Fever induction by localized subcutaneous inflammation in guinea pigs: the role of cytokines and prostaglandins

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Ross, Günter, Thomas Hübschle, Ulrich Pehl, Hans-Albert Braun, Karlheinz Voigt, Rüdiger Gerstberger, and Joachim Roth. Fever induction by localized subcutaneous inflammation in guinea pigs: the role of cytokines and prostaglandins. J Appl Physiol 94: 1395–1402, 2003. —In guinea pigs, dose-dependent febrile responses can be induced by injection of a high (100 μg/kg) or low (10 μg/kg) dose of bacterial lipopolysaccharide (LPS) into artificial subcutaneously implanted Teflon chambers. In this fever model, LPS does not enter the systemic circulation from the site of localized tissue inflammation in considerable amounts but causes a local induction of the proinflammatory cytokines tumor necrosis factor (TNF) and interleukin-6 (IL-6), which can be measured in lavage fluid collected from the chamber area. Only in response to the high LPS dose, small traces of TNF are measurable in blood plasma. A moderate increase of circulating IL-6 occurs in response to administration of both LPS doses. To investigate the putative roles of TNF and prostaglandins in this fever model, a neutralizing TNF binding protein (TNF-bp) or a nonselective inhibitor of cyclooxygenases (diclofenac) was injected along with the high or low dose of LPS into the subcutaneous chamber. In control groups, both doses of LPS were administered into the chamber along with the respective vehicles for the applied drugs. The fever response to the high LPS dose remained unimpaired by treatment with TNF-bp despite an effective neutralization of bioactive TNF in the inflamed tissue area. In response to the low LPS dose, there was an accelerated defervescence under the influence of TNF-bp. Blockade of prostaglandin formation with diclofenac completely abolished fever in response to both LPS doses. In conclusion, prostaglandins seem to be essential components for the manifestation of fever in this model.

IN EXPERIMENTAL ANIMALS, FEVER can be induced by systemic administration of bacterial lipopolysaccharide (LPS) which, in turn, causes the appearance of a cascade of proinflammatory cytokines in the bloodstream. These circulating cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 are traditionally regarded as endogenous mediators of the LPS-induced febrile response (4, 17). Fever and other brain-controlled sickness responses sometimes seem to occur in the absence of a substantial rise of circulating concentrations of cytokines (39). Therefore, an alternative mechanism by which fever signals from the activated immune system are transported to the brain might be the stimulation of peripheral sensory nerves (4, 10, 29, 30, 39). In this context, an experimental fever model has been established recently in rats in which LPS is administered locally, rather than systemically, into a subcutaneous air pouch (8, 9, 21, 22) that does not lead to LPS appearance in the circulation (7). In contrast to intraperitoneal or intravenous injection of LPS, this model nicely mimics localized infection or inflammation. Recently, a similar experimental fever model was established and evaluated in guinea pigs; the air pouch was replaced with a subcutaneously implanted artificial Teflon chamber (30).

Despite the absence of LPS in the systemic circulation after its injection into a subcutaneous air pouch (7) or a subcutaneous chamber (see Does LPS enter the circulation from the site of injection? under RESULTS), an elevation of IL-6 concentrations in the circulation can be observed. Cartmell et al. (9) provided convincing evidence that the rise of circulating IL-6 in response to LPS injection into the subcutaneous air pouch in rats is due to a spillover of this cytokine from the local site of inflammation into the systemic circulation and that the increase of IL-6 in the bloodstream, albeit moderate, is involved in the manifestation of fever. It has, however, to be noted that peripheral in contrast to central IL-6 has only a moderate pyrogenic activity (3, 14, 33). Because of this fact, injection of a relatively high dose of IL-6 alone into the subcutaneous air pouch in rats was not pyrogenic but did induce fever when coincjected with a small dose of IL-1β (9). It thus seems that IL-6 needs cofactors, possibly produced at the site of localized tissue inflammation, to be able to develop pyrogenic properties. We therefore tried to obtain evidence for possible participation of TNF and prostaglandins in the febrile response that develops after injection of LPS into a subcutaneous chamber. Biological...
activity of TNF or of prostaglandins was antagonized by injecting a neutralizing TNF binding protein (TNF-bp; 32, 37) or diclofenac, a potent nonselective inhibitor of cyclooxygenases (31), directly into the subcutaneous chamber along with LPS, and we evaluated the effects of the treatment of guinea pigs with both drugs on the febrile response.

MATERIALS AND METHODS

Animals. This study was performed in male guinea pigs (Cavia aperea porcellus) with a body weight of 370–390 g at the beginning of the experiments (day of surgery). The animals were housed in individual cages at 22°C and a 12:12-h light-dark cycle (light off at 7:00 PM). The animals had access to food and water ad libitum. Twice a week, the reservoirs were filled with fresh pelleted food and water, and at the same time the cages were changed. About 10 days before the fever experiment, the animals were prepared surgically (see below) and habituated at least twice to the experimental handling procedures. The national guidelines for experiments with vertebrate animals have been followed, and for the experimental protocols approval by the local ethics committee has been obtained (reference numbers GI 20/1-1/96 and GI 18/2-42/00).

Surgery. About 10 days before the experiment, the animals were chronically implanted with intra-arterial catheters for blood sampling, artificial subcutaneous Teflon (polytetrafluoroethylene) chambers equipped with catheters for injection of drugs and collection of lavage, and radiotransmitters for measurement of body temperature. Briefly, the guinea pigs were anesthetized with 100 mg/kg ketamine hydrochloride (Pharmacia Upjohn, Erlangen, Germany) and 4 mg/kg xylazine (Bayer, Leverkusen, Germany). A polyethylene catheter (Portex, Kent, UK; reference number 800/140/100; 0.4 mm ID, 0.8 mm OD) was inserted through the left carotid artery until it reached the aortic arch. Slow aspiration of blood with the syringe indicated the correct position of the catheters. The catheter was then fixed with two sutures. The distal end of the catheter was tunneled subcutaneously to the interscapular region of the back, where it was exteriorized and again fixed with two sutures. The muscle layer and the skin were closed separately with sutures. Finally, the catheter was flushed with sterile heparinized saline and sealed by heating.

The artificial chambers were implanted into subcutaneous cavities that were formed with a cylindrical Plexiglas stick after a cutaneous incision. The cavities that existed after removal of the Plexiglas stick had about the same diameter and size as the artificial subcutaneous chamber, which was open at both sides. The open side of the chambers had close contact to the skin tissue. The subcutaneous chamber was placed laterally to the dorsal midline, caudally to the scapulae of the anesthetized guinea pigs. The chambers were equipped with catheters for administration of drugs and sampling of lavage, which were also tunneled subcutaneously to the interscapular region of the back, caudally to the arterial catheter and sealed by heating. Then the skin was closed with sutures. A schematic diagram of the subcutaneous chamber providing information about its shape and size is shown in Fig. 1.

After surgical placement of arterial catheter and subcutaneous chamber, a biotelemetry transmitter for measurement of core temperature was implanted intraperitoneally.

Substances. Bacterial LPS (derived from Escherichia coli, O111:B4, Sigma Chemical, St. Louis, MO) was suspended in sterile pyrogen-free 0.9% saline at a concentration of 100 µg/ml (high dose) or 10 µg/ml (low dose). Doses of 100 or 10 µg/kg were used for injections into the subcutaneous chamber.

TNF-bp, a synthetic dimeric polyethyleneglycol-linked form of the type 1 soluble receptor of TNF [PEG-(rsTNF-R1)2], was kindly provided by Dr. Dave Martin (AMGEN, Boulder, CO). This substance effectively neutralizes bioactive TNF (32, 37). TNF-bp was dissolved in sterile pyrogen-free saline at a concentration of 5 mg/ml (stock solution). The stock solution was diluted with sterile saline to a final concentration of 1 mg/ml, and aliquots of 100 µg of TNF-bp were prepared from this solution. An amount of 100 µg of TNF-bp in a volume of 300 µl per animal was injected into the subcutaneous chamber along with LPS or 0.9% sterile saline.

Diclofenac-sodium (Calbiochem, La Jolla, CA) was dissolved at a concentration of 5 mg/ml in a vehicle that consisted of 95% sterile saline and 5% ethanol. The drug was injected into the subcutaneous chamber with LPS or an equivalent volume of the vehicle for diclofenac at a dose of 5 mg/kg.

Collection of plasma and lavage fluid. During the experiments, blood samples (~0.5 ml per sample) were slowly drawn from the intra-arterial catheter into sterile heparinized syringes and were immediately centrifuged. After each blood sampling procedure, the catheter was flushed with a small volume (~0.1 ml) of heparinized saline (~80 IU of heparin) and closed by heating. To obtain lavage fluid from the artificial subcutaneous chamber, 1 ml of sterile saline was slowly injected into the chamber through the catheter, left there for ~30 s, withdrawn again into a sterile syringe, and immediately centrifuged. Thereafter the catheter was flushed with a small volume (0.1 ml) of sterile pyrogen-free saline and closed by heating. Plasma and lavage supernatant were stored at ~70°C for later determination of cytokines. Within 2–3 days before the experiment, the animals were at least twice accustomed to the procedures for sampling of blood and lavage fluid, which then did not cause excitement or stress-induced changes of body temperature. On the day of the experiment, it was tested whether there was an accumulation of fluid in the chamber by aspiration with a syringe through the catheter. The experiment was performed only if there was <0.1 ml of fluid in the syringe.

In the experiment, plasma and lavage fluid were collected 1 h before as well as 1 and 3 h after administration of drugs.
into the subcutaneous chamber. The first sample was collected to obtain information about basal levels of the investigated cytokines. The collection times after injection of drugs were chosen because LPS-induced peak values of TNF and IL-6 can be detected 1 h (TNF) and 3 h (IL-6) after systemic LPS administration in guinea pigs (31).

Experimental protocol. Twelve groups of guinea pigs (n = 5–6 per group) were used for the experiments. Four groups were given 100 μg/kg LPS and either 100 μg/animal TNF-bp, 5 mg/kg diclofenac, or equivalent volume of vehicles for TNF-bp (sterile saline) or diclofenac (95% saline and 5% alcohol), respectively. Another four groups of animals were given 10 μg/kg LPS along with the same amounts of TNF-bp, diclofenac, or the vehicles for both drugs. Finally, four control groups of animals were given an equivalent volume of sterile saline instead of LPS, and the same amounts of TNF-bp, diclofenac, or vehicles for both drugs were injected. During the experiment, blood samples and lavage fluid from the subcutaneous chamber were collected 1 h before, as well as 1 or 3 h after injection of the above-mentioned solutions into the subcutaneous chamber. Body core temperature was evaluated by subcutaneously implanted battery-operated biotelemetry transmitters (VM-FH-discs or PDT-4000 E-mitter; Mini-Mitter, Sunriver, OR). Output (frequency in Hz) was monitored by an antenna placed under each cage (RA 1000 or ER-4000 radioreceivers, Mini-Mitter). A data-acquisition system (Vital View, Mini-Mitter) was used for automatic control of data collection and analysis. Body temperature was monitored and recorded at 5-min intervals. For the analysis and graphical documentation, temperature data at time intervals of 15 min were used.

Measurement of body temperature. Abdominal temperature was measured by use of intraperitoneally implanted battery-operated biotelemetry transmitters (VM-FH-discs or PDT-4000 E-mitter; Mini-Mitter, Sunriver, OR). Output (frequency in Hz) was monitored by an antenna placed under each cage (RA 1000 or ER-4000 radioreceivers, Mini-Mitter). A data-acquisition system (Vital View, Mini-Mitter) was used for automatic control of data collection and analysis. Body temperature was monitored and recorded at 5-min intervals. For the analysis and graphical documentation, temperature data at time intervals of 15 min were used.

Bioassays for TNF and IL-6. Determination of TNF was performed by a bioassay based on the cytotoxic effect of TNF on the mouse fibrosarcoma cell line WEHI 164 subclone 13 (11). The assay was performed in sterile, 96-well microtiter plates. Serial dilutions of biological samples or different concentrations of a murine TNF-standard (code 88/532, National Institute for Biological Standards and Control, South Mimms, UK) were incubated for 24 h in wells that had been seeded with 50,000 actinomycin D-treated WEHI cells. The number of surviving cells after 24 h was measured by use of the dimethylthiazol-diphenyl tetrazolium bromide colorimetric assay (15). Plasma samples were prediluted so that serial dilution of samples and standard dilution curves were parallel. The detection limit of the assay, after the dilution of samples into the assays was considered, was 6 pg/ml TNF.

Determination of IL-6 was performed by a bioassay based on the dose-dependent growth stimulation of IL-6 on the B9 hybridoma cell line (1). The assay was performed in sterile, 96-well microtiter plates. In each well, 5,000 B9 cells were incubated for 72 h with serial dilutions of biological samples or with different concentrations of a human IL-6 standard (code 69/545, National Institute for Biological Standards and Control). The number of cells in each well was measured by use of the dimethylthiazol-diphenyl tetrazolium bromide assay (see above). The detection limit of the assay, after the dilution of samples into the assays was considered, was 3 IU IL-6/ml.

Endotoxin assay. To test whether LPS enters the systemic circulation from the site of injection (artificial subcutaneous chamber), LPS in blood plasma was measured by a sensitive limulus amebocyte lysate assay (QCL-1000, license no. 709, Bio-Whittaker, Walkersville, MD). A standard curve was created with endotoxin standard (E. coli 0111:B4) in the range of 0–200 pg/ml. Plasma samples collected from three groups of guinea pigs 60 min after intrachamber injection of 100 or 10 μg/kg LPS or sterile saline were assayed for LPS. In a study in rats using a subcutaneous air pouch, this time point corresponded to the peak of LPS measurable in the pouch (7).

Evaluation and statistics. In graphs of the thermal responses to LPS injections, the mean changes of abdominal temperatures were plotted against time. At each time point, abdominal temperatures were expressed as means ± SE. An analysis of variance (ANOVA), followed by Scheffe’s post hoc test, was used to compare thermal responses of groups of animals. Circulating levels of TNF-α or IL-6 were also compared by ANOVA and Scheffe’s test. Because the values for cytokine concentrations are not normally distributed, a log-transformation of the cytokine values was performed before the statistical calculation.

All calculations were carried out on an Apple Macintosh computer using the software package StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Does LPS enter the circulation from the site of injection? One hour after injection of sterile saline or 100 or 10 μg/kg LPS, blood samples were collected and assayed for endotoxin. In one guinea pig injected with the high dose of LPS, small traces of endotoxin (69 pg/ml) were detected in blood plasma. In all other animals plasma concentrations of LPS were below the detection limit of the assay.

Effects of treatment with TNF-bp on fever in response to LPS-injection into the subcutaneous chamber. Figure 2 shows the thermal responses of guinea pigs to injections into the subcutaneous chamber of sterile saline or 100 μg TNF-bp dissolved in sterile saline (A), 100 μg/kg LPS with saline or with TNF-bp (B), or 10 μg/kg LPS with saline or with TNF-bp (C).

TNF-bp per se had no significant influence on abdominal temperature of guinea pigs compared with animals injected with sterile saline. The febrile response of guinea pigs to intrachamber injections of the high LPS dose (100 μg/kg) was not altered by coadministration of TNF-bp instead of saline. In response to the low LPS dose, there was an accelerated defervescence under the influence of TNF-bp. From 210 to 360 min after injection of 10 μg/kg LPS, body temperature of guinea pigs treated with TNF-bp was significantly lower than that of animals injected with LPS and saline (P = 0.0014; ANOVA).

To test the neutralizing capacity of TNF-bp, TNF (and IL-6) were measured in lavage fluid and in plasma at selected stages of this experiment. The concentrations of bioactive TNF and IL-6 in lavage fluid and blood plasma that were collected before and after injection of 100 μg/kg LPS and saline or LPS and TNF-bp into the subcutaneous chamber are shown in Fig. 3.
One hour before the injection of LPS, basal levels of TNF (range: 100–1,000 pg/ml) and IL-6 (range: 700–7,000 IU/ml) were measured in lavage fluid of subcutaneous chambers. Treatment with LPS caused a pronounced and significant increase of concentrations of both cytokines in lavage fluid (TNF peak: 39,300 – 11,760 pg/ml; IL-6 peak: 80,490 – 30,420 IU/ml; both measured 180 min after injection of LPS into the chamber). Coadministration of TNF-bp significantly decreased the LPS-induced levels of bioactive TNF, not of IL-6, in the lavage fluid collected from the subcutaneous chambers. Just traces of bioactive TNF (10–20 pg/ml) were detected in guinea pigs treated with LPS plus TNF-bp. These data indicated the powerful TNF-neutralizing capacity of TNF-bp. In both groups, there was a significant increase of bioactive IL-6 in lavage fluid, but no significant differences of IL-6 concentrations were calculated between both groups.

In blood plasma, basal levels of IL-6, but not of TNF, were detected. In response to administration of LPS, small amounts of TNF appeared in the blood stream, again significantly lower in animals treated with TNF-bp. Three hours after injection of LPS, circulating IL-6 rose significantly, but again there was no difference in LPS-induced plasma levels of IL-6 between both groups.

The concentrations of bioactive TNF and IL-6 in lavage fluid and blood plasma that were collected before and after injection of 100 μg/kg LPS and saline or...
LPS and TNF-bp into the subcutaneous chamber are shown in Fig. 4.

Generally, the pattern of LPS-induced increase of cytokines in lavage fluid and blood in response to the low LPS dose (10 μg/kg) was very similar to the profile of the experiment in which the high LPS dose (100 μg/kg) was used. However, the absolute peak values of both cytokines were lower than in response to the high LPS dose. Administration of LPS caused a significant rise of TNF in the lavage fluid and of IL-6 in both lavage fluid and blood plasma. Again, LPS-induced increase of TNF in the lavage fluid was significantly depressed by coinjection of TNF-bp 1 h as well as 3 h after administration of 10 μg/kg LPS. Treatment with TNF-bp had no apparent influence on levels of IL-6 in lavage fluid or plasma, although the basal (preinjection) values of IL-6 were higher in the group treated with 10 μg/kg LPS and TNF-bp compared with animals injected with LPS and sterile saline (lavage fluid: \( P = 0.0004 \); blood plasma: \( P = 0.023 \)). Bioactive TNF was not detected in the circulation at any stage of this experiment.

In summary, the potent neutralization of LPS-induced bioactive TNF had only moderate (low LPS dose) or even no influence (high LPS dose) on the manifestation of a febrile response in this experimental model of localized tissue inflammation.

Effects of treatment with a nonselective cyclooxygenase inhibitor on fever in response to LPS-injection into the subcutaneous chamber. Figure 5 shows the thermal responses of guinea pigs to injections into the subcutaneous chamber of 0.9 % NaCl and vehicle (95 % sterile saline and 5 % alcohol) or 5 mg/kg diclofenac (A), 100 μg/kg LPS with vehicle or with diclofenac (B), or 10 μg/kg LPS with vehicle or with diclofenac (C).

Diclofenac per se had no apparent influence on abdominal temperature of guinea pigs compared with animals injected with vehicle. The febrile response of guinea pigs to intrachamber injections of the high LPS dose (Fig. 5B) or the low LPS dose (Fig. 5C) was, however, completely suppressed by treatment with diclofenac. From 45 to 360 min after injection of 100 μg/kg LPS (\( P = 0.0015 \); ANOVA) and from 60 to 360 min after injection of 10 μg/kg LPS (\( P = 0.0102 \); ANOVA), body temperature of guinea pigs treated with LPS and diclofenac was significantly lower than that of animals injected with LPS and vehicle, indicating an important function of cyclooxygenase products in the induction and maintenance of fever in this experimental model.

DISCUSSION

In humans and animals, peripheral administration of LPS induces a characteristic array of brain-mediated illness responses including fever, sickness behavior, and neuroendocrine modifications, predominantly activation of the hypothalamic-pituitary-adrenal axis (2, 4, 10, 17, 31). These responses are thought to be mediated by endogenously produced proinflammatory cytokines. Those cytokines are the mediators of humoral signal pathways from the periphery to those parts of the brain that control the aforementioned signs of sickness. The humoral signal pathways can be activated by intravenous, intra-arterial, intramuscular, or intraperitoneal administrations of LPS, which all result in a pronounced rise of plasma levels of TNF-α and IL-6 and in the appearance of small traces of IL-1β in the systemic circulation depending on the injected LPS dose (12, 16–18, 31, 32). Lenczowski et al. (18) reported that intraperitoneally administered LPS reaches the general circulation and postulated in line with their own results that the appearance of endotoxin in the blood is a prerequisite for the appearance of the cytokine IL-6 in the general circulation, as well as for the activation of the hypothalamic-pituitary-adrenal axis. On the other hand, Cartmell et al. (7) injected 100 μg/kg LPS into a subcutaneous air pouch of rats and were able to recover virtually all of the injected LPS from the pouch within several hours by injecting and collecting lavage
Fluid, whereas LPS was undetectable in blood plasma. In the present study, we confirmed in guinea pigs that 1 h after administration of 10 or 100 μg/kg LPS into subcutaneous chambers LPS cannot be detected in the bloodstream, whereas there is constantly an increase of circulating IL-6. In a study in rats (9), it could be demonstrated that circulating IL-6 derives from the site of inflammation, in this case a subcutaneous air pouch, and that systemic (intraperitoneal) treatment with antibodies directed against rat IL-6 suppresses the febrile response to localized intrapouch injections of LPS. These results suggest that IL-6 that enters the systemic circulation from the site of local subcutaneous inflammation is crucial for the development of fever in this model. Neutralizing antibodies against guinea pig IL-6 are, at least in our hands, not available. We were therefore unable to confirm that the rise of IL-6 in plasma, which is measurable in response to injection of LPS into the subcutaneous chamber, is, indeed, also essential for the febrile response in guinea pigs. Another interesting aspect of the study by Cartmell et al. (9) in rats is that IL-6 alone is not able to induce fever when injected at a high dose into the subcutaneous air pouch, but rather it enhances pyrogenic properties of other factors as shown for IL-1β. In other words, IL-6 seems to activate fever-inducing brain mechanisms by acting in concert with other pyrogenic molecules. Therefore, we tested whether TNF or prostaglandins, which both are implicated in LPS-induced fever, might play a role in the febrile response that develops in guinea pigs after injection of LPS into a subcutaneous chamber.

Bioactive TNF was present in lavage fluid collected from the subcutaneous chamber even under basal conditions, indicating a local inflammatory response to the implanted chamber. However, no febrile increase of body temperature and no other obvious signs of sickness (i.e., retarded growth, reduced food or water consumption) was obvious in our experimental animals. After injection of LPS into the chamber, there was an increase of bioactive TNF in the lavage fluid that was effectively neutralized by treatment with TNF-bp. The neutralization of locally produced TNF had no influence on fever induced by the high LPS dose. After injection of the low LPS dose, we observed a faster defervescence under the influence of local treatment with TNF-bp. This means that the blockade of TNF affected the late rather than the early phase of the febrile response to the low LPS dose. Why was fever in response to the high LPS dose not affected in the same way? As already mentioned above, the spillover of a sufficiently high amount of IL-6 from the site of inflammation into the circulation seems to be a critical component in this fever model. In response to the low LPS dose, circulating levels of IL-6 were lower than in animals treated with LPS + NaCl C (low LPS dose): thermal effects of injections of 10 μg/kg LPS + Diclo (n = 6; □, Tb = 39.13 ± 0.11°C) or 100 μg/kg LPS + vehicle (n = 6; ●, Tb = 39.07 ± 0.16°C) into subcutaneous chambers. From 45 to 360 min after injection, ΔTb of guinea pigs treated with LPS + Diclo was significantly lower than in animals treated with LPS + NaCl C (low LPS dose): thermal effects of injections of 10 μg/kg LPS + Diclo (n = 5; □, Tb = 38.86 ± 0.12°C) or 10 μg/kg LPS + vehicle (n = 6; ●, Tb = 38.83 ± 0.21°C) into subcutaneous chambers. From 60 to 360 min after injection, ΔTb of guinea pigs treated with LPS + Diclo was significantly lower than in animals treated with LPS + vehicle. Symbols represent means, bars indicate SE; stars indicates a significant difference between both groups (P < 0.05).
might be of interest to consider cyclooxygenase products (i.e., prostaglandins), the role of which has not yet been evaluated in fever models of local subcutaneous inflammation. Administration of diclofenac, a non-selective cyclooxygenase inhibitor, into the subcutaneous chamber along with LPS resulted in a complete suppression of fever. This observation strongly suggests a participation of prostaglandins as humoral mediators for fever induction was ignored or rejected for quite a long time. It should, however, be noted that a more recent study showed that intravenous or intra-arterial administration of prostaglandins (17, 24), the idea of a role for circulating (peripheral) prostaglandins as humoral mediators for fever induction was ignored or rejected for quite a long time. Intravenous injection of PGE2 that is bound to albumin, in contrast to free PGE2, induces inflammatory response (13, 29) or the dose of type of inflammation, or whether diclofenac enters the circulation from the site of injection and even suppresses formation of prostaglandins within the brain. Because of the failure to induce fever by intravenous or intra-arterial administration of prostaglandins (17, 24), the idea of a role for circulating (peripheral) prostaglandins as humoral mediators for fever induction was ignored or rejected for quite a long time.

Alternatively, LPS-induced PGE2 might act as a pyrogenic signal locally at the site of the implanted chamber. In this context it seems to be of interest that activation of afferent nerve fibers might contribute to the manifestation of fever (10, 39) depending on the type of inflammatory response (13, 29) or the dose of the injected pyrogen (4, 28). In a previous study, we demonstrated that a part of the febrile response that is induced by injecting the low LPS dose (10 μg/kg) into the subcutaneous chamber can be abrogated by coadministration of a local anesthetic (30), a finding that supports the view that afferent nerve fibers might, indeed, participate in the generation of fever in this model. What kinds of cutaneous afferent nerve fibers are involved in temperature regulation and could thus be involved in the febrile resetting of body temperature? From the skin there is a predominant input of peripheral cold sensors. Peripheral cold sensors, stimulated by cold or menthol (34), can initiate direct cold defense reactions such as peripheral vasoconstriction and an increased heat production, responses that are also observed during a febrile rise of body temperature. Recently, the cold thermoregulation process has been characterized on the basis of recordings of ionic currents and calcium signals (25, 26, 38). Interestingly, one of the research groups who recently investigated the peripheral cold receptors at a cellular and molecular level provided evidence that peripheral cold-sensitive neurons are selectively activated by PGE2 (19). This finding might, in part, explain why administration of diclofenac into the subcutaneous chamber completely abolished LPS-induced fever. The suppressed formation of PGE2 could have resulted in a lack of activation of cold sensors in the inflamed skin area with the consequence that this part of the febrile message was abolished. At present, the possible participation of peripheral cold sensors, stimulated by PGE2, in the manifestation of fever in response to cutaneous tissue inflammation is still speculative. The investigation of such a novel mechanism is, however, an exciting perspective and clearly merits further studies on this topic.

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