Arginine vasopressin does not mediate the attenuated febrile response to intravenous IL-1β in pregnant rats

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Eliason, Heather L., and James E. Fewell. Arginine vasopressin does not mediate the attenuated febrile response to intravenous IL-1β in pregnant rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R450–R454, 1999.—Rats have an attenuated febrile response to intravenous endogenous pyrogen [e.g., interleukin-1β (IL-1β)] near the term of pregnancy. The present experiments were carried out on 25 nonpregnant and 32 pregnant rats to test the hypothesis that arginine vasopressin functioning as an endogenous antipyretic substance in the central nervous system mediates this attenuated febrile response. An intravenous injection of recombinant rat IL-1β (rrIL-1β) after intracerebroventricular vehicle produced a significant increase in core temperature in both nonpregnant and pregnant animals, the magnitude and duration of which was greater in the nonpregnant than in the pregnant rats. In pregnant rats, intravenous rrIL-1β after intracerebroventricular vasopressin V1-receptor antagonist accentuated the core temperature response compared with that observed with intravenous rrIL-1β after intracerebroventricular vehicle. In pregnant animals, however, intravenous rrIL-1β after intracerebroventricular vasopressin V1-receptor antagonist produced a decrease in core temperature rather than an increase in core temperature, which was observed with intravenous rrIL-1β after intracerebroventricular vehicle. Thus our data do not support the hypothesis that a pregnancy-related activation of arginine vasopressin as an endogenous antipyretic substance in the central nervous system attenuates the febrile response to intravenous rrIL-1β near the term of pregnancy in rats.

endogenous antipyretic; endogenous pyrogen; interleukin

METHODS

Experiments were carried out on 25 nonpregnant and 32 pregnant Sprague-Dawley rats (Charles River Laboratories) undergoing their first pregnancy, weighing 237 ± 9 and 267 ± 14 g, respectively, at the time of surgery and 245 ± 8 and 303 ± 13 g, respectively, at the time of experiment. The rats were housed individually in Plexiglas cages in a temperature- and humidity-controlled 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. No less than five 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 (VM-FH, Mini-Mitter Company) was inserted into the peritoneal cavity for later measurement of core temperature. In addition, a catheter (PhysioCath, Data Sciences International) was inserted to the superior vena cava via the left jugular vein for administration of recombinant rat IL-1β (rrIL-1β). The catheter was tunneled under the skin and exteriorized on the dorsal scapular area. Between surgery and experiments, 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.

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The animal's head was 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 using the coordinates anterior-posterior – 0.6 mm, lateral 2.0 mm in relation to the bregma, and 2.0 mm below the surface of the brain (28). J ewler's screws and
dental acrylic were used to fix the guide cannula to the skull. A 25-gauge stainless steel styllet was placed into the guide
cannula between surgery and 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 observations. During the experiment, each animal was studied in its home cage in an environmental chamber. Ambient temperature within the chamber was maintained at 22 ± 1°C. Each cage was placed over a platform antenna (PhysioTel CTR 86, Data Sciences International) that received the output frequency from the biotelemetry device and interfaced with a peripheral processor (Dataquest IV, Data Sciences International) for determination of core temperature.

Experimental protocol. Twenty-five nonpregnant and thirty-two pregnant rats were randomly allocated to four experimental groups based on the combination of injectate 1 (intracerebroventricular vehicle or vasopressin V1-receptor antagonist) and injectate 2 (intravenous vehicle or rrlIL-1β), and each animal was studied only once. Pregnant animals were studied on day 19, 20, or 21 of gestation (term ~22 days).

On the day before each experiment, the animal was removed from its cage, weighed, and then returned to its cage in the environmental chamber. On the day of the experiment, after a suitable control period, the rat was returned from its cage and given an intracerebroventricular injection of either 10 µl vehicle (artificial cerebrospinal fluid (aCSF)) or 1.0 nmol vasopressin V1-receptor antagonist dissolved in 10 µl aCSF. A suitable control period was defined as one in which five consecutive 2-min measurements of core temperature did not vary by >0.2°C. The rat was returned to its cage for 30 min, during which core temperature was recorded at 10-min intervals. Then the animal was removed from its cage and intravenously injected with either 0.2 ml vehicle (phosphate-buffered saline containing 1% bovine serum albumin) or 0.2 µg/kg rrlIL-1β dissolved in 0.2 ml of vehicle. The catheter was flushed with 0.2 ml of sterile normal saline, making the total injected volume 0.4 ml in all rats. The animal was then returned to its home cage, and core temperature was recorded at 10-min intervals for 6 h.

After each experiment the rat was anesthetized with pentobarbital sodium. The injection cannula was reinserted into the guide cannula, and 10 µl of black ink was injected into the ventricle via gravity flow. 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 brain was then removed and sectioned. The presence of ink in the cerebroventricular system verified correct placement of the injection cannula.

IL-1β. Recombinant rat IL-1β was purchased from R & D Systems as a lyophilized sample from a sterile filtered solution in phosphate-buffered saline containing 50 µg 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 ~0.1-ml aliquots and stored in sterile plastic vials at –70°C. 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 0.2 ml. The dose of rrlIL-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. Vehicle was phosphate-buffered saline containing 1% bovine serum albumin, and all injections were followed by 0.2 ml sterile saline to flush the catheter.

Vasopressin V1-receptor antagonist. A selective vasopressin V1-receptor antagonist (Pmp1-O-Me-Tyr2-[Arg6]vasopressin) was purchased as powder from Peninsula Laboratories. The powder was dissolved in aCSF (in mM: 128 Na+, 2.5 K+, 1.3 Ca2+, 1.0 Mg2+, and 135 Cl– (19)) to make a working solution of 0.2 nmol/µl. This solution was divided into 0.25-ml aliquots and stored in sterile plastic vials at –70°C. At the time of injection, the desired solution was removed from the freezer and the injection cannula was filled with the appropriate volume of vasopressin V1-receptor antagonist and vehicle to make a total injected volume of 0.1 µl. A dose of 1.0 nmol vasopressin V1-receptor antagonist was selected because we have previously shown that this dose restores the febrile response to an intracerebroventricular injection of PGE1 in near-term pregnant rats (13). Vehicle was aCSF.

Statistical analysis. Statistical analysis was carried out using a three-factor ANOVA for repeated measures followed by a Newman-Keuls multiple comparison test to determine if state (nonpregnant or pregnant), injectate (vehicle or vasopressin V1-receptor antagonist), or time influenced the average change in core temperature from control expressed as degrees Celsius per hour for the 6-h period after injection 2. All data are presented as means ± SD, and P < 0.05 was considered to be of statistical significance.

RESULTS

After intracerebroventricular administration of vehicle, intravenous administration of rrlIL-1β produced significant increases in core temperature in both nonpregnant and pregnant animals (Fig. 1). The core temperature response, however, was significantly delayed and attenuated in both magnitude and duration in pregnant compared with nonpregnant animals. The average change in core temperature was significantly lower in pregnant rats than in nonpregnant rats after vehicle/rrIL-1β (Fig. 2). There were no significant effects of intravenous administration of vehicle after intracerebroventricular administration of vehicle on core temperature in either nonpregnant or pregnant rats.

After intracerebroventricular administration of vasopressin V1-receptor antagonist, intravenous administration of rrlIL-1β produced a significant increase in core temperature in nonpregnant rats and a short-lived but significant decrease in core temperature in pregnant rats (Fig. 3). Furthermore, the average change in core temperature was significantly greater in nonpregnant rats after vasopressin V1-receptor antagonist/rrIL-1β than after vehicle/rrIL-1β (Fig. 2). In contrast, the average change in core temperature was lower in pregnant rats after vasopressin V1-receptor antagonist/
rrIL-1β than after vehicle/rrIL-1β. There were no significant effects of intravenous administration of vehicle after intracerebroventricular administration of a vasopressin V1-receptor antagonist on core temperature in either nonpregnant or pregnant rats.

**DISCUSSION**

Our experiments provide new information about pregnancy and fever in rats. Novel findings of the study were as follows. 1) Intravenous injection of rrIL-1β after an intracerebroventricular injection of vehicle produced a significant increase in core temperature in both nonpregnant and pregnant animals, but the magnitude and duration of this increase were significantly greater in nonpregnant compared with pregnant rats. 2) In nonpregnant animals, intravenous injection of rrIL-1β after an intracerebroventricular injection of a vasopressin V1-receptor antagonist produced an increase in core temperature that was significantly greater in magnitude and duration than that observed with an intravenous injection of rrIL-1β after an intracerebroventricular injection of vehicle. 3) In pregnant animals, intravenous injection of rrIL-1β after an intracerebroventricular injection of a vasopressin V1-receptor antagonist produced a decrease in core temperature rather than an increase in core temperature, which was observed with an intravenous injection of rrIL-1β after an intracerebroventricular injection of vehicle. Thus our data do not support the hypothesis that a pregnancy-related activation of arginine vasopressin as an endogenous antipyretic substance in the central nervous system attenuates the febrile response to rrIL-1β near the term of pregnancy in rats.

Beeson (1) identified endogenous pyrogen in 1948 as a substance produced and released by circulating leukocytes and fixed macrophages in response to exogenous pyrogens (i.e., bacterial endotoxin, lipopolysaccharide), and which produces an increase in body temperature. This substance was later renamed IL-1 and its release, as well as the release of other endogenous pyrogens such as IL-6, tumor necrosis factor, and interleukins-α and -γ, constitute an essential step in the genesis of fever after exposure to exogenous pyrogens (10). Because IL-1β fails to accumulate in the brain in significant quantities after peripheral injection (4), it has been postulated that endogenous pyrogens induce fever through production of one or more end mediators such as prostaglandins of the E series (2, 7, 18, 24). These end mediators act directly or indirectly in the central nervous system to activate heat-conserving and heat-producing mechanisms, the relative contributions of
which depend on the pyrogen type and dose, ambient and core temperature, and the age and size of the host (3). Therefore, fever is a complex process involving multiple steps, each of which could be influenced by the programmed rheostatic (25) changes in physiology that accompany the maternal adaptation to pregnancy.

Simrose and Fewell (30) were the first to show that pregnancy alters the febrile response to endogenous pyrogen in rats. In their experiments, the intravenous administration of a half-maximal effective dose (ED₅₀) of recombinant human IL-1β (i.e., 0.1 µg/kg as determined in nonpregnant Sprague-Dawley rats) produced an increase in core temperature on day 13 of gestation but produced a decrease in core temperature on days 17 and 21 of gestation. In the present study, rats that were given an intracerebroventricular injection of vehicle before an intravenous ED₅₀ of rrIL-1β developed a small but significant fever on days 19, 20, and 21 of gestation. These differences most likely result from the pyrogenic effects of recombinant human vs. rat IL-1β given to the latter species.

In nonpregnant rats, intravenous injection of rrIL-1β after an intracerebroventricular injection of a vasopressin V₁-receptor antagonist produced an increase in core temperature that was significantly greater in magnitude and duration than that observed with an intravenous injection of rrIL-1β after an intracerebroventricular injection of vehicle. The increased magnitude and duration of the febrile response of nonpregnant rats are consistent with the V₁-receptor antagonist preventing the endogenous antipyretic effect of arginine vasopressin and are consistent with previous experiments carried out on rats (9, 26, 27). More difficult to explain are the results obtained in the pregnant rats, that is, an intravenous injection of rrIL-1β after an intracerebroventricular injection of a vasopressin V₁-receptor antagonist produced a decrease in core temperature rather than an increase in core temperature, which was observed with an intravenous injection of rrIL-1β after an intracerebroventricular injection of vehicle. This hypothermic response, however, appears to be due to an interaction between the V₁-receptor antagonist and rrIL-1β because the V₁-receptor antagonist had no effect on core temperature in either nonpregnant or pregnant rats when it was followed by vehicle. Regardless of the mechanism, our data do not support the hypothesis that a pregnancy-related activation of arginine vasopressin as an endogenous antipyretic substance in the central nervous system attenuates the febrile response to rrIL-1β near the term of pregnancy in rats.

Fever is a highly regulated process involving the synthesis and release of both pyrogens and antipyretics. Endogenous antipyretics are produced and released by the body during the course of the febrile response and effectively limit the magnitude and duration of the core temperature response. Among the substances that have been identified as endogenous antipyretics are arginine vasopressin (8, 9), α-melanocyte-stimulating hormone (21, 22), IL-1 receptor antagonist (32), and IL-10 (20). Our current experiments provide evidence that arginine vasopressin does not mediate the attenuated febrile response to intravenous injection as it does to intracerebroventricular injection of PGE₁ (13) near the term of pregnancy in rats. Perhaps intravenous administration of rrIL-1β does not elicit a normal end mediator response (i.e., a normal prostaglandin E response) in pregnant animals as it does in nonpregnant animals because there is an alteration in the number or properties of cytokine receptors near the term of pregnancy, or, alternatively, there may be increases in the circulating levels of IL-1 receptor antagonist in rats as there is in humans near the term of pregnancy (29). Alternatively, plasma levels of α-melanocyte-stimulating hormone increase near the term of pregnancy in rats (35) and thus may play a role in mediating the attenuated febrile response to endogenous pyrogen. These possibilities warrant further investigation.

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