Fever response to intravenous prostaglandin E₂ is mediated by the brain but does not require afferent vagal signaling

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Submitted 2 October 2007; accepted in final form 22 January 2008

Ootsuka Y, Blessing WW, Steiner AA, Romanovsky AA. Fever response to intravenous prostaglandin E₂ is mediated by the brain but does not require afferent vagal signaling. Am J Physiol Regul Integr Comp Physiol 294: R1294–R1303, 2008. First published January 30, 2008; doi:10.1152/ajpregu.00709.2007.—PGE₂ produced in the periphery triggers the early phase of the febrile response to infection and may contribute to later phases. It can be hypothesized that peripherally synthesized PGE₂ transmits febrigenic signals to the brain via vagal afferent nerves. Before testing this hypothesis, we investigated whether the febrigenic effect of intravenously administered PGE₂ is mediated by the brain and is not the result of a direct action of PGE₂ on thermoeffector cells. In anesthetized rats, intravenously injected PGE₂ (100 μg/kg) caused an increase in sympathetic discharge to interscapular brown adipose tissue (iBAT), as well as increases in iBAT thermogenesis, end-expired CO₂, and colonic temperature (Tc). All these effects were prevented by inhibition of neuronal function in the raphe region of the medulla oblongata using an intra-raphe microinjection of muscimol. We then asked whether the brain-mediated PGE₂ fever requires vagal signaling and answered this question by conducting two independent studies in rats. In a study in anesthetized rats, acute bilateral cervical vagotomies did not affect the effects of intravenously injected PGE₂ (100 μg/kg) on iBAT sympathetic discharge and Tc. In a study in conscious rats, administration of PGE₂ (280 μg/kg) via an indwelling jugular catheter caused tail skin vasoconstriction, tended to increase oxygen consumption, and increased Tc; none of these responses was affected by total truncal subdiaphragmatic vagotomy performed 2 wk before the experiment. We conclude that the febrile response to circulating PGE₂ is mediated by the brain, but that it does not require vagal afferent signaling.

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Materials and Methods

Experiments in Anesthetized Rats

Animals and surgery. Experiments were conducted in male Sprague-Dawley rats (210–580 g) under protocols approved by the Flinders University Animal Welfare Committee. After a rat was anesthetized with isoflurane via a nasal mask, its trunk and limbs were shaved, and an endotracheal tube was inserted via a tracheotomy. For vagotomy experiments, both cervical vagi were exposed, and thin threads were looped around the intact nerves. The right femoral artery and vein were cannulated for measurement of systemic arterial pressure and for intravenous drug administration, respectively. Isoflurane anesthesia was then replaced with urethane (400–800 mg/kg iv) and α-chloralose (40–80 mg/kg iv); α-chloralose was dissolved in 10% 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich, St. Louis, MO).

Preparation of PGE2. PGE2 was initially dissolved in absolute ethanol and kept as a stock solution at −20°C. On the experimental day, PGE2 solution was freshly prepared by diluting the PGE2 stock solution with water (water for injections BP, AstraZeneca, North Ryde, NSW, Australia) and ethanol. The final concentration of ethanol was 4% for all PGE2 doses. The dose of PGE2 used was chosen based on separate experiments (data not shown). The dose of 100 µg/kg was found to readily activate iBAT thermogenesis and cause fever; this dose was used for all experiments in anesthetized rats. Administration of the thermally inactive dose of 4 µg/kg was used as a control. Other recent studies of fever (35, 74) also used a subpyrogenic dose of a pyrogen as a control treatment.

Histological examination of medullary injection sites. On completion of the experiments involving microinjections of muscimol into the raphe, each rat was anesthetized with pentobarbital sodium (over 80 mg/kg iv), and formaldehyde/glutaraldehyde solution was perfused transcardially into the ascending aorta. The brain was removed for the histological demonstration of injection sites by visualization of horseradish peroxidase reaction product.

Data analysis. All data were digitized with MacLab (ADInstruments, Bella Vista, NSW, Australia). The iBAT SNA was digitized at 400 Hz; the iBAT temperature, Tsk-trunk, and Tc were digitized at 10 Hz. The amplitude of iBAT SNA was expressed as log total power spectral density between 0 and 20 Hz from the autospectra of sequential 5.12-s segments of nerve activity. Group data were analyzed by ANOVA with repeated measures; postinjection values were compared with preinjection values. Fisher’s protected t-test was used to determine significant differences between individual means, and the significance threshold was set at the 0.05 level. All data are shown as means ± SE.

Experiments in Conscious Rats

Animals and surgery. The experiments were conducted under protocols approved by the St. Joseph’s Hospital Animal Care and Use Committee (Phoenix, AZ). Male Wistar rats were obtained from Harlan (Indianapolis, IN). They were housed in cages kept in a rack equipped with a Smart Bio-Pack ventilation system and Thermo-Pak temperature control system (Allentown Caging Equipment, Allentown, NJ); the temperature of the incoming air was maintained at 28°C. Standard rat food and tap water were available ad libitum. The rats were housed in a room with a 12:12-h light-dark cycle (lights on at 7:00 AM). Each rat was extensively handled and habituated to
staying inside wire-mesh conical confiners; the confiners were used later in the experiment.

On day 0, the rats were subjected to total subdiaphragmatic truncal vagotomy. Following an overnight food deprivation, the rats were anesthetized with ketamine-xylazine-acpomazine (55.6, 5.5, and 1.1 mg/kg ip) and given an antibiotic (enrofloxacin, 1.1 mg/kg sc). The stomach was accessed via a middle upper laparotomy and gently pulled to expose the esophagus. The ventral and dorsal vagal trunks running along the esophagus were identified and cut immediately below the diaphragm; for certainty, the hepatic vagal branch was also cut separately. In sham-vagotomized rats, the viscera were handled, but no nerves were cut. To alleviate the gastrointestinal complications of vagotomy, the vagotomized (but not sham-vagotomized) rats received a highly palatable liquid diet (PMI Micro-stabilized Rodent Liquid Diet LD101; TestDiet, Richmond, IN) for days 1–9. Placing the animals on a liquid diet prevents excessive body mass loss and other complications of vagotomy (1, 66, 70) that are subjected to underlie some contradictory results of fever experiments in vagotomized animals (62). On day 9, the body mass difference between vagotomized (342 ± 15 g) and sham-operated (385 ± 15 g) rats was less than 12%. All rats were then fed regular pelleted food for the rest of the study.

On day 11, all rats were subjected to venous catheterization under ketamine-xylazine-acpomazine anesthesia and enrofloxacin protection. A small longitudinal incision was made on the ventral surface of the neck, left of the trachea. The left jugular vein was exposed, freed from its surrounding connective tissue, and ligated. A silicone catheter (ID 0.5 mm, OD 0.9 mm) filled with heparinized (10 U/ml) saline was passed into the superior vena cava through the jugular vein and secured in place with ligatures. The free end of the catheter was knotted, tunneled under the skin to the nape, and exteriorized. The skin wound was sutured. The catheter was flushed with heparinized saline on days 12 and 14.

Instrumentation and thermometry. On day 15, the experiments were performed in the thermocouple-respirometry setup (84). The rats were placed in confiners and equipped with copper-constantan thermocouples for recording Tc and tail skin temperature (Tsk-tail). The thermocouples were plugged into a digital thermometer (Cole-Parmer, Vernon Hills, IL). Each rat in the confiner was then placed inside a cylindrical Plexiglas chamber (Sable Systems), which was sealed and continuously ventilated; the airflow was maintained at 600 ml/min with the help of a mass flow controller (Sierra Instruments, Monterey, CA). The air leaving each chamber was automatically sampled, dried, and passed through an O2 analyzer (Sable Systems). This measure was justified elsewhere (67). The O2 data were digitized and captured using a Sable Systems system and software. V˙O2 was calculated by comparing the O2 fraction (F) in the air exiting a chamber containing a rat (Fca) to that exiting an empty chamber (Fcb). Formula 2 was then employed: V˙O2 = [A × (Fca - Fcb)]/[1 - (1 - RQ) × Fcb]/M, where A is air flow, RQ is respiratory quotient, and M is rat body mass. The term that includes RQ (Formula 2) accounts for the fact that CO2 produced by the rat was not extracted from the air passing through the O2 analyzer in our experimental setup; RQ was considered to be 0.71. The Tc, Tsk-tail, and V˙O2 responses were compared across treatments and time points by two-way ANOVA for repeated measures using Statistica AX'99 (StatSoft, Tulsa, OK). The data are reported as means ± SE.

RESULTS

Experiments in Anesthetized Rats

Effects of intravenous PGE2 in intact anesthetized rats. In “intact” anesthetized rats, a pyrogenic dose of PGE2 (100 μg/kg) administered intravenously increased all of the parameters monitored, that is, activated thermogenesis and caused fever (P < 0.01 for iBAT SNA, iBAT temperature, end ExpCO2, and Tc; Figs. 1 and 2), whereas a subpyrogenic dose of PGE2 (4 μg/kg) did not cause significant changes in any of these parameters (Fig. 2). The thermogenic effect of PGE2 was rapid (latency of the iBAT SNA response was 162 ± 6 s). The infusion of PGE2 also caused even more rapid, transient falls in blood pressure (data not shown) and Tc, but both parameters started to recover within 2 min after the PGE2 administration. These instantaneous, short-lived, low-magnitude responses were outside the focus of the present study.

Effects of intravenous PGE2 after injection of muscimol into the raphe. To investigate whether the iBAT effects of intravenously administered PGE2 are mediated by the brain, we injected muscimol in the medullary raphe. After the recording from sympathetic iBAT nerves was validated by the appropriate response to cooling (Fig. 3A), the rat was rewarmed to reduce iBAT SNA to precooling levels. Muscimol (1 nmol in 100 nl) was then microinjected into the raphe, and PGE2 (100 μg/kg iv) was administered 5 min later. In this circumstance,
no thermogenic response occurred, and $T_c$ tended to fall, even though the rat was warmed (Fig. 3B). When the truncal skin was subsequently cooled, there was no increase in any thermogenic parameter (Fig. 3C), thus confirming inactivation of the raphe neuronal function by the muscimol injection (47). Postmortem histological examination confirmed that injection sites were in the raphe region (Fig. 4).

**Effects of intravenous PGE2 after bilateral cervical vagotomy.** Bilateral cervical vagotomy did not alter the magnitude of the thermogenic effects of PGE2 (100 $\mu$g/kg iv) administered 15 min after the nerve transection (Fig. 5). The increase in iBAT SNA in response to cooling was also observed in vagotomized animals (data not shown).

**Experiments in Conscious Rats**

**Effects of intravenous PGE2 in conscious rats with or without bilateral subdiaphragmatic vagotomy.** In a second study (conducted in conscious rats), injection of the vehicle (BSA) did not cause any significant thermoregulatory effect in either sham-operated or vagotomized rats (Fig. 6). Compared with the corresponding BSA-treated controls, sham-operated rats infused with PGE2 (280 $\mu$g/kg iv) displayed a significant rise in $T_c$ ($P < 0.05, 10–120$ min); within 30 min, their $T_c$ increased from 38.1 $\pm$ 0.2 to 38.8 $\pm$ 0.2°C ($n = 6$) (Fig. 6). In association with this rise, the HLI transiently decreased ($P < 0.05, 20–30$ min) from 0.5 $\pm$ 0.1 to 0.2 $\pm$ 0.1 ($n = 6$), suggesting thermoregulatory vasoconstriction of the tail artery bed. Also, in association with the $T_c$ rise, there was a strong tendency ($P = 0.056, 20$ min) for an increase in $V_{O_2}$, an index of metabolic heat production. None of the effects of PGE2 was attenuated by vagotomy. In fact, vagotomy significantly enhanced the effect of PGE2 on $V_{O_2}$ ($P < 0.05, 20–30$ min).

**DISCUSSION**

*The Febrigenic Effect of Circulating PGE2 is Mediated by the Brain*

The fever response to centrally administered PGE1 involves both skin vasoconstriction and BAT thermogenesis (12, 89).
The present study shows that the fever response to intravenously administered PGE2 also involves both of these major autonomic thermoeffectors. We demonstrate a reduction in the heat loss from the tail (confirmation of our recent study, Ref. 82) and an increased iBAT thermogenesis (a novel observation). This patterned activation of effectors does not agree with the idea of intravenous PGE2-induced fever being due to a local action of PGE2 in a single thermoeffector tissue. Rather, it suggests a brain-mediated mechanism to recruit multiple effectors. Furthermore, the expected local action of PGE2 in the skin is vasodilation rather than vasoconstriction (23, 50). As for local actions in the BAT, several studies show that PGE1 and E2 suppress BAT thermogenesis (3, 20, 36), although one study suggests that PGE2 can activate thermogenesis in brown adipocytes (43).

In addition to being indirectly supported by the patterned activation of thermoeffectors, the view that intravenous PGE2-induced fever is a brain-mediated response is supported directly by our experiments involving microinjections of muscimol into the raphe. Neurons in the medullary raphe region—the neurons inactivated in the present study—constitute an essential lower brain stem relay for the central outflow to spinal iBAT and tail artery sympathetic preganglionic neurons (8, 42, 45, 91). Blockade of neurons in the raphe/parapyramidal region has been shown to abolish iBAT thermogenesis elicited by intrabrain PGE2 (34, 46, 48). Our present study demonstrates that similar blockade of these neurons entirely prevents the intravenous PGE2-elicited iBAT SNA and the increase in end ExpCO2. Increased expiration of CO2 implies an increase in metabolic heat production; in paralyzed animals, such an increase results almost exclusively from nonshivering thermogenesis. Thus, our results show that the febrigenic action of intravenously administered PGE2 is mediated by the brain.

**Intravenous PGE2-Induced Fever Can Occur Independently of Vagal Afferents**

It is known that vagotomized animals respond with normal fevers to intrabrain PGE2 (38, 86). Ours is the first study to...
investigate whether vagotomy alters the febrile response to intravenously administered PGE₂. Even though the febrigenic effect of intravenous PGE₂ proved to be brain mediated (i.e., requires transduction of febrigenic information from the periphery to the brain), this effect was not reduced by partial or complete elimination of vagal afferent pathways. Truncal subdiaphragmatic vagotomy did not attenuate the increase in Tₑ caused by intravenous PGE₂. This surgery results in total subdiaphragmatic vagal denervation, including hepatic afferent vagal denervation, but it does not eliminate supradiaphragmatic (e.g., pulmonary) innervation. Together with the liver, the lungs are the major sources of peripheral PGE₂ synthesis during fever (25, 82), and PGE₂ has been shown to affect pulmonary vagal afferents in a receptor-specific manner (95). To eliminate the possibility that supradiaphragmatic vagus could convey febrigenic signals to the brain, we performed experiments with bilateral cervical vagotomy. Although rats normally do not survive this surgery, anesthetized rats after remaining under anesthesia do survive and can be maintained in an adequate physiological condition. After this procedure, we found no change in the amplitude of the intravenous PGE₂-elicited fever, thus confirming that the integrity of neither subdiaphragmatic nor supradiaphragmatic vagal afferents is critical for this response.

Another important difference between our subdiaphragmatic vagotomy experiments in conscious rats and our cervical vagotomy experiments in anesthetized rats is the effector responses recruited in the intravenous PGE₂ fever recorded. This is important, because more and more evidence is being accumulated that suggests the thermoregulatory system functions not as a unified control system but as a “federation” of relatively independent thermoeffector loops (63). In conscious rats exposed to a thermoneutral environment, PGE₂-induced fever was due to tail skin vasoconstriction, and whereas Vₒ₂ tended to increase, there was no statistically significant change in Vₒ₂. Vagotomy did not attenuate the tail skin vasculature response. In anesthetized rats, only one thermoeffector mechanism, BAT thermogenesis, was studied. Anesthesia typically induces strong dissociation between thresholds for activation of autonomic thermoeffectors (57, 77), and depending on deep
body and skin temperatures of an anesthetized rat preparation, only one thermoeffector can typically be studied, either BAT thermogenesis or tail skin vasoconstriction (S. F. Morrison, personal communication). In our experiments, intravenous PGE2-induced fever was due to, at least partially, increased BAT thermogenesis (increases in iBAT SNA, iBAT temperature, and ExpCO2). Cervical vagotomy attenuated neither this thermogenic response nor the resultant PGE2 fever in anesthetized rats.

The biological fate and physiological effects of amphipathic substances (such as PGE2) strongly depend on serum albumin (59). Up to 99% of circulating PGE2 is albumin bound (92). Albumin also plays an important transport role in the nervous tissue as it accounts for 80% of total protein in the cerebrospinal fluid (59). Furthermore, blood-borne (not synthesized in tissue as it accounts for 80% of total protein in the cerebrospinal fluid) albumin is present in peripheral nerves (59). Not surprisingly, the same amphipathic lipid can cause different physiological effects depending on the form in which it is delivered: aggregated free, monomeric free, monomeric albumin-bound, or monomeric other protein-bound (24, 28, 65). The solutions of PGE2 used in the present experiments in anesthetized vs. conscious rats were prepared differently: a hydroalcoholic solution of PGE2 vs. a PGE2-BSA complex in saline, respectively. In agreement with the literature (11, 15, 58, 65, 79, 80, 82), both preparations caused comparable fevers at similar doses, thus confirming that physiologically active (monomeric) PGE2 was successfully delivered to the receptors triggering the fever response. Furthermore, vagotomy had no effect on the febrile response to either preparation of PGE2. Hence, in no paradigm studied in our experiments did the febrile effect of intravenous PGE2 depend on the integrity of vagal afferent nerves.

**How Does Intravenous PGE2 Activate Brain Thermoregulatory Pathways?**

How circulating PGE2 conveys pyrogenic information to the brain and causes fever remains speculative. That intravenous PGE2 readily causes a variety of effects (e.g., fever, urination, and defecation; see Ref. 65) shows that it can avoid rapid enzymatic inactivation in the lungs and other tissues and reach distal sites; PGE2 binding to albumin is thought to play a protective role (59). Once dissociated from albumin at a site near or at the BBB, PGE2 could be carried into the brain tissue (13) by transporters expressed at the BBB (27, 29).

Circumventricular organs (including the organum vasculosum laminae terminalis, or OVLT) represent another potential way to enter the brain. Because OVLT lesions had been reported to block LPS fever (5), this route was thought to be crucial for pyrogenic signaling. However, OVLT lesioning causes multiple “side effects” that can decrease febrile responsiveness without interfering with the processes of febrigenic signaling to the brain (74). These multiple “side effects” create interpretational problems and compromise the direct support of the OVLT signaling theory that lesion experiments were thought to provide (64). Moreover, several studies show that PGE2 triggers fever by acting solely or at least principally on the EP3 receptor (30, 52, 93), perhaps EP3-α (94), but attempts to demonstrate EP3 receptors in the circumventricular structures have failed so far (44, 53).

Activation of EP3 receptors is thought to inhibit the discharge of the neurons on which these receptors are located (49). Thus, the relevant PGE2-sensitive neurons may normally act as an inhibitory brake on hypothalamic or brain stem neurons that activate descending thermoregulatory pathways (41, 64); the action of PGE2 would remove the brake and disinhibit these pathways, thus allowing fever to develop. There is a concentration of EP3-positive neurons in the median preoptic nucleus of the anterior hypothalamus (44, 53), and selective genetic deletion of EP3 receptors in this hypothalamic structure in mice prevents the fever response to intracerebroventricular PGE2 or intraperitoneal LPS (30). EP3 receptors are also expressed in other brain regions, including the raphe/parapyramidal area (44, 53), but local application of PGE2 in the raphe affects neither iBAT nor tail artery SNA (90). Clearly, more studies are needed to trace the pathway(s) by which circulating PGE2 conveys febrigenic signals to thermoeffector circuitry.

**Perspectives and Significance**

The last decade has witnessed a rise and an apparent decline of the vagal theory of triggering the febrile response (for detailed history, see Refs. 62 and 64). Although surgical vagotomy was initially found by many to attenuate or completely block some or even all febrile phases in rats, this surgery can lead to severe “side effects” (such as malnutrition and inability to mount a thermogenic response) that can atten-
valuate the febrile response independent of the febrigenic signaling (70). Many earlier studies ignored this issue. When caution was exercised to prevent malnutrition and associated disorders and to produce vagotomized animals fully capable of increasing their body temperature (38, 69, 70, 85), surgical vagotomy was found to cause no attenuation of any phase of the polyphasic febrile response of rats to LPS (72). The complete ineffectiveness of surgical vagotomy to attenuate polyphasic LPS or IL-1β fever in rats was then confirmed by several studies (9, 21, 22, 33, 87), some of which (21, 22, 33) were conducted by the same groups that had reported blockade of fever by surgical vagotomy in their earlier papers. Another procedure, capsaicin desensitization (sometimes referred to as “chemical vagotomy”), has been repeatedly found to attenuate the first phase of LPS fever in rats (87, 88), but this effect has been attributed to a nonneural action of capsaicin (14, 60) and, hence, is likely unrelated to the proposed vagal signaling. It seems safe to conclude that the polyphasic LPS- and IL-1β-induced fevers do not require vagal signaling, at least in the rat.

As for very small, just above the pyrogenic threshold doses of LPS (~1 μg/kg), they induce a monophasic fever, which is characterized by a longer latency, shorter duration, and a different shape of the body temperature curve (64). Similar to the polyphasic fever, the monophasic fever is totally PGE2-mediated (COX-2-dependent) (83). Different from the polyphasic fever, the monophasic febrile response to LPS has been reported to be attenuated by either total subdiaphragmatic vagotomy (70, 72) or selective transection of the hepatic vagal branch (but not of the gastric or celiac branches) (78). Furthermore, monophasic fever responses to small, just above the pyrogenic threshold, doses of IL-1β (100–500 ng/kg)—but not higher doses of this cytokine—have also been shown to be attenuated by subdiaphragmatic vagotomy (22). It should be noted, however, that monophasic fevers occur in a very narrow range of pyrogen doses, have a low magnitude (sometimes just a few tenths of a degree), and are less reproducible than polyphasic fevers. Hence, it may be prudent to carefully reexamine the effects of vagotomies on monophasic fevers (22, 70, 72, 78) before drawing a firm conclusion, especially because the majority of data comes from a single laboratory. There is also a study in guinea pigs showing that subdiaphragmatic vagotomy blocks both febrile phases of the biphasic response to a single dose of LPS (76), but this report lacks methodological details (liquid diet, body mass, confirmation of the vagotomized guinea pigs used were fully capable of mounting a thermogenic response. Again, it would be prudent to confirm the reported effect of vagotomy before categorically accepting that LPS fever requires vagal signaling in the guinea pig. It should be noted, however, that parenchymal vagal innervation of the liver is denser in the guinea pig than in the rat (37), and, therefore, a greater vagal involvement in fever in the guinea pig cannot be ruled out.

Conclusion

Studies of polyphasic febrile responses of rats to LPS and IL-1β overwhelmingly support the conclusion that these fevers do not require vagal signaling (9, 14, 21, 22, 33, 60, 72). Because LPS-induced fever in rats has been shown to be triggered by peripheral PGE2 (82) and because PGE2 has widely been suspected to act on the vagus nerve to trigger fever (4, 6, 62, 64, 68), we examined whether the febrile response to intravenous PGE2 critically depends on the integrity of the vagus. We performed two sets of independent experiments in different rat preparations that involved different types of vagotomy, different methods of PGE2 delivery, and different measures of thermoeffector activity. We have found that intravenous PGE2 causes fever in rats via a brain-mediated mechanism, but that vagotomy does not affect this response. Hence, the present experiments further support the notion that vagal signaling is unlikely to be an important mechanism for blood-brain febrigenic signaling.

ACKNOWLEDGMENTS

The authors thank M. Quinlan, R. Flook, and D. L. Oliveira for technical assistance and J. M. Turko for editing the paper. Present address for A. A. Steiner: Department of Pharmaceutical Sciences, Albany College of Pharmacy, Albany, New York.

GRANTS

The study was funded by the National Health and Medical Research Council of Australia, the Flinders Medical Foundation, the National Institute of Neurological Disorders and Stroke (Grant R01NS041233), and the St. Joseph’s Foundation.

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