Am J Physiol Regul Integr Comp Physiol 294: R1294–R1303, 2008.
First published January 30, 2008; doi:10.1152/ajpregu.00709.2007.

Fever response to intravenous prostaglandin E_2 is mediated by the brain but does not require afferent vagal signaling

Youichirou Ootsuka,[1] William W. Blessing,[1] Alexandre A. Steiner,[2] and Andrej A. Romanovsky[2]

[1]*Department of Human Physiology, Centre for Neuroscience, Flinders University, Adelaide, Australia; and* [2]*Systemic Inflammation Laboratory, Trauma Research, St. Joseph's Hospital and Medical Center, Phoenix, Arizona*

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

Ootsuka Y, Blessing WW, Steiner AA, Romanovsky AA. Fever response to intravenous prostaglandin E_2 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.—PGE_2 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 PGE_2 transmits febrigenic signals to the brain via vagal afferent nerves. Before testing this hypothesis, we investigated whether the febrigenic effect of intravenously administered PGE_2 is mediated by the brain and is not the result of a direct action of PGE_2 on thermoeffectors. In anesthetized rats, intravenously injected PGE_2 (100 μg/kg) caused an increase in sympathetic discharge to interscapular brown adipose tissue (iBAT), as well as increases in iBAT thermogenesis, end-expired CO_2, and colonic temperature (T_c). 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 PGE_2 fever requires vagal signaling and answered this question by conducting two independent studies in rats. In a study in anesthetized rats, acute bilateral cervical vagotomy did not affect the effects of intravenously injected PGE_2 (100 μg/kg) on iBAT sympathetic discharge and T_c. In a study in conscious rats, administration of PGE_2 (280 μg/kg) via an indwelling jugular catheter caused tail skin vasoconstriction, tended to increase oxygen consumption, and increased T_c; 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 PGE_2 is mediated by the brain, but that it does not require vagal afferent signaling.

temperature; thermoregulation; febrile; vagotomy; raphe; brown adipose tissue

TARGETED DISRUPTION OF GENES affecting the synthesis of prostaglandin (PG) E_2 or its receptors suggests that this lipid plays a crucial role in the febrile response to endogenous pyrogens (such as the proinflammatory cytokines, e.g., IL-1β) or to exogenous pyrogens (such as LPS) (17, 30, 31, 52, 83, 93). Whereas centrally produced PGE_2 is crucial for later phases of fever, peripherally produced PGE_2 plays a critical role in the early (first) phase of the polyphasic fever induced by LPS (for a review, see Refs. 4, 6, 26, 64). In the early phase, expression of several enzymes involved in PGE_2 synthesis, including cyclooxygenase (COX)-2, and the number of cells displaying COX-2 immunoreactivity increases in the liver and lungs, but not in the brain (25, 82). Importantly, nearly all hepatic and pulmonary cells that express COX-2 during the early phase are macrophages (82), and the early febrile phase depends entirely on cells of hematopoietic origin (81), most likely macrophages (73). As the result of the rapid increase in PGE_2 synthesis, the concentration of circulating PGE_2 surges early during the fever response, both in venous and arterial blood (39, 75, 80, 82). Furthermore, inhibitors of PG synthesis, even those that poorly cross the blood-brain barrier (BBB), block fever (40, 75). Neutralization of circulating PGE_2 with anti-PGE_2 antibodies (which do not cross the BBB) also delays and attenuates (82) or even completely blocks (32) the early phase of LPS fever. Not surprisingly, intravenous or intracarotid PGE_2 or PGE_1 readily causes fever when administered in a monomeric form, that is, either with a sufficient amount of an organic solvent (such as ethanol) (11, 15, 58, 79, 80) or as a complex with serum albumin, a principal endogenous carrier of circulating PGE_2 (65, 82). For comparison, several studies that used a low concentration of ethanol (or no ethanol) and/or a high concentration of PGE_2 or PGE_1 in the suspension injected intravenously or in the carotid artery failed to induce fever, presumably because the drug was administered in an aggregated form (see Ref. 65).

The liver is an important site of LPS-induced PGE_2 synthesis (25, 82), and selective transection of the hepatic vagal branch (a small, predominantly afferent nerve servicing the liver and its portal vein) has been reported to attenuate the fever response to small doses of LPS (78). On the basis of this and other evidence (32, 51), several authors have proposed that peripherally produced (perhaps hepatic) PGE_2 may transmit febrigenic signals to the brain by activating the peripheral processes of vagal afferent nerves (4, 6, 68), possibly via the action on the EP3 subtype of PGE receptors present on vagal neurons (16). However, the hypothesis that the integrity of the vagal nerves is required for the febrigenic effects of peripheral PGE_2 has never been tested directly in an experiment. To test this hypothesis in two independent studies in rats using different techniques of PGE_2 administration and different modes of surgical vagotomy was the main purpose of the present work.

Although it is widely believed that the brain mediates all fevers, a direct action of pyrogenic mediators on thermoeffector tissues cannot be excluded. For example, PGE_2 has been shown to be produced in the brown adipose tissue (BAT) (61) and to increase the calcium concentration and O_2 consumption ($\dot{V}O_2$) in rat brown adipocytes via a direct action (43). Furthermore, PGE_2 and other pyrogens (LPS) can induce expression of uncoupling proteins in brown adipocytes and hepatocytes, also via direct actions (2, 10). Hence, before testing the hypothesis that peripheral PGE_2 causes fever by signaling the

Address for reprint requests and other correspondence: Y. Ootsuka, Dept. of Human Physiology, School of Medicine, Flinders Univ., Bedford Park, SA 5042 Australia (e-mail: youichirou.ootsuka@flinders.edu.au).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

brain via a vagal mechanism, we needed to answer decisively the question of whether brain signaling is involved, that is, whether this fever is a brain-mediated response. We did so by studying the effect of inhibition of the raphe region of the medulla oblongata (by a local injection of muscimol) on the fever response to the intravenous administration of PGE_2. Neurons in the raphe/parapyramidal region respond to stimulation of vagal afferents (18) and control spinal sympathetic outflow to the two major autonomic thermoeffectors in the rat: BAT (including interscapular BAT, iBAT) and the heat-exchanging cutaneous vascular beds (including the tail skin) (7, 41).

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). The level of anesthesia was maintained at a depth sufficient to abolish withdrawal reflexes.

The rat was then mounted prone in a stereotaxic frame, and a water jacket was placed around the thorax and abdomen to maintain colonic temperature (T_c; a measure of deep body temperature) at 36–37°C and to alter the temperature of the trunk skin ($T_{sk\text{-}trunk}$) when needed. A burr-hole craniotomy was performed, and the dorsal surface of the cerebellum was exposed for stereotaxic access to the medullary raphe. To record iBAT sympathetic nerve activity (SNA), the sympathetic nerves innervating iBAT were dissected as described previously (57), and nerve signals were recorded with bipolar silver electrodes (NL100 preamplifier; Digitimer, Welwyn Garden City, UK), amplified (gain 10,000; NL104 amplifier; Digitimer) and filtered (1–1000 Hz; NL125 filter; Digitimer). The rat was paralyzed with gallamine triethiodide (initially, 6 mg iv, bolus; thereafter, 4 mg iv every 1–1.5 h) and then ventilated artificially with 100% O_2. The animal was allowed to recover from paralysis between doses so that adequate anesthesia could be confirmed before paralysis was reestablished. End expiratory CO_2 concentration ($ExpCO_2$) was monitored continuously (Normocap; Datex, Helsinki, Finland) and maintained between 3.5 and 4.5% (resting condition) by adjusting ventilation volume. The iBAT temperature, $T_{sk\text{-}trunk}$, and T_c were measured with thermocouples and an amplifier (TC-2000; Sable Systems, Las Vegas, NV).

Experimental procedures. Before all PGE_2 injections, the truncal skin was cooled by perfusing cold (5–10°C) water through the jacket for a short (2–3 min) time period. The consequent increase in iBAT SNA was used to confirm that recording was from the nerves supplying iBAT (56); only animals displaying an adequate iBAT SNA response to skin cooling were used in further experiments. Warm (37–50°C) water was then reintroduced into the jacket to rewarm the truncal skin. After PGE_2 or its vehicle was infused intravenously over 1 min, all parameters were monitored for at least 30 min.

In vagotomy experiments, both cervical vagi were cut after the skin-cooling and rewarming procedure, and PGE_2 or its vehicle was administered 15 min thereafter. The nerves were cut by pulling the threads that had been previously placed around them.

In the muscimol experiments, the skin cooling and rewarming procedure was followed by a microinjection of muscimol (1 nmol in 100 nl Ringer) into the raphe (centered at the midline, 11.5 mm posterior to bregma, 10.0 mm deep from the dorsal surface of cerebellum) using a glass micropipette (tip diameter, 30–50 μm). The same dose of muscimol was used in our previous studies to cause strong, long-lasting inhibitory effects (54, 55). The micropipette was connected to a syringe filled with air; the microinjection was made over 20 s by monitoring movement of a meniscus in the micropipette. PGE_2 was administered ~5 min after the microinjection. A second skin cooling procedure was then performed to document a loss of the iBAT response to cooling, thereby, confirming the effectiveness of muscimol injection into the raphe. A small amount of horseradish peroxidase (type VI) was added to the muscimol solution to allow for visualization of the injection site at post mortem histological examination.

At the end of all the experiments (except for the experiments involving microinjections of muscimol into the raphe), the skin-cooling procedure was performed again. After the iBAT SNA increased, a ganglionic blockade with chlorisondamine chloride (10 mg/kg iv) was attempted to confirm a loss of iBAT SNA and thus to ensure that nerve recording was from postganglionic sympathetic axons.

Preparation of PGE_2. PGE_2 was initially dissolved in absolute ethanol and kept as a stock solution at −20°C. On the experimental day, PGE_2 solution was freshly prepared by diluting the PGE_2 stock solution with water (water for injections BP, AstraZeneca, North Ryde, NSW, Australia) and ethanol. The final concentration of ethanol was 4% for all PGE_2 doses. The dose of PGE_2 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, $T_{sk\text{-}trunk}$, and T_c 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-acepromazine (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 suspected 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-acepromazine 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 T_c and tail skin temperature ($T_{sk\text{-}tail}$). 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 O_2 analyzer (Sable Systems). This measure was used to calculate $\dot{V}O_2$. The Plexiglas chamber containing the rat was kept inside a climatic chamber (model 3940; Forma Scientific, Marietta, OH), which was set to maintain an ambient temperature of 27°C. The venous catheter was connected to a PE-50 extension filled with saline. The extension was passed through a port of the Plexiglas chamber, and the port was sealed with paraffin. The extension was then passed through a port of the climatic chamber and connected to a syringe.

The rats were allowed to acclimatize to the experimental conditions for at least 3 h, after which period they were infused intravenously with PGE_2 (280 μg/kg delivered over 10 min) or its vehicle (saline-containing bovine serum albumin). T_c, $T_{sk\text{-}tail}$, and $\dot{V}O_2$ were monitored for at least 120 min. At the end of the recording period, the rats were euthanized with pentobarbital sodium (100 mg/kg iv), and their stomachs were excised and weighed (together with stomach contents). Confirming the effectiveness of the vagotomy (66, 70, 78), the stomach mass was significantly ($P < 0.001$) higher in the vagotomized rats (9.8 ± 1.7 g) than in the sham-operated rats (3.0 ± 0.3 g).

Preparation of PGE_2. On the day of the experiment, a 2:1 (molar ratio) PGE_2-albumin complex was freshly prepared by adding PGE_2 (Sigma-Aldrich) and bovine serum albumin (BSA; Sigma-Aldrich) to pyrogen-free saline, and then sonicating this system for 3 min and incubating it at 37°C for 1 h. The dose of PGE_2 used (280 μg/kg) was chosen based on our recent study (82) in which this dose produced a moderate fever and clear tail skin vasoconstriction. It appeared to be reasonably close to the dose used to activate thermogenesis (100 μg/kg) in experiments in anesthetized rats. Importantly, both febrigenic doses of PGE_2 used in this study caused fevers that were similar in magnitude to the first phase of LPS fever (which is mediated by peripheral PGE_2; see Ref. 82) in both Wistar (~0.8°C; Ref. 71) and Sprague-Dawley rats (~0.7°C; Ref. 71). Furthermore, both doses were likely to produce physiologically relevant blood concentrations of PGE_2. On the basis of the assumptions that PGE_2 is evenly distributed in the extracellular compartment (20% of the body mass) and that its half-life is 1 min (19), it can be estimated that a dose of 100–280 mg/kg results in an extracellular PGE_2 concentration of 15–43 pg/ml after 15 min of its administration, a time when the fever induced by PGE_2 is starting to develop in either anesthetized or conscious rats. Such estimated concentrations are similar to the PGE_2 concentrations measured in the plasma of rats at the onset of LPS-induced fever (82).

Data processing and analysis. The T_c, $T_{sk\text{-}tail}$, and ambient temperature (T_a) data were digitized and captured using a digital thermometer (Cole-Parmer). The absolute value of T_c was used as an index of deep body temperature. To evaluate thermoeffector responses of tail skin vasculature, the heat loss index (HLI) was calculated according to *Formula 1*: $HLI = (T_{sk\text{-}tail} - T_a)/(T_c - T_a)$.

Formula 1 was justified elsewhere (67). The O_2 data were digitized and captured using a Sable Systems system and software. $\dot{V}O_2$ was calculated by comparing the O_2 fraction (F) in the air exiting a chamber containing a rat (F_{rat}) to that exiting an empty chamber (F_{ch}). *Formula 2* was then employed: $\dot{V}O_2 = [A \times (F_{ch} - F_{rat})]/ [1 - (1 - RQ) \times F_{ch}]/M$, 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 CO_2 produced by the rat was not extracted from the air passing though the O_2 analyzer in our experimental setup; RQ was considered to be 0.71. The T_c, HLI, and $\dot{V}O_2$ 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 PGE_2 in intact anesthetized rats. In "intact" anesthetized rats, a pyrogenic dose of PGE_2 (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 $ExpCO_2$, and T_c; Figs. 1 and 2), whereas a subpyrogenic dose of PGE_2 (4 μg/kg) did not cause significant changes in any of these parameters (Fig. 2). The thermogenic effect of PGE_2 was rapid (latency of the iBAT SNA response was 162 ± 6 s). The infusion of PGE_2 also caused even more rapid, transient falls in blood pressure (data not shown) and T_c, but both parameters started to recover within 2 min after the PGE_2 administration. These instantaneous, short-lived, low-magnitude responses were outside the focus of the present study.

Effects of intravenous PGE_2 after injection of muscimol into the raphe. To investigate whether the iBAT effects of intravenously administered PGE_2 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. 3*A*), the rat was rewarmed to reduce iBAT SNA to precooling levels. Muscimol (1 nmol in 100 nl) was then microinjected into the raphe, and PGE_2 (100 μg/kg iv) was administered 5 min later. In this circumstance,

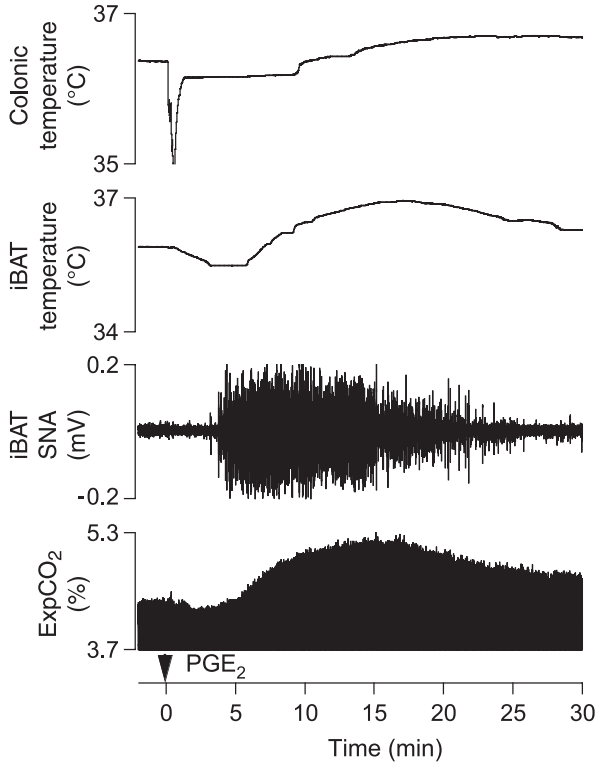

Fig. 1. Effect of intravenous administration (arrow) of PGE_2 (100 μg/kg) on colonic temperature (T_c), interscapular brown adipose tissue (iBAT) temperature, iBAT sympathetic nerve activity (SNA), and end $ExpCO_2$ in an "intact" anesthetized rat.

no thermogenic response occurred, and T_c tended to fall, even though the rat was warmed (Fig. 3*B*). When the truncal skin was subsequently cooled, there was no increase in any thermogenic parameter (Fig. 3*C*), 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 PGE_2 after bilateral cervical vagotomy. Bilateral cervical vagotomy did not alter the magnitude of the thermogenic effects of PGE_2 (100 μg/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 PGE_2 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 PGE_2 (280 μg/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 ± 0.2 to 38.8 ± 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 ± 0.1 to 0.2 ± 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 $\dot{V}O_2$, an index of metabolic heat production. None of the effects of PGE_2 was attenuated by vagotomy. In fact, vagotomy significantly enhanced the effect of PGE_2 on $\dot{V}O_2$ ($P < 0.05$, 20–30 min).

DISCUSSION

The Febrigenic Effect of Circulating PGE_2 is Mediated by the Brain

The fever response to centrally administered PGE_1 involves both skin vasoconstriction and BAT thermogenesis (12, 89).

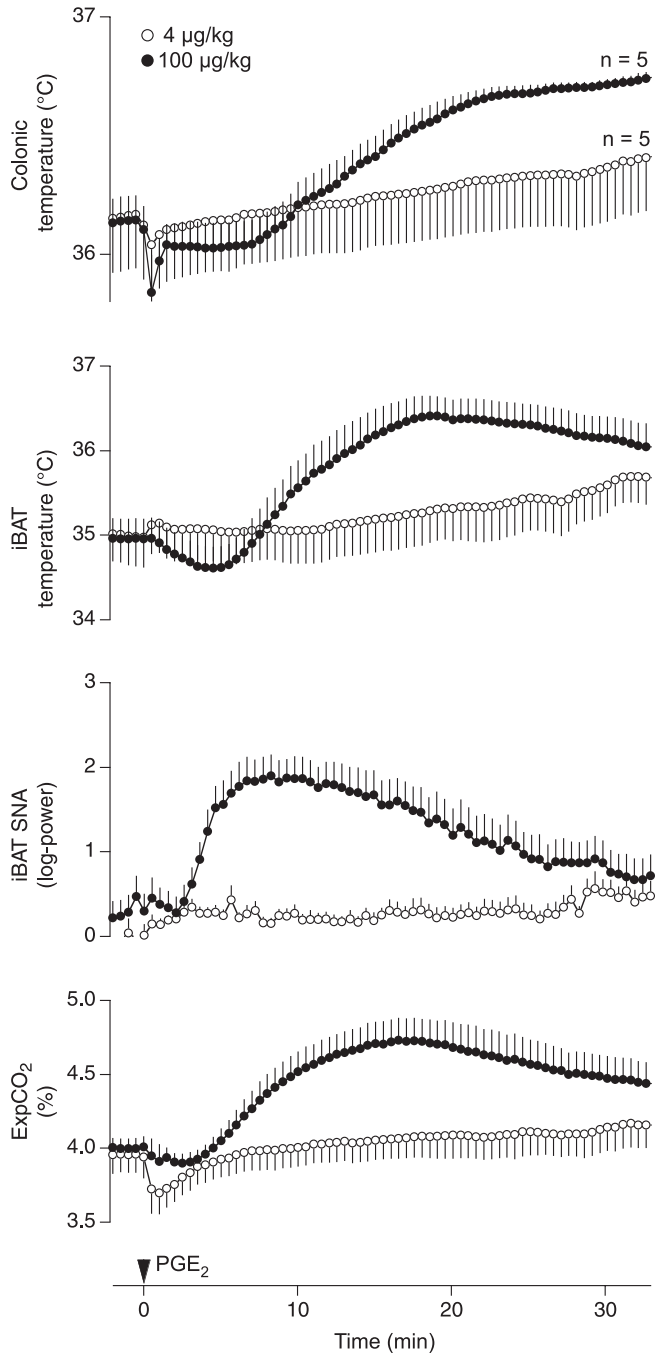

Fig. 2. Thermoregulatory responses to intravenous administration (arrow) of PGE_2 at a subpyrogenic dose (4 μg/kg; control) or at a pyrogenic dose (100 μg/kg) in anesthetized rats; group data are expressed as means ± SE.

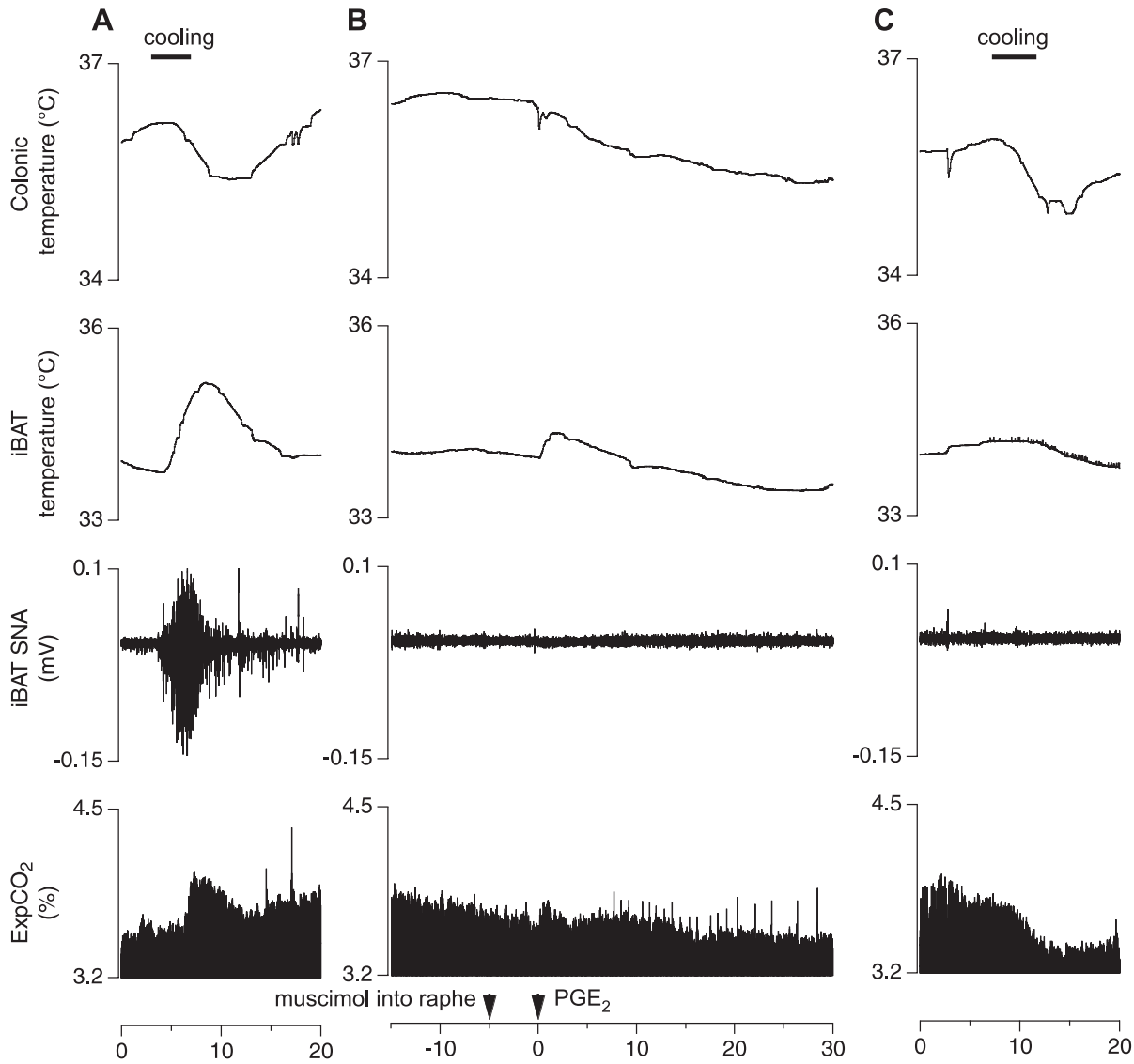

Fig. 3. Effects of inhibition of raphe neurons with muscimol on the thermoregulatory effects of PGE_2 (100 μg/kg iv; the right arrow) and on cooling skin in an anesthetized rat. Traces in *A*, *B*, and *C* represent sequential time periods. Muscimol microinjection (1 nmol in 100 nl) was performed at the left arrow.

The present study shows that the fever response to intravenously administered PGE_2 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 PGE_2-induced fever being due to a local action of PGE_2 in a single thermoeffector tissue. Rather, it suggests a brain-mediated mechanism to recruit multiple effectors. Furthermore, the expected local action of PGE_2 in the skin is vasodilation rather than vasoconstriction (23, 50). As for local actions in the BAT, several studies show that PGE_1 and E_2 suppress BAT thermogenesis (3, 20, 36), although one study suggests that PGE_2 can activate thermogenesis in brown adipocytes (43).

In addition to being indirectly supported by the patterned activation of thermoeffectors, the view that intravenous PGE_2-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 PGE_2 (34, 46, 48). Our present study demonstrates that similar blockade of these neurons entirely prevents the intravenous PGE_2-elicited iBAT SNA and the increase in end $ExpCO_2$. Increased expiration of CO_2 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 PGE_2 is mediated by the brain.

Intravenous PGE_2-Induced Fever Can Occur Independently of Vagal Afferents

It is known that vagotomized animals respond with normal fevers to intrabrain PGE_2 (38, 86). Ours is the first study to

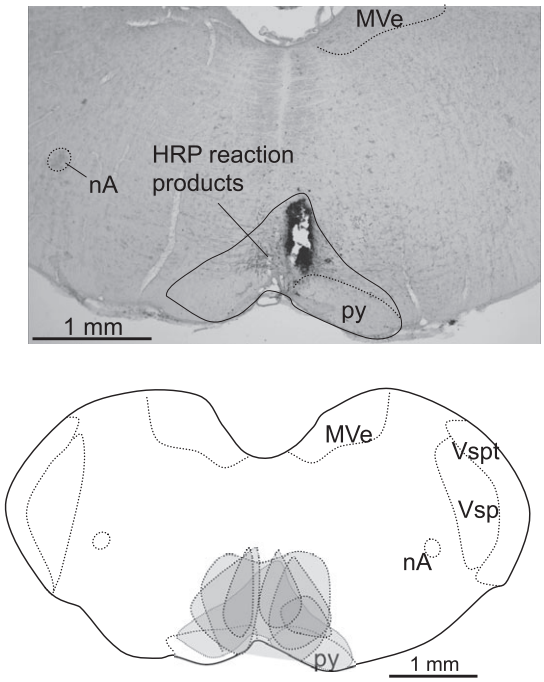

Fig. 4. A photograph of the rostral medulla oblongata (coronal section) showing a representative injection site marked by a horseradish peroxidase reaction product (*top*) and a schematic showing the location of all injection sites (superimposed shadowed areas) used in the muscimol experiments (*bottom*). MVe, medial vestibular nucleus; nA, nucleus ambiguous; py, pyramidal tract; Vsp, spinal nucleus of the trigeminal nerve; Vspt, spinal tract of the trigeminal nerve.

investigate whether vagotomy alters the febrile response to intravenously administered PGE_2. Even though the febrigenic effect of intravenous PGE_2 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 T_c caused by intravenous PGE_2. 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 PGE_2 synthesis during fever (25, 82), and PGE_2 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 PGE_2-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 PGE_2 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, PGE_2-induced fever was due to tail skin vasoconstriction, and whereas $\dot{V}O_2$ tended to increase, there was no statistically significant change in $\dot{V}O_2$. 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

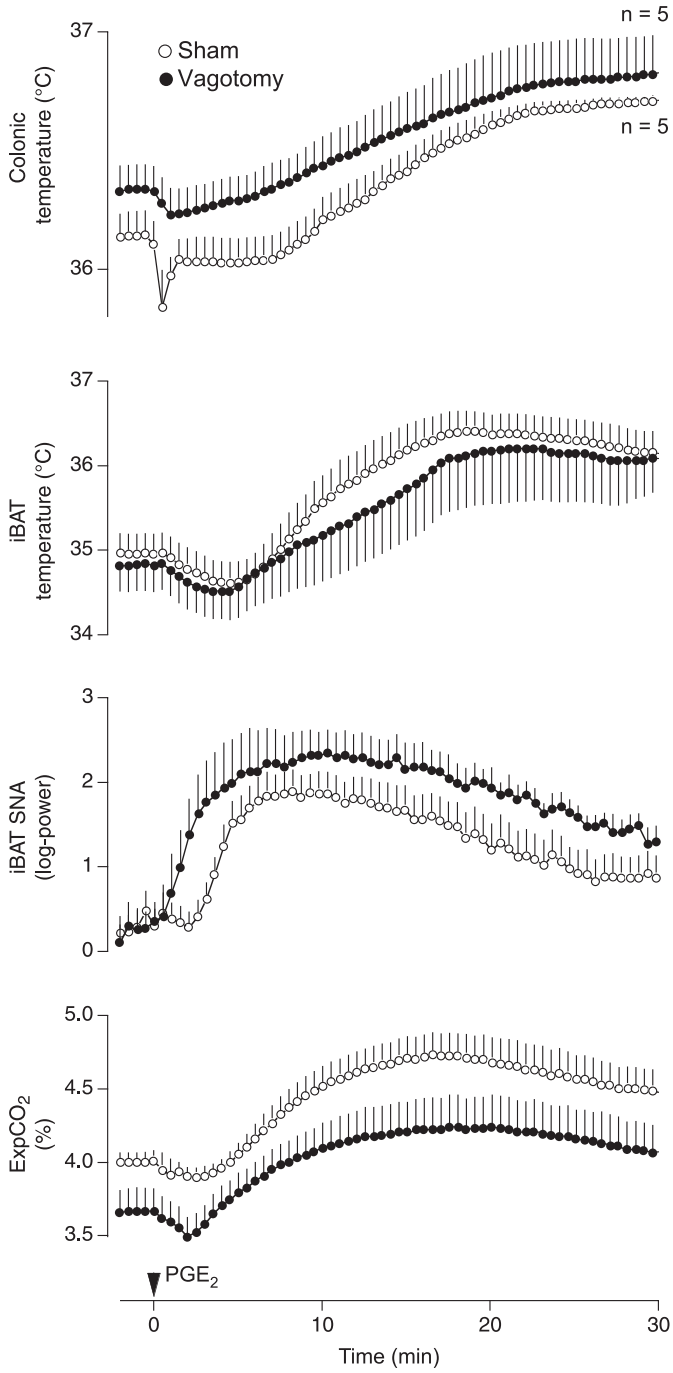

Fig. 5. Thermoregulatory effects of intravenous administration (arrow) of PGE_2 (100 μg/kg) in anesthetized rats after sham vagotomy or bilateral cervical vagotomy; group data are expressed as means ± SE.

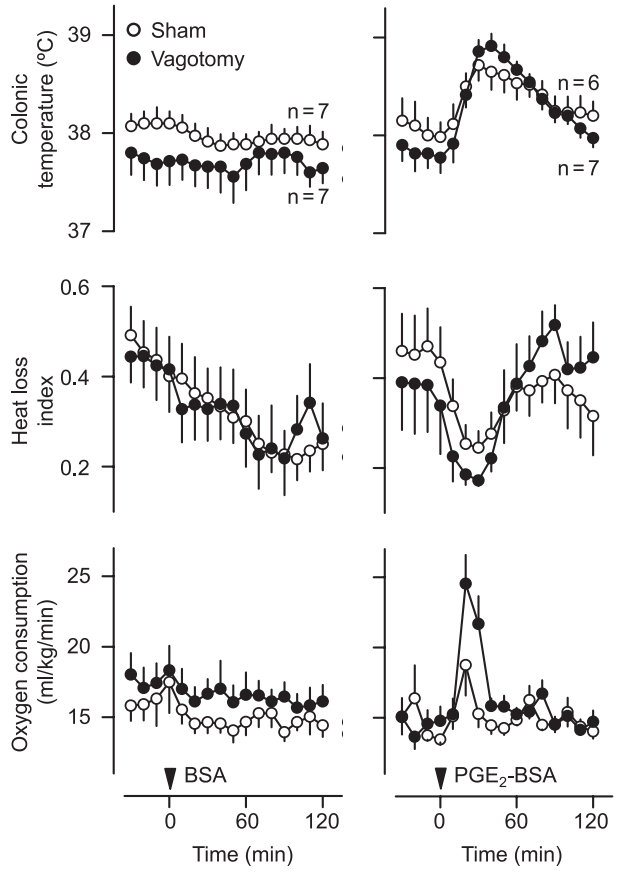

Fig. 6. Thermoregulatory effects of intravenous injection (arrows) of vehicle (BSA; left column) or PGE_2 (PGE_2-BSA complex, 280 μg/kg; right column) in conscious rats with sham vagotomy or with bilateral subdiaphragmatic vagotomy; group data are expressed as means ± SE.

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 PGE_2-induced fever was due to, at least partially, increased BAT thermogenesis (increases in iBAT SNA, iBAT temperature, and $ExpCO_2$). Cervical vagotomy attenuated neither this thermogenic response nor the resultant PGE_2 fever in anesthetized rats.

The biological fate and physiological effects of amphipathic substances (such as PGE_2) strongly depend on serum albumin (59). Up to 99% of circulating PGE_2 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 situ) albumin is present in peripheral nerves (59). Not surprisingly, the same amphipathic lipid can cause different physiological (including thermoregulatory) 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 PGE_2 used in the present experiments in anesthetized vs. conscious rats were prepared differently: a hydroalcoholic solution of PGE_2 vs. a PGE_2-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) PGE_2 was successfully delivered to the receptors triggering the fever response. Furthermore, vagotomy had no effect on the febrile response to either preparation of PGE_2. Hence, in no paradigm studied in our experiments did the febrile effect of intravenous PGE_2 depend on the integrity of vagal afferent nerves.

How Does Intravenous PGE_2 Activate Brain Thermoregulatory Pathways?

How circulating PGE_2 conveys pyrogenic information to the brain and causes fever remains speculative. That intravenous PGE_2 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; PGE_2 binding to albumin is thought to play a protective role (59). Once dissociated from albumin at a site near or at the BBB, PGE_2 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 PGE_2 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 PGE_2-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 PGE_2 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 PGE_2 or intraperitoneal LPS (30). EP3 receptors are also expressed in other brain regions, including the raphe/parapyramidal area (44, 53), but local application of PGE_2 in the raphe affects neither iBAT nor tail artery SNA (90). Clearly, more studies are needed to trace the pathway(s) by which circulating PGE_2 conveys febrigenic signals to thermoeffector circuitries.

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-

uate 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 fevers 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 PGE_2-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 a lack of thermoeffector insufficiency) that would suggest that 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 PGE_2 (82) and because PGE_2 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 PGE_2 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 PGE_2 delivery, and different measures of thermoeffector activity. We have found that intravenous PGE_2 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-to-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.

REFERENCES

1. **Andrews PL, Rothwell NJ, Stock MJ.** Effects of subdiaphragmatic vagotomy on energy balance and thermogenesis in the rat. *J Physiol* 362: 1–12, 1985.
2. **Armstrong MB, Towle HC.** Polyunsaturated fatty acids stimulate hepatic UCP-2 expression via a PPARα-mediated pathway. *Am J Physiol Endocrinol Metab* 281: E1197–E1204, 2001.
3. **Ball KT, Takeuchi M, Yoneyama Y, Power GG.** Role of prostaglandin I_2 and prostaglandin E_2 in the initiation of nonshivering thermogenesis during the simulation of birth in utero. *Reprod Fertil Dev* 7: 399–403, 1995.
4. **Blatteis CM.** Endotoxic fever: new concepts of its regulation suggest new approaches to its management. *Pharmacol Ther* 111: 194–223, 2006.
5. **Blatteis CM, Bealer SL, Hunter WS, Llanos QJ, Ahokas RA, Mashburn TA Jr.** Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res Bull* 11: 519–526, 1983.
6. **Blatteis CM, Li S, Li Z, Feleder C, Perlik V.** Cytokines, PGE_2 and endotoxic fever: a re-assessment. *Prostaglandins Other Lipid Mediat* 76: 1–18, 2005.
7. **Blessing WW.** Lower brainstem pathways regulating sympathetically mediated changes in cutaneous blood flow. *Cell Mol Neurobiol* 23: 527–538, 2003.
8. **Blessing WW, Nalivaiko E.** Raphe magnus/pallidus neurons regulate tail but not mesenteric arterial blood flow in rats. *Neuroscience* 105: 923–929, 2001.
9. **Caldwell FT Jr, Graves DB, and Wallace BH.** Humoral versus neural pathways for fever production in rats after administration of lipopolysaccharide. *J Trauma* 47: 120–129, 1999.
10. **Cannon B, Houstek J, Nedergaard J.** Brown adipose tissue. More than an effector of thermogenesis? *Ann NY Acad Sci* 856: 171–187, 1998.
11. **Coceani F, Bishai I, Engelberts D, House RV, Adamson SL.** Response of newborn and adult sheep to pyrogens: relation between fever and brain eicosanoid changes. *Brain Res* 700: 191–204, 1995.
12. **Crawshaw LI, Stitt JT.** Behavioural and autonomic induction of prostaglandin E_1 fever in squirrel monkeys. *J Physiol* 244: 197–206, 1975.
13. **Davidson J, Abul HT, Milton AS, Rotondo D.** Cytokines and cytokine inducers stimulate prostaglandin E_2 entry into the brain. *Pflügers Arch* 442: 526–533, 2001.
14. **Dogan MD, Patel S, Rudaya AY, Steiner AA, Szekely M, Romanovsky AA.** Lipopolysaccharide fever is initiated via a capsaicin-sensitive mechanism independent of the subtype-1 vanilloid receptor. *Br J Pharmacol* 143: 1023–1032, 2004.
15. **Eguchi N, Hayashi H, Urade Y, Ito S, Hayaishi O.** Central action of prostaglandin E_2 and its methyl ester in the induction of hyperthermia after

their systemic administration in urethane-anesthetized rats. *J Pharmacol Exp Ther* 247: 671–679, 1988.
16. **Ek M, Kurosawa M, Lundeberg T, Ericsson A.** Activation of vagal afferents after intravenous injection of interleukin-1β: role of endogenous prostaglandins. *J Neurosci* 18: 9471–9479, 1998.
17. **Engblom D, Saha S, Engstrom L, Westman M, Audoly LP, Jakobsson PJ, Blomqvist A.** Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. *Nat Neurosci* 6: 1137–1138, 2003.
18. **Evans AR, Blair RW.** Responses of medullary raphe neurons to electrical and chemical activation of vagal afferent nerve fibers. *J Neurophysiol* 70: 1950–1961, 1993.
19. **Forstermann U, Neufang B.** Elimination from the circulation of cats of 6-keto-prostaglandin E_1 compared with prostaglandins E_2 and I_2. *J Pharm Pharmacol* 35: 724–728, 1983.
20. **Gunn TR, Ball KT, Gluckman PD.** Withdrawal of placental prostaglandins permits thermogenic responses in fetal sheep brown adipose tissue. *J Appl Physiol* 74: 998–1004, 1993.
21. **Hansen MK, Daniels S, Goehler LE, Gaykema RP, Maier SF, Watkins LR.** Subdiaphragmatic vagotomy does not block intraperitoneal lipopolysaccharide-induced fever. *Auton Neurosci* 85: 83–87, 2000.
22. **Hansen MK, O'Connor KA, Goehler LE, Watkins LR, Maier SF.** The contribution of the vagus nerve in interleukin-1β-induced fever is dependent on dose. *Am J Physiol Regul Integr Comp Physiol* 280: R929–R934, 2001.
23. **Holzer P, Jocic M, Peskar BA.** Mediation by prostaglandins of the nitric oxide-induced neurogenic vasodilatation in rat skin. *Br J Pharmacol* 116: 2365–2370, 1995.
24. **Ivanov AI, Patel S, Kulchitsky VA, Romanovsky AA.** Platelet-activating factor: a previously unrecognized mediator of fever. *J Physiol* 553: 221–228, 2003.
25. **Ivanov AI, Pero RS, Scheck AC, Romanovsky AA.** Prostaglandin E_2-synthesizing enzymes in fever: differential transcriptional regulation. *Am J Physiol Regul Integr Comp Physiol* 283: R1104–R1117, 2002.
26. **Ivanov AI, Romanovsky AA.** Prostaglandin E_2 as a mediator of fever: synthesis and catabolism. *Front Biosci* 9: 1977–1993, 2004.
27. **Ivanov AI, Scheck AC, Romanovsky AA.** Expression of genes controlling transport and catabolism of prostaglandin E_2 in lipopolysaccharide fever. *Am J Physiol Regul Integr Comp Physiol* 284: R698–R706, 2003.
28. **Ivanov AI, Steiner AA, Patel S, Rudaya AY, Romanovsky AA.** Albumin is not an irreplaceable carrier for amphipathic mediators of thermoregulatory responses to LPS: compensatory role of α_1-acid glycoprotein. *Am J Physiol Regul Integr Comp Physiol* 288: R872–R878, 2005.
29. **Kis B, Isse T, Snipes JA, Chen L, Yamashita H, Ueta Y, Busija DW.** Effects of LPS stimulation on the expression of prostaglandin carriers in the cells of the blood-brain and blood-cerebrospinal fluid barriers. *J Appl Physiol* 100: 1392–1399, 2006.
30. **Lazarus M, Yoshida K, Coppari R, Bass CE, Mochizuki T, Lowell BB, Saper CB.** EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. *Nat Neurosci* 10: 1131–1133, 2007.
31. **Li S, Wang Y, Matsumura K, Ballou LR, Morham SG, Blatteis CM.** The febrile response to lipopolysaccharide is blocked in cyclooxygenase-$2^{-/-}$, but not in cyclooxygenase-$1^{-/-}$ mice. *Brain Res* 825: 86–94, 1999.
32. **Li Z, Perlik V, Feleder C, Tang Y, Blatteis CM.** Kupffer cell-generated PGE_2 triggers the febrile response of guinea pigs to intravenously injected LPS. *Am J Physiol Regul Integr Comp Physiol* 290: R1262–R1270, 2006.
33. **Luheshi GN, Bluthe RM, Rushforth D, Mulcahy N, Konsman JP, Goldbach M, Dantzer R.** Vagotomy attenuates the behavioural but not the pyrogenic effects of interleukin-1 in rats. *Auton Neurosci* 85: 127–132, 2000.
34. **Madden CJ, Morrison SF.** Excitatory amino acid receptor activation in the raphe pallidus area mediates prostaglandin-evoked thermogenesis. *Neuroscience* 122: 5–15, 2003.
35. **Matsumura S, Shibakusa T, Fujikawa T, Yamada H, Matsumura K, Inoue K, Fushiki T.** Intracisternal administration of transforming growth factor-β evokes fever through the induction of cyclooxygenase-2 in brain endothelial cells. *Am J Physiol Regul Integr Comp Physiol* 294: R266–R275, 2008.
36. **McMahon KK, Schimmel RJ.** Apparent absence of α_2 adrenergic receptors from hamster brown adipocytes. *Life Sci* 30: 1185–1192, 1982.
37. **Metz W, Forssmann WG.** Innervation of the liver in guinea pig and rat. *Anat Embryol (Berl)* 160: 239–252, 1980.
38. **Milligan ED, McGorry MM, Fleshner M, Gaykema RP, Goehler LE, Watkins LR, Maier SF.** Subdiaphragmatic vagotomy does not prevent fever following intracerebroventricular prostaglandin E_2: further evidence for the importance of vagal afferents in immune-to-brain communication. *Brain Res* 766: 240–243, 1997.
39. **Morimoto A, Morimoto K, Watanabe T, Sakata Y, Murakami N.** Does an increase in prostaglandin E_2 in the blood circulation contribute to a febrile response in rabbits? *Brain Res Bull* 29: 189–192, 1992.
40. **Morimoto A, Murakami N, Nakamori T, Watanabe T.** Evidence for separate mechanisms of induction of biphasic fever inside and outside the blood-brain barrier in rabbits. *J Physiol* 383: 629–637, 1987.
41. **Morrison SF.** Central pathways controlling brown adipose tissue thermogenesis. *News Physiol Sci* 19: 67–74, 2004.
42. **Morrison SF, Sved AF, Passerin AM.** GABA-mediated inhibition of raphe pallidus neurons regulates sympathetic outflow to brown adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 276: R290–R297, 1999.
43. **Nagai M, Tuchiya K, Kojima H.** Prostaglandin E_2 increases the calcium concentration in rat brown adipocytes and their consumption of oxygen. *Prostaglandins* 51: 377–386, 1996.
44. **Nakamura K, Kaneko T, Yamashita Y, Hasegawa H, Katoh H, Negishi M.** Immunohistochemical localization of prostaglandin EP3 receptor in the rat nervous system. *J Comp Neurol* 421: 543–569, 2000.
45. **Nakamura K, Matsumura K, Hubschle T, Nakamura Y, Hioki H, Fujiyama F, Boldogkoi Z, Konig M, Thiel HJ, Gerstberger R, Kobayashi S, Kaneko T.** Identification of sympathetic premotor neurons in medullary raphe regions mediating fever and other thermoregulatory functions. *J Neurosci* 24: 5370–5380, 2004.
46. **Nakamura K, Matsumura K, Kaneko T, Kobayashi S, Katoh H, Negishi M.** The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. *J Neurosci* 22: 4600–4610, 2002.
47. **Nakamura K, Morrison SF.** Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 292: R127–R136, 2007.
48. **Nakamura K, Morrison SF.** A thermosensory pathway that controls body temperature. *Nat Neurosci* 11: 62–71, 2008.
49. **Negishi M, Sugimoto Y, Ichikawa A.** Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim Biophys Acta* 1259: 109–119, 1995.
50. **Neisius U, Olsson R, Rukwied R, Lischetzki G, Schmelz M.** Prostaglandin E_2 induces vasodilation and pruritus, but no protein extravasation in atopic dermatitis and controls. *J Am Acad Dermatol* 47: 28–32, 2002.
51. **Niijima A.** The afferent discharges from sensors for interleukin 1β in the hepatoportal system in the anesthetized rat. *J Auton Nerv Syst* 61: 287–291, 1996.
52. **Oka T, Oka K, Kobayashi T, Sugimoto Y, Ichikawa A, Ushikubi F, Narumiya S, Saper CB.** Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP_1 and EP_3 receptors. *J Physiol* 551: 945–954, 2003.
53. **Oka T, Oka K, Scammell TE, Lee C, Kelly JF, Nantel F, Elmquist JK, Saper CB.** Relationship of EP(1–4) prostaglandin receptors with rat hypothalamic cell groups involved in lipopolysaccharide fever responses. *J Comp Neurol* 428: 20–32, 2000.
54. **Ootsuka Y, Blessing WW.** Inhibition of medullary raphe/parapyramidal neurons prevents cutaneous vasoconstriction elicited by alerting stimuli and by cold exposure in conscious rabbits. *Brain Res* 1051: 189–193, 2005.
55. **Ootsuka Y, Blessing WW, McAllen RM.** Inhibition of rostral medullary raphe neurons prevents cold-induced activity in sympathetic nerves to rat tail and rabbit ear arteries. *Neurosci Lett* 357: 58–62, 2004.
56. **Ootsuka Y, Heidbreder CA, Hagan JJ, Blessing WW.** Dopamine D_2 receptor stimulation inhibits cold-initiated thermogenesis in brown adipose tissue in conscious rats. *Neuroscience* 147: 127–135, 2007.
57. **Ootsuka Y, McAllen RM.** Comparison between two rat sympathetic pathways activated in cold defense. *Am J Physiol Regul Integr Comp Physiol* 291: R589–R595, 2006.
58. **Parrott RF, Vellucci SV, Forsling ML, Goode JA.** Hyperthermic and endocrine effects of intravenous prostaglandin administration in the pig. *Domest Anim Endocrinol* 12: 197–205, 1995.
59. **Peters TJ.** All about albumin: biochemistry, genetics, and medical applications. San Diego, CA: Academic Press, 1996.
60. **Petervari E, Garami A, Pakai E, Szekely M.** Effects of perineural capsaicin treatment of the abdominal vagus on endotoxin fever and on a non-febrile thermoregulatory event. *J Endotoxin Res* 11: 260–266, 2005.
61. **Portet R, de Marco F, Zizine L, Senault C, Bertin R.** Regulation of prostaglandin E_2 and F_α productions by rat brown fat during the perinatal period; effects of ambient temperature. *Biochimie* 64: 523–526, 1982.

62. **Romanovsky AA.** Signaling the brain in the early sickness syndrome: are sensory nerves involved? *Front Biosci* 9: 494–504, 2004.
63. **Romanovsky AA.** Thermoregulation: some concepts have changed. *Functional* architecture of the thermoregulatory system. *Am J Physiol Regul Integr Comp Physiol* 292: R37–R46, 2007.
64. **Romanovsky AA, Almeida MC, Aronoff DM, Ivanov AI, Konsman JP, Steiner AA, Turek VF.** Fever and hypothermia in systemic inflammation: recent discoveries and revisions. *Front Biosci* 10: 2193–2216, 2005.
65. **Romanovsky AA, Ivanov AI, Karman EK.** Blood-borne, albumin-bound prostaglandin E_2 may be involved in fever. *Am J Physiol Regul Integr Comp Physiol* 276: R1840–R1844, 1999.
66. **Romanovsky AA, Ivanov AI, Lenczowski MJ, Kulchitsky VA, Van Dam AM, Poole S, Homer LD, Tilders FJ.** Lipopolysaccharide transport from the peritoneal cavity to the blood: is it controlled by the vagus nerve? *Auton Neurosci* 85: 133–140, 2000.
67. **Romanovsky AA, Ivanov AI, Shimansky YP.** Selected contribution: Ambient temperature for experiments in rats: A new method for determining the zone of thermal neutrality. *J Appl Physiol* 92: 2667–2679, 2002.
68. **Romanovsky AA, Ivanov AI, Szekely M.** Neural route of pyrogen signaling to the brain. *Clin Infect Dis* 31 *Suppl* 5: S162–S167, 2000.
69. **Romanovsky AA, Kulchitsky VA, Simons CT, Sugimoto N, Szekely M.** Cold defense mechanisms in vagotomized rats. *Am J Physiol Regul Integr Comp Physiol* 273: R784–R789, 1997.
70. **Romanovsky AA, Kulchitsky VA, Simons CT, Sugimoto N, Szekely M.** Febrile responsiveness of vagotomized rats is suppressed even in the absence of malnutrition. *Am J Physiol Regul Integr Comp Physiol* 273: R777–R783, 1997.
71. **Romanovsky AA, Simons CT, Kulchitsky VA.** "Biphasic" fevers often consist of more than two phases. *Am J Physiol Regul Integr Comp Physiol* 275: R323–R331, 1998.
72. **Romanovsky AA, Simons CT, Szekely M, Kulchitsky VA.** The vagus nerve in the thermoregulatory response to systemic inflammation. *Am J Physiol Regul Integr Comp Physiol* 273: R407–R413, 1997.
73. **Romanovsky AA, Steiner AA, Matsumura K.** Cells that trigger fever. *Cell Cycle* 5: 2195–2197, 2006.
74. **Romanovsky AA, Sugimoto N, Simons CT, Hunter WS.** The organum vasculosum laminae terminalis in immune-to-brain febrigenic signaling: a reappraisal of lesion experiments. *Am J Physiol Regul Integr Comp Physiol* 285: R420–R428, 2003.
75. **Rotondo D, Abul HT, Milton AS, Davidson J.** Pyrogenic immunomodulators increase the level of prostaglandin E_2 in the blood simultaneously with the onset of fever. *Eur J Pharmacol* 154: 145–152, 1988.
76. **Sehic E, Blatteis CM.** Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. *Brain Res* 726: 160–166, 1996.
77. **Sessler DI.** Perioperative thermoregulation and heat balance. *Ann NY Acad Sci* 813: 757–777, 1997.
78. **Simons CT, Kulchitsky VA, Sugimoto N, Homer LD, Szekely M, Romanovsky AA.** Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis? *Am J Physiol Regul Integr Comp Physiol* 275: R63–R68, 1998.
79. **Siren AL.** Central cardiovascular and thermal effects of prostaglandin E_2 in rats. *Acta Physiol Scand* 116: 229–234, 1982.
80. **Skarnes RC, Brown SK, Hull SS, McCracken JA.** Role of prostaglandin E in the biphasic fever response to endotoxin. *J Exp Med* 154: 1212–1224, 1981.
81. **Steiner AA, Chakravarty S, Rudaya AY, Herkenham M, Romanovsky AA.** Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoietic cells. *Blood* 107: 4000–4002, 2006.
82. **Steiner AA, Ivanov AI, Serrats J, Hosokawa H, Phayre AN, Robbins JR, Roberts JL, Kobayashi S, Matsumura K, Sawchenko PE, Romanovsky AA.** Cellular and molecular bases of the initiation of fever. *PLoS Biol* 4: e284, 2006.
83. **Steiner AA, Rudaya AY, Robbins JR, Dragic AS, Langenbach R, Romanovsky AA.** Expanding the febrigenic role of cyclooxygenase-2 to the previously overlooked responses. *Am J Physiol Regul Integr Comp Physiol* 289: R1253–R1257, 2005.
84. **Steiner AA, Turek VF, Almeida MC, Burmeister JJ, Oliveira DL, Roberts JL, Bannon AW, Norman MH, Louis JC, Treanor JJ, Gavva NR, Romanovsky AA.** Nonthermal activation of transient receptor potential vanilloid-1 channels in abdominal viscera tonically inhibits autonomic cold-defense effectors. *J Neurosci* 27: 7459–7468, 2007.
85. **Sugimoto M, Arai I, Futaki N, Hashimoto Y, Honma Y, Nakaike S.** COX-1 inhibition enhances scratching behaviour in NC/Nga mice with atopic dermatitis. *Exp Dermatol* 15: 582–588, 2006.
86. **Sugimoto N, Simons CT, Romanovsky AA.** Vagotomy does not affect thermal responsiveness to intrabrain prostaglandin E_2 and cholecystokinin octapeptide. *Brain Res* 844: 157–163, 1999.
87. **Szekely M, Balasko M, Kulchitsky VA, Simons CT, Ivanov AI, Romanovsky AA.** Multiple neural mechanisms of fever. *Auton Neurosci* 85: 78–82, 2000.
88. **Szekely M, Balasko M, Romanovsky AA.** Peripheral neural inputs. Their role in fever development. *Ann NY Acad Sci* 813: 427–434, 1997.
89. **Szelenyi Z, Bartho L, Szekely M, Romanovsky AA.** Cholecystokinin octapeptide (CCK-8) injected into a cerebral ventricle induces a fever-like thermoregulatory response mediated by type B CCK-receptors in the rat. *Brain Res* 638: 69–77, 1994.
90. **Tanaka M, McAllen RM.** A subsidiary fever center in the medullary raphe? *Am J Physiol Regul Integr Comp Physiol* 289: R1592–R1598, 2005.
91. **Tanaka M, Nagashima K, McAllen RM, Kanosue K.** Role of the medullary raphe in thermoregulatory vasomotor control in rats. *J Physiol* 540: 657–664, 2002.
92. **Unger WG.** Binding of prostaglandin to human serum albumin. *J Pharm Pharmacol* 24: 470–477, 1972.
93. **Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, Tanaka T, Yoshida N, Narumiya S.** Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. *Nature* 395: 281–284, 1998.
94. **Vasilache AM, Andersson J, Nilsberth C.** Expression of PGE_2 EP_3 receptor subtypes in the mouse preoptic region. *Neurosci Lett* 423: 179–183, 2007.
95. **Zhang G, Lee LY.** Prostaglandin E_2 enhances the sensitizing effect of hyperthermia on pulmonary C-fibers in rats. *Respir Physiol Neurobiol* 156: 241–249, 2007.