenous pyrogen, the present experiments were carried out to determine whether PGEs increase in the area surrounding the organum vasculosum laminae terminalis (peri-OVLT) of near-term pregnant (P) rats as in nonpregnant (NP) rats after intravenous (iv) administration of recombinant rat interleukin-1β (rrIL-1β). Core temperature was measured by telemetry and peri-OVLT interstitial fluid was sampled in 12 NP and 12 P chronically instrumented, Sprague-Dawley rats by microdialysis for determination of total PGEs by radioimmunoassay. Basal core temperatures were higher in NP compared with P rats (NP 37.9°C ± 0.5, P 36.9°C ± 0.4; P < 0.05), but basal peri-OVLT PGEs were similar in both groups (NP 260 ± 153 pg/ml, P 278 ± 177 pg/ml; P = not significant). Intravenous administration of rrIL-1β to NP rats produced a significant increase in core temperature with a latency, magnitude, and duration of 10 min, 0.87°C, and at least 170 min, respectively; peri-OVLT PGEs were increased significantly by 30 min and averaged 270% above basal levels throughout the experiment. In P rats, however, neither core temperature nor peri-OVLT PGEs increased significantly after iv administration of rrIL-1β. Intravenous administration of vehicle did not significantly alter core temperature or peri-OVLT PGEs in either group of rats. Thus peri-OVLT PGEs do not increase in P rats as they do in NP rats after iv administration of rrIL-1β. The mechanism of this interesting component of the maternal adaptation to pregnancy, which likely plays a major role in mediating the attenuated febrile response to endogenous pyrogen near the term of pregnancy, warrants further investigation.

METHODS

Experiments were carried out on 12 nonpregnant and 12 pregnant Sprague-Dawley rats (Charles River Laboratories) undergoing their first pregnancy. The rats were housed individually in Plexiglas cages containing Aspen-Chip Laboratory bedding (Northeastern Products) in a humidity-con-
trolled environmental chamber at an ambient temperature of 22 ± 1°C in a 12:12-h light-dark cycle (lights on at 0700) and were handled several times before an experiment to familiarize the animal with the investigator. All animals had continuous access to food (Lab Diet 5001) and tap water.

**Surgical preparation.** Five days before an experiment, each rat was anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). By means of a paramedian laparotomy, a free-floating battery-operated biotelemetry device (VM-FH, Mini-Mitter) was inserted into the peritoneal cavity for later measurement of core temperature. In addition, a sterile and endotoxin-free catheter (PhysioCath, Data Sciences International) was inserted into the superior vena cava via the right jugular vein for administration of rrIL-1β. The catheter was then tunneled under the skin and exteriorized on the dorsal scapular area. Between surgery and an experiment, the catheter was filled with a sterile heparin solution (1,000 U/ml), and a 25-gauge stainless steel wire was inserted into the end to seal the catheter.

The animal’s head was then placed in a stereotaxic frame with an associated micromanipulator (SAS-4100, Bioanalytical Systems [BAS]), and the skull was exposed by means of a midline scalp incision. A sterile and endotoxin-free intracebral guide cannula (MD-2250, BAS) for a BR-2 brain microdialysis probe (MD-2200, BAS) was placed such that its tip rested ~2 mm above the OVLT by using the coordinates anterior-posterior 0.6 mm, lateral 0.0 mm in relation to the bregma, and 6.9 mm below the surface of the brain as described and verified by Komaki et al. (31). Jeweler’s screws and dental acrylic were used to fix the guide cannula to the skull. A stylet (MD-2250, BAS) was placed into the guide cannula between surgery and an experiment. The skin was sutured to close the wound, and a topical antibiotic (Topazone, Austin) and spray adhesive bandage (OpSite, Smith + Nephew) were applied.

All surgical and experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

**Conditions of observations.** During an experiment, each animal was studied in a BAS containment system for awake animals (MD-1575); key components of this system include a round-bottomed containment bowl (MD-1500), a counterbalanced arm (MD-1501), a liquid swivel (MD-1505), a tether assembly with vial support (MD-1509), and a system table (MD-1504) that accommodates a variable-speed infusion pump (CMA-100). This system allows in vivo sampling experiments to be carried out on conscious animals with minimal handling or restraint stress in that the subjects have some freedom of movement, comfortable bedding, and free access to food and water during an experiment. Ambient temperature was maintained at 22 ± 1°C, and each containment bowl was placed over a platform antenna (PhysioTel CTR 86, Data Sciences International) that received the output frequency (Hz) from the biotelemetry device; each platform antenna was interfaced with a peripheral processor (Dataquest iv; Data Sciences International) for determination of core temperature.

**Experimental protocol.** Twelve nonpregnant and 12 pregnant rats were allocated to two experimental groups on the basis of whether they received an iv injection of rrIL-1β (nonpregnant n = 6; pregnant n = 7) or vehicle (nonpregnant n = 6; pregnant n = 5), and each animal was studied only once. Pregnant animals were studied on day 19, 20, or 21 of gestation (term ~22 days). All experiments were carried out during the light cycle and began between 1000 and 1100 to avoid any circadian effects on the measured variables.

On the day before an experiment, each animal was removed from its cage, weighed, and then returned to its cage in the environmental chamber. On the day of an experiment, the rat was placed in the BAS containment system for awake animals, it was secured to the liquid swivel by a neck collar, and the stylet was removed from the guide cannula. A BR-2 microdialysis probe was then inserted into the guide cannula, and microdialysis was initiated at a flow rate of 2.0 μl/min. This flow rate produced the most consistent PGE recovery rates from 30 in vitro experiments testing flow rates of 0.5–4.0 μl/min and PGE concentrations of 0–2.5 ng/ml; the average PGE recovery rate at a flow rate of 2.0 μl/min was 11.5%.

A control period of 1 h was allowed, during which time two dialysate samples were collected on ice, one over the first 30 min and one over the second 30 min. After the control period, rrIL-1β or vehicle was injected via the venous catheter and dialysate samples were collected over 30-min intervals for 180 min. Dialysate samples were stored at ~70°C until they were analyzed for total PGE content with use of a radioimmunoassay. Core temperature was recorded throughout the experiment at 10-min intervals.

After an experiment, the rat was anesthetized with pentobarbital sodium. The chest was then opened, and the vascular system was perfused through the heart with normal saline, followed by 10% buffered formalin to fix the brain tissue. The location of the tip of the dialysis probe was then verified histologically by R. N. Auer (hematoxylin and eosin-stained 6-μm sections) in 12 randomly selected rats out of the 24 studied to lie within the peri-OVLT region (i.e., within 1 mm of the OVLT).

rrIL-1β. rrIL-1β was purchased from R & D Systems as a lyophilized sample from a sterile, filtered solution in phosphate-buffered saline, containing 50 μg of bovine serum albumin per 1 μg of cytokine. The sample was reconstituted by adding sterile phosphate-buffered saline containing 1% bovine serum albumin to the vial to make a stock solution of 10 μg/ml. This solution was divided into ~100-μl aliquots and stored at ~70°C in sterile plastic vials. On the day of the experiment, a sample of stock solution was thawed and diluted to the appropriate dose in phosphate-buffered saline containing 1% bovine serum albumin to make a total injected volume of 200 μl. The dose of rrIL-1β (i.e., 0.2 μg/kg) used in our experiments was the dose that produced a half-maximal core temperature response in experimental series testing doses from 0.1 to 2.0 μg/kg in nonpregnant animals (22). In previous experimental series testing doses of IL-1β from 0 to 6.4 μg/kg in near-term pregnant animals, our laboratory did not observe significant increases in core temperature (60). Vehicle was phosphate-buffered saline containing 1% bovine serum albumin, and all injections were followed by 0.2 ml of sterile saline to flush the catheter.

**PGE radioimmunoassay.** Radioimmunoassay of PGE in microdialysates was performed according to the method of Van Orden et al. (57) with minor modifications as follows. Microdialysates were not subjected to chromatography but were added directly to the assay tubes, and PGE1 125Iiodotyrosine methyl ester was used in place of tritiated PGE2. Sensitivity, defined as the least amount of PGE distinguished from 0 at the 95% confidence level, was 1.0 pg. Specificity of the PGE antibody as well as intra- and interassay coefficient of variations have been previously reported (57).

**Statistical analysis.** Statistical analysis was carried out by using a three-factor analysis of variance for repeated measures followed by a Newman-Keuls multiplecomparison test.
to determine whether state (pregnant or nonpregnant), injectate (vehicle or rrIL-1β), or time influenced core temperature or PGE levels. In addition, a two-factor ANOVA followed by a Newman-Keuls multiple-comparison test was carried out to determine whether state or injectate influenced the fever index (15) expressed as area under the core temperature-time curve for the 3 h after iv administration of vehicle or rrIL-1β. All results are presented as means ± SD; *P < 0.05 was considered to be of statistical significance.

RESULTS

As we have previously reported (20, 23), basal core temperatures were significantly (P < 0.05) higher in nonpregnant rats (37.9 ± 0.5°C) compared with near-term pregnant rats (36.9 ± 0.4°C). The iv administration of rrIL-1β to nonpregnant rats produced a significant increase in core temperature with a latency, magnitude, and duration of 10 min, 0.87°C, and at least 170 min, respectively (Figs. 1 and 2). There were no significant effects of iv administration of rrIL-1β on core temperature in near-term pregnant rats or of iv administration of vehicle in either group of rats.

Basal PGE levels were similar (not significant) in dialysate fractions collected from nonpregnant (260 ± 153 pg/ml) and pregnant (278 ± 177 pg/ml) rats. PGE levels in the interstitial fluid surrounding the OVLT increased significantly after iv administration of rrIL-1β in nonpregnant rats, averaging 270% of control values throughout the experiment (Fig. 3). PGE levels did not change significantly after iv administration of rrIL-1β in pregnant rats or after administration of vehicle in either group of rats.

DISCUSSION

Our experiments provide new information about potential mechanisms of the attenuated febrile response to endogenous pyrogen near the term of pregnancy in rats (52). Novel findings were that iv administration of rrIL-1β produced significant increases in peri-OVLT PGEs and core temperature in nonpregnant rats but not in near-term pregnant rats. Thus our data support the hypothesis that pregnancy impairs the synthesis and release of PGEs into the interstitial fluid of the peri-OVLT region after iv administration of rrIL-1β.
The mechanism of this interesting component of the maternal adaptation to pregnancy, which likely plays a major role in mediating the attenuated febrile response to endogenous pyrogen near the term of pregnancy, is currently unknown.

As we have previously observed and reported (20, 23), basal core temperatures were significantly higher in nonpregnant rats compared with near-term pregnant rats. The “regulated” decrease in core temperature near the term of pregnancy most likely results from the hormonal changes that occur at this time of gestation in rats. For example, Yoshinaga et al. (61) showed that ovarian venous levels of estrogen are low during the first two-thirds of gestation, increase slowly from day 14 or 15 of gestation toward term, and then increase precipitously from day 20 of gestation to the time of parturition. To the contrary, ovarian venous and systemic levels of progesterone increase slowly during the first two-thirds of gestation, decrease slowly from day 14 or 15 of gestation toward term, and then decrease precipitously from day 19 or 20 of gestation to the time of parturition (27, 43). Given that administration of progesterone or estradiol raises metabolic rate and core temperature in ovariecotmized rats (34, 37), progesterone appears to be the most likely candidate for mediating the changes in basal core temperature near the term of pregnancy in this species. Progesterone has long been known to have thermogenic effects (25, 47) and recently has been shown to influence firing patterns of preoptic thermosensitive neurons (46). This postulate requires further investigation.

In 1948, Beeson (3) identified endogenous pyrogen as a fever-inducing substance produced and released by circulating leukocytes and fixed macrophages in response to exogenous pyrogens (i.e., bacterial endotoxin, lipopolysaccharide). This substance was later renamed IL-1, and its release as well as the release of other endogenous pyrogens, such as IL-6 and tumor necrosis factor-α, constitutes an essential step in the genesis of fever after exposure to exogenous pyrogens (17). Classically, IL-1 is thought to act within the OVLT to evoke the synthesis and release of PGEs that serve to activate heat-producing and heat-conserving mechanisms to increase core temperature (30, 55).

It was Milton and Wendlandt (41) who first presented evidence that prostaglandins play a role in fever when they reported that intracerebroventricular (icv) administration of PGE1 in conscious cats elicited an increase in core temperature along with shivering, piloerection, and peripheral vasoconstriction and with the animals assuming a “curled-up” position; comparable results were later obtained after icv administration of PGE2 (42). Similar observations have been made in rats (36, 50), and numerous studies have shown that prostaglandins are released into the cerebrospinal fluid (4, 12, 48) during pyrogen-induced fevers. Furthermore, congenital absence of the PGE-receptor subtype EP3 impairs the febrile response to exogenous and endogenous pyrogens in mice (56). Moreover, Komaki et al. (31) showed that iv injection of IL-1β causes increased levels of PGE2 in the interstitial fluid of the OVLT and the medial preoptic area of the hypothalamus in rats. This is relevant to febrigenesis because Scammell et al. (51) recently showed that neuroanatomic sites clustered along the ventromedial aspect of the preoptic area just anterior to the OVLT are the most pyrogenic in response to microinjection of PGE2; this area has a high concentration of PGE2-binding sites (39, 59).

We have previously shown that pregnant as well as nonpregnant rats activate both behavioral and autonomic thermoregulatory effectors to increase core temperature after icv injection of the pyrogen PGE1 (19); the duration of activation, however, is abbreviated in pregnant compared with nonpregnant rats, and this appears to limit the magnitude and duration of the febrile response. Subsequent experiments have shown that near-term pregnant rats develop “normal” core temperature responses to an icv injection of PGE1 (21) when it follows an icv injection of a vasopressin V1-receptor antagonist. This suggests that, if exposure to exogenous or endogenous pyrogen elicits a normal PGE response in near-term pregnant animals, arginine vasopressin acting as an endogenous antipyretic would serve to limit the magnitude and duration of the core temperature response. Interestingly, icv injection of a vasopressin V1-receptor antagonist does not “normalize” the core temperature response of near-term pregnant rats to an iv injection of rrIL-1β (22). This led us to speculate, as demonstrated in the present study, that iv administration of rrIL-1β does not elicit a nor-
minal PGE response in pregnant animals as it does in nonpregnant animals.

Although our present experiments were not designed to investigate the mechanism of the attenuated PGE response in near-term pregnant rats after exposure to blood-borne pyrogen, there are at least three possibilities. First, it is possible that blood-borne rrIL-1β does not elicit a normal end-mediator response because there is an alteration in the number or properties of cytokine receptors near the term of pregnancy. Alternatively, there may be an increase in the circulating levels of IL-1-receptor antagonist that competes with IL-1β for occupancy of type I and type II IL receptors on a variety of cells (2). This has been shown to occur in humans near the term of pregnancy (49). Second, it is possible that corticosterone mediates the altered PGE response in near-term pregnant rats. Corticosterone, which modulates fever after exposure to lipopolysaccharide (40, 45), is elevated near the term of pregnancy in rats (18, 53). Glucocorticoids (e.g., corticosterone) are antipyretic (10, 13) and are known to stimulate the response in near-term pregnant rats. Corticosterone, humans near the term of pregnancy (49). Second, it is possible that blood-borne rrIL-1β increases in core temperature. A moderate increase in core temperature during the latter part of gestation may be detrimental to the fetus not only by increasing oxygen demand but also by causing a rightward shift of the oxyhemoglobin dissociation curve, thereby decreasing oxygen affinity and oxygen saturation. Studies in primates have shown that hyperthermia in the absence of infection is associated directly with the Development of fetal hypoxia, metabolic acidosis, and hypotension (44). Furthermore, in conditions where fetal oxygen availability is severely limited (e.g., asphyxia during birth), an increase in core temperature may exacerbate newborn neurological injury (6, 33) and increase perinatal morbidity and mortality.

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