Influence of pregnancy on the febrile response to ICV administration of PGE\(_1\) in rats studied in a thermoline

HEATHER L. ELIASON AND JAMES E. FEWELL

Department of Physiology and Biophysics, Health Sciences Centre, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Eliason, Heather L., and James E. Fewell. Influence of pregnancy on the febrile response to ICV administration of PGE\(_1\) in rats studied in a thermoline. J. Appl. Physiol. 82(5): 1453–1458, 1997.—Rats near term of pregnancy have an attenuated febrile response to ICV injection of prostaglandin E\(_1\) (PGE\(_1\)) when they are studied at an ambient temperature below their thermoneutral zone. Given that nonshivering thermogenesis in brown adipose tissue is impaired in rodents near term of pregnancy, it is possible that the attenuated febrile response is forced by impairment of this component of the autonomic thermoregulatory response. If this were the case, then near-term pregnant rats should develop a “normal” fever after PGE\(_1\) administration if they were studied in a thermoline where they could utilize behavioral as well as autonomic thermoregulatory effectors to increase their body core temperature (T\(_{bc}\)). Experiments were, therefore, carried out on 13 nonpregnant and 14 pregnant chronically instrumented rats in a thermoline (temperature gradient 10–40°C) to investigate their T\(_{bc}\) responses to ICV injection of PGE\(_1\). ICV injection of 0.2 µg PGE\(_1\) produced significant increases in T\(_{bc}\) and fever index in both nonpregnant and pregnant animals (day 19 of gestation); the increases, however, were significantly attenuated in the pregnant compared with the nonpregnant rats. Behavioral (e.g., selected ambient temperature) and autonomic (e.g., oxygen consumption) thermoregulatory effectors were activated to increase T\(_{bc}\) after ICV PGE\(_1\) in both groups of animals, but the duration of activation was shortened in pregnant compared with nonpregnant rats. The abbreviated thermoregulatory effector responses and the resultant attenuated febrile response to PGE\(_1\) in the pregnant rats may have resulted from a pregnancy-related activation of an endogenous antipyretic system.

NUMEROUS PHYSIOLOGICAL CHANGES occur during the maternal adaptation to pregnancy. In rats, these changes include reversible alterations in thermoregulatory control. For example, baseline body core temperature (T\(_{bc}\)) decreases as gestation advances and then increases around the time of parturition (12, 19). Furthermore, there are different thermoregulatory responses to cold (17) and to pyrogens such as bacterial endotoxin (25), interleukin-1ß (35), and prostaglandin E\(_1\) (PGE\(_1\)) (39) in near-term pregnant rats compared with those observed in nonpregnant rats. The mechanism(s) of these pregnancy-induced changes is presently unknown.

Previous experiments in our laboratory designed to investigate the influence of pregnancy on the thermoregulatory response to PGE\(_1\) (39) were carried out at an ambient temperature of 22 ± 1°C, which is several degrees below the thermoneutral zone of nonpregnant rats (14). In these experiments, an intracerebroventricular (ICV) injection of 0.2 µg of PGE\(_1\) produced significant increases in T\(_{bc}\) in both nonpregnant and near-term pregnant animals. The increase in T\(_{bc}\) was, however, significantly attenuated in near-term pregnant compared with nonpregnant rats. Nonshivering thermogenesis in brown adipose tissue, which is an important autonomic thermoregulatory effector for heat production during the development of fever in rats studied at an ambient temperature below their thermoneutral zone (13), is impaired in rodents near term of pregnancy (2, 41). Thus it is possible that the attenuated febrile response observed in near-term pregnant rats was forced by an impairment of this component of the autonomic thermoregulatory response such that T\(_{bc}\) did not increase to reach the new central nervous system thermoregulatory set point after PGE\(_1\) administration. If this were the case, then we would expect near-term pregnant rats to develop a “normal” fever after PGE\(_1\) administration if they were placed in a thermoline where they could utilize behavioral as well as autonomic thermoregulatory effectors to increase their T\(_{bc}\) (5, 24). Therefore, our present experiments were carried out to test the hypothesis that nonpregnant and near-term pregnant rats would develop similar fevers after ICV administration of PGE\(_1\) when they are studied in a thermoline.

METHODS

Experiments were carried out on 13 nonpregnant and 14 pregnant Sprague-Dawley rats (weighing 224 ± 15 and 226 ± 11 g, respectively, at the time of surgery and 243 ± 21 and 313 ± 17 g at the time of experiment) undergoing their first pregnancy (Charles River Laboratories). The rats were housed in individual cages at 22 ± 1°C in a light-dark cycle with lights on from 0700 to 1900 and were handled every alternate day to familiarize the animal with the investigator. All animals had continuous access to food (Lab Diet 5001) and tap water.

Surgical preparation. No sooner than 7 days before an experiment, each rat was anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). A paramedian laparotomy was done, and a free-floating battery-operated biotelemetry device (PhysioTel TA10ETA-F20, Data Sciences International) was inserted into the peritoneal cavity for later measurement of T\(_{bc}\). The skin was then sutured to close the incision.

The animal was then placed in a stereotaxic frame, and the skull was exposed by means of a midline scalp incision. A stainless steel guide cannula (1.5 cm long, 20-gauge thin-walled tubing; Small Parts) was placed 1 mm above the left lateral ventricle by using the coordinates −0.6 mm anterior to the bregma and 2.0 mm lateral in relation to the bregma. Stainless steel guide cannula (1.5 cm long, 20-gauge thin-walled tubing; Small Parts) was placed 1 mm above the left lateral ventricle by using the coordinates −0.6 mm anterior to the bregma and 2.0 mm lateral in relation to the bregma. Stainless steel guide cannula (1.5 cm long, 20-gauge thin-walled tubing; Small Parts) was placed 1 mm above the left lateral ventricle by using the coordinates −0.6 mm anterior to the bregma and 2.0 mm lateral in relation to the bregma. Stainless steel guide cannula (1.5 cm long, 20-gauge thin-walled tubing; Small Parts) was placed 1 mm above the left lateral ventricle by using the coordinates −0.6 mm anterior to the bregma and 2.0 mm lateral in relation to the bregma.
dental acrylic were used to fix the guide cannula to the skull; the skin was then sutured to dose the incision. A 25-gauge stainless steel stylet was placed in the guide cannula between surgery and an experiment.

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 observation. The experiments were carried out with the animals in a thermocline, which consisted of a platform containing a 200-cm sealed perspex cylinder with an internal diameter of 11.5 cm. A linear temperature gradient of 10–40°C was produced in the thermocline by circulating hot and cold water into copper coils wrapped around the cylinder (Endocal Refrigerated Circulating Bath RTE-8DD, Neslab). Selected ambient temperature was determined by monitoring the position of the animal in the thermocline and recording the corresponding ambient temperature. For measurement of Tbc, the thermocline was placed over a series of platform antennas (PhysioTel CTR 86, Mini-Mitter), which received the output frequency (Hz) from the biotelemetry device; these were interfaced with a peripheral processor (Dataquest III, Data Sciences), which was connected to an IBM computer. Oxygen consumption was calculated from the difference in oxygen concentrations between the inflow and outflow gases (Ametek-Applied Electrochemistry S-3A/1 O2 analyzer) and the flow rate (2.00 l/min).

Experimental protocol. The rats were divided into two groups, with each animal being studied only once. The rats were given an ICV injection of either PGE1 [0.2 µg in 10 µl of artificial cerebrospinal fluid (CSF)] or vehicle (10 µl artificial CSF). Within each of these experimental groups, both nonpregnant rats and near-term pregnant rats (day 19 or 20 of gestation) were studied. On the day of an experiment, the animal was brought into the laboratory in the morning, removed from its cage, and placed in the thermocline. After at least 1 h had passed, control measurements were made at 2-min intervals. A suitable control period was defined as one in which Tbc was stable (i.e., ±0.2°C) for five consecutive measurements. After the 10-min control period, the rat was removed from the thermocline and given an ICV injection of either PGE1 or vehicle. For each ICV injection, a 25-gauge injection cannula was placed into the guide cannula, and the solution of choice was allowed to flow via gravity into the lateral ventricle over 30 s. After an injection, the animal was placed back into the thermocline, and oxygen consumption was measured at 10-min intervals for 2 h. Fever index was expressed as area under the Tbc curve in degrees Celsius per hour.

After an experiment, each rat was anesthetized, and the injection cannula was reinserted into the previously used guide cannula. Ten microliters of black ink were then injected via gravity flow. The chest was opened, and the cerebral vascular system was perfused through the heart with 10% buffered Formalin to firm up the brain tissue. The brain was then removed and sectioned. Correct placement of the injection cannula was verified by the presence of ink in the cerebroventricular system.

Prostaglandin. PGE1 was purchased as Prostin (ampule of 500 µg/ml in absolute ethanol, Upjohn) and divided into 25-µl portions and stored in sterile plastic vials at −70°C. Artificial CSF [128 mM Na+, 2.5 mM K+, 1.3 mM Ca2+, 1.0 mM Mg2+, and 135 mM Cl− (22)] was added to the vial to make a working solution of 50 µg/ml immediately before the injection. The choice of dose (50% effective dose) was based on previous ICV PGE1 dose-Tbc response experiments in nonpregnant rats reported by Marques et al. (24). Marques et al. and Stitt (38) have shown that this ICV dose of PGE1 produces an increase in Tbc between 1.6 and 1.8°C. We have previously found that the concentration of ethanol in the injectate does not cause changes in Tbc in either nonpregnant or pregnant rats when mixed with artificial CSF alone (unpublished observations).

Statistical analysis. Statistical analysis was carried out by using a three-factor multivariate analysis of variance for repeated measures, followed by a Newman-Keuls multiple-comparison test to determine whether state (pregnant or nonpregnant), injectate (vehicle or PGE1), or time influenced the measured or calculated variables. A two-factor multivariate analysis of variance followed by a Newman-Keuls multiple-comparison test was used to determine whether drug or state influenced the fever index and whether state or time influenced the change in Tbc from control after ICV administration of PGE1. All results are presented as means ± SD, with the exception of selected ambient temperature, which is presented as the mode; P < 0.05 was considered to be of statistical significance unless otherwise indicated.

RESULTS

ICV injection of 0.2 µg PGE1 produced a significant increase in Tbc in both nonpregnant and pregnant animals (Fig. 1). The increase, however, was significantly greater at 30 min in the nonpregnant rats compared with the pregnant rats (Fig. 2). In addition, the fever index was significantly greater in nonpregnant (0.45 ± 0.19°C/h) than in pregnant (0.26 ± 0.13°C/h) animals after ICV administration of PGE1. In the nonpregnant rats, Tbc increased by 10 min, peaked at 30 min, and remained elevated for 40 min. In the pregnant rats, Tbc increased by 10 min, peaked at 20 min, and remained elevated for 50 min. Artificial CSF did not significantly affect Tbc in either group of animals.

Pregnant and nonpregnant rats selected warmer ambient temperatures of similar magnitude after ICV injection of PGE1 (Fig. 3). In the nonpregnant rats, selected ambient temperature increased by 10 min and remained elevated for 20 min. In the pregnant rats, selected ambient temperature increased by 10 min and remained elevated for 10 min. In both groups of animals, selected ambient temperature decreased transiently during febrility. Interestingly, after ICV injection of vehicle, selected ambient temperature decreased in both groups of animals, the duration of which was increased in the nonpregnant rats compared with the pregnant rats.

Pregnant and nonpregnant rats increased their oxygen consumption by similar amounts after ICV injection of PGE1 (Fig. 4). In the nonpregnant rats, oxygen consumption increased by 10 min and remained elevated for 20 min. In the pregnant rats, oxygen consumption increased by 10 min and remained elevated for 10 min. After ICV injection of vehicle, oxygen consumption increased in both groups of animals, the duration of which was increased in the nonpregnant rats compared with the pregnant rats.

DISCUSSION

Our experiments provide new and important information about pregnancy and fever in rats. Novel findings
in our study were the following: 1) ICV injection of 0.2 µg PGE1 produced a significant increase in Tbc in both nonpregnant and pregnant animals studied in a thermonecline; the increase, however, was significantly greater in nonpregnant compared with pregnant rats and 2) both nonpregnant and pregnant rats activated behavioral (i.e., they selected a warmer ambient temperature) and autonomic (i.e., they increased their total body oxygen consumption) thermoregulatory effectors during febrigenesis; the duration of their activation, however, was shortened in pregnant compared with nonpregnant rats. Since in the absence of shivering, the increase in total body oxygen consumption after ICV administration of PGE1 provides an indirect estimate of heat production by nonshivering thermogenesis, our data do not support the hypothesis that an impairment of this component of the autonomic thermoregulatory response mediates the attenuated febrile response in rats near term of pregnancy.

Considerable evidence has accumulated that prostaglandins of the E series play a role in mediating the febrile response to exogenous and endogenous pyrogens (37). More than 20 years ago, Milton and Wendlandt (26) showed that ICV administration of prostaglandin E produced a dose-dependent increase in deep body temperature in cats. Similar observations have been made in other species, including rabbits (27) and rats (24, 33), and numerous studies have shown that prostaglandins are released into the CSF during pyrogen-induced fevers (3, 11, 32). Furthermore, Komaki et al. (20) have shown that an intravenous injection of interleukin-1β causes release of prostaglandin E2 (PGE2) into the interstitial fluid of the organum vasculosum of the lamina terminalis and the medial preoptic area of the hypothalamus in rats. Although it is generally acknowledged that PGE2 is likely to be the "natural" prostaglandin mediator of fever, there is no evidence that PGE1, as used in our experiments, acts in any way differently from PGE2 (28).

Fever, which is defined as a regulated increase in Tbc (36), is achieved by activation of heat-conserving and heat-producing mechanisms, the relative contributions of which depend on the pyrogen dose and type, the ambient temperature, and the age and size of the host (4, 6, 30, 40). Previous experiments in our laboratory have shown that the febrile response to ICV administration of PGE1 (39) is attenuated in pregnant rats compared with nonpregnant rats when they were studied at an ambient temperature below their thermoneutral zone. Given that nonshivering thermogenesis in brown adipose tissue, which is an important autonomic thermoregulatory effector for heat production during the development of fever in rats studied at an ambient temperature below their thermoneutral zone (13), is impaired in rodents near term of pregnancy (2, 41), it is...
possible that the attenuated febrile response was forced by an impairment of this component of the autonomic thermoregulatory response such that $T_{bc}$ did not increase to reach the new central nervous system thermoregulatory set point after PGE$_1$ administration. If this were indeed the case, then we would expect near-term pregnant rats to develop a normal fever after PGE$_1$ administration if they were placed in a thermocline where they could utilize behavioral as well as autonomic thermoregulatory effectors to increase their $T_{bc}$ (5, 24). This did not occur despite activation of both behavioral and autonomic thermoregulatory effectors after ICV injection of PGE$_1$. Although we observed an activation of both behavioral and autonomic thermoregulatory effectors after ICV injection of PGE$_1$, the duration of their activation was abbreviated in pregnant compared with nonpregnant rats. The nonpregnant rats selected a warmer ambient temperature and increased their total body oxygen consumption for 30 min after ICV administration of PGE$_1$, whereas the pregnant rats selected a warmer ambient temperature for only 20 min and increased their total body oxygen consumption for only 10 min. During activation, however, the magnitudes of the behavioral and autonomic

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**Fig. 3.** Selected ambient temperatures before and after administration of prostaglandin E$_1$ or vehicle (arrows) in nonpregnant and pregnant rats. A: nonpregnant-vehicle. B: pregnant-vehicle. C: nonpregnant-prostaglandin E$_1$. D: pregnant-prostaglandin E$_1$. Values are modes.

**Fig. 4.** Oxygen consumption before and after administration of prostaglandin E$_1$ or vehicle (arrows) in nonpregnant and pregnant rats. A: nonpregnant-vehicle. B: pregnant-vehicle. C: nonpregnant-prostaglandin E$_1$. D: pregnant-prostaglandin E$_1$. Values are means ± SD. *P < 0.05 vs. C.
thermoregulatory effector responses were similar in nonpregnant and pregnant animals. The shortened thermoregulatory effector response in pregnant rats appeared to limit the magnitude of the febrile response after ICV injection of PGE\textsubscript{1}.

It is possible that the abbreviated thermoregulatory effector response observed in rats near term of pregnancy resulted from the activation of an endogenous antipyretic system. Arginine vasopressin, which functions as an endogenous antipyretic substance in the central nervous system (18), is elevated in plasma (22) and in a number of hypothalamic nuclei in rats near term of pregnancy (9, 22). Furthermore, Ruwe et al. (34) have shown that administration of arginine vasopressin into the ventral septal area of the rat limits the increase in T\textsubscript{bc} evoked by the ICV injection of PGE\textsubscript{2}. Thus it is possible that a pregnancy-related activation of arginine vasopressin as an endogenous antipyretic substance may have limited the febrile response to ICV PGE\textsubscript{1} in our experiments. This requires further investigation.

Perspectives. Regardless of the mechanism of the altered febrile response to pyrogen in rats near term of pregnancy, what are the possible consequences for the fetus? From the standpoint of oxygen supply and demand, it may be advantageous to the fetus for the mother not to develop fever for several reasons. One reason is that fever may cause circulatory adjustments such that blood flow from internal body organs, including the uterus and placenta (7), shifts toward thermogenic organs (e.g., brown adipose tissue). Under conditions of maximal stimulation, brown adipose tissue, which usually represents <1% of body weight, can receive up to 60% of the cardiac output (29). An ensuing decrease in uteroplacental blood flow could compromise placental gas exchange, with a resulting decrease in fetal oxygen supply (10). Another reason is that during fever, fetal body temperature, which is normally 0.4–0.8°C higher than maternal body temperature (1), increases in parallel (1, 15), or exceeds (21) the rise in maternal body temperature, with a resulting increase in oxygen demand secondary to the temperature coefficient of metabolism (i.e., Q\textsubscript{10}). If the Q\textsubscript{10} in humans is \(~2.3\) (16), then metabolic rate increases \(~10%\) for each 1°C increase in body temperature. A moderate increase in body 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. Furthermore, in conditions where fetal oxygen availability is severely limited (e.g., asphyxia during birth), an increase in body temperature may exacerbate neuronal injury (8, 23) and increase perinatal morbidity and mortality.

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Address for reprint requests: J. E. Fewell, Heritage Medical Research Bldg., Univ. of Calgary, 206, 3330 Hospital Dr., N.W., Calgary, AB, Canada T2N 4N1 (E-mail: fewell@acs.ucalgary.ca).

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