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Rapid Report

Platelet-activating factor: a previously unrecognized mediator of fever

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Lipopolysaccharide (LPS)-induced systemic inflammation is accompanied by either hypothermia (prevails when the ambient temperature (T_a) is subneutral) or fever (prevails when T_a is neutral or higher). Because platelet-activating factor (PAF) is a proximal mediator of LPS inflammation, it should mediate both thermoregulatory responses to LPS. That PAF possesses hypothermic activity and mediates LPS-induced hypothermia is known. We asked whether PAF possesses pyrogenic activity (Expt 1) and mediates LPS fever (Expt 2). The study was conducted in Long-Evans rats implanted with jugular catheters. A complex with bovine serum albumin (BSA) was infused as a physiologically relevant form of PAF; free (aggregated) PAF was used as a control. In Expt 1, either form of PAF caused hypothermia when infused (83 pmol kg^{-1} min^{-1}, 60 min, I.V.) at a subneutral T_a of 20 °C, but the response to the PAF–BSA complex (−4.5 ± 0.5 °C, nadir) was ~4 times larger than that to free PAF. At a neutral T_a of 30 °C, both forms caused fever preceded by tail skin vasoconstriction, but the febrile response to PAF–BSA (1.0 ± 0.1 °C, peak) was > 2 times higher than that to free PAF. Both the hypothermic (at 20 °C) and febrile (at 30 °C) responses to PAF–BSA started when the total amount of PAF infused was extremely small, < 830 pmol kg^{-1}. In Expt 2 (conducted at 30 °C), the PAF receptor antagonist BN 52021 (29 μmol kg^{-1}, I.V.) had no thermal effect of itself. However, it strongly (~2 times) attenuated the febrile response to PAF (5 nmol kg^{-1}, I.V.), implying that this response involves the PAF receptor and is not due to a detergent-like effect of PAF on cell membranes. BN 52021 (but not its vehicle) was similarly effective in attenuating LPS (10 μg kg^{-1}, I.V.) fever. It is concluded that PAF is a highly potent endogenous pyrogenic substance and a mediator of LPS fever.

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Earlier studies proposed that histamine release from sensitized leukocytes involved a soluble mediator, which was named platelet-activating factor (PAF) and subsequently identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (for review, see Benveniste, 1990). It was soon discovered that this phospholipid is a key player in the pathogenesis of thrombosis, acute inflammation, anaphylaxis, asthma, transplant rejection, and many other diseases and conditions (Braquet *et al.* 1987). It was also found that PAF exerts many of its actions at concentrations as low as picomolar (Montrucchio *et al.* 2000), and that PAF is the most potent endogenous agent capable of inducing circulatory shock (Feuerstein & Hallenbeck, 1987). After Bessin *et al.* (1983) noticed multiple similarities between PAF-induced shock and that occurring in response to bacterial lipopolysaccharide (LPS, endotoxin), data were rapidly accumulated showing that PAF is a mediator of experimental LPS-induced shock as well as of the related clinical conditions, viz. systemic inflammatory response syndrome (SIRS), sepsis and septic shock (for reviews, see Braquet *et al.* 1987; Feuerstein & Hallenbeck, 1987; Ayala & Chaudry, 1996; Rabinovici *et al.* 1999; Montrucchio *et al.* 2000).

In brief, PAF synthesis is activated by LPS and inflammatory cytokines [such as tumour necrosis factor (TNF)-α and interleukin (IL)-1β] both *in vitro* and *in vivo*; it is also activated in SIRS (Siren *et al.* 1992; Qu *et al.* 1998; Han *et al.* 2002). Furthermore, systemic administration of PAF to experimental animals reproduces most symptoms of LPS- and TNF-α-induced shock (Bessin *et al.* 1983; Terashita *et al.* 1985; Huang *et al.* 1994). Finally, transgenic mice overexpressing the PAF receptor respond to LPS with increased mortality (Ishii *et al.* 1997), whereas a variety of chemically unrelated PAF receptor antagonists attenuate, completely block, or reverse many symptoms and outcomes of experimental systemic inflammation, including arterial

hypotension, pulmonary oedema, reduced cardiac output, hypoxia and mortality (Terashita *et al.* 1985; Giral *et al.* 1996; Beyer *et al.* 1998). Because PAF mediates such a wide range of responses to LPS and symptoms of sepsis, it is considered a proximal mediator of the inflammatory cascade (Kuijpers *et al.* 1994). The proximal position of PAF agrees with its ability to activate nuclear factor (NF)-κB, a transcription factor crucial for the production of several inflammatory cytokines, including IL-1β, IL-6, and TNF-α (Han *et al.* 1999, 2002).

Among other vital functions, thermoregulation is affected in systemic inflammation, and a deflection of body temperature from its normal level (fever or hypothermia) is listed as an obligatory criterion in all clinical definitions of SIRS, sepsis and septic shock. In SIRS, hypothermia occurs only in the most severe cases and is 10 times less common than fever (Clemmer *et al.* 1992). Similar to clinical systemic inflammation, administration of LPS to laboratory animals causes either fever or hypothermia. The hypothermia occurs in most severe cases (larger doses of LPS) and at an ambient temperature (T_a) below thermoneutrality (Romanovsky *et al.* 1998; Romanovsky, 2000). As a proximal mediator of septic and LPS-induced shock (Kuijpers *et al.* 1994), PAF should mediate both thermoregulatory symptoms of systemic inflammation: fever and hypothermia.

That PAF is involved in the hypothermic response has been established. It has been shown that CV-3988, a PAF receptor antagonist, attenuates LPS-induced hypothermia in rats (Ephgrave *et al.* 1997), whereas α_1-antitrypsin, a non-specific inhibitor of PAF release, prevents LPS hypothermia in mice (Libert *et al.* 1996). It has also been shown that rats respond with hypothermia to systemic administration of PAF (Ephgrave *et al.* 1997; Million *et al.* 1997). In contrast to its hypothermic activity, the pyrogenic activity of peripherally administered PAF has never been observed. However, both the studies that demonstrated the hypothermic effect of PAF in rats, viz. by Ephgrave *et al.* (1997) and Million *et al.* (1997), were conducted at an unspecified T_a ('room temperature'), which is substantially below the thermoneutral zone (29–32 °C; see Romanovsky *et al.* 2002) for this species. In the case of LPS, a subneutral T_a creates optimal conditions for the development of hypothermia (Romanovsky *et al.* 1998; Romanovsky, 2000), whereas LPS is highly pyrogenic when administered under thermoneutral conditions. We hypothesized that not only LPS-induced hypothermia, but also LPS fever, is mediated by PAF. We further conjectured that the proposed involvement of PAF in both thermoregulatory responses reflects its dual action on thermoregulation: in addition to causing hypothermia in a cool environment, PAF may also cause fever at thermoneutrality. This hypothesis was tested in the present study.

To conduct this study, the following methodological problem had to be solved. As an amphipathic substance, PAF readily self-aggregates in aqueous solutions (Kramp *et al.* 1984). In the aggregated state, many biological activities of amphipaths are drastically reduced (Heirwegh, 1984). The very low (micromolar) critical concentration of micelle formation for PAF (Kramp *et al.* 1984) constitutes an obstacle for preparing aqueous solutions in which PAF remains in a non-aggregated, monomeric form. According to Benveniste (1990), a similar obstacle was faced in the process of the discovery of PAF and overcome by using bovine serum albumin (BSA) as a carrier. Albumin binds PAF with a high affinity (K_d = 100 nM; Clay *et al.* 1990). Because in the plasma more then 80 % of PAF is bound to albumin (Kulikov & Bergel'son, 1984), the PAF–albumin complex is the most physiologically relevant form of PAF. In the present study, both the BSA-bound (monomeric) and free (aggregated) forms of PAF were used.

METHODS

Animals

Eighty-two male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 280–320 g at the time of the experiments were used. The animals were housed individually in a rack equipped with a Smart Bio-Pack ventilation system (model SB4100) and Thermo-Pak temperature control system (model TP2000; Allentown Caging Equipment, Allentown, NJ, USA); the temperature of the incoming air was maintained at 28 ± 1 °C. Standard rat chow (Harlan Teklad, Madison, WI, USA) and tap water were available *ad libitum*. The room was on a 12:12 h light–dark cycle (lights on at 7.00 h). The cage space was enriched with artificial 'rat holes' (cylindrical confiners made of stainless steel wire). In addition to spending time in the confiners voluntarily, the rats were systematically habituated to them (10 training sessions). The same confiners were used later in the experiments. The protocols were approved by the Legacy Health System (Portland, OR, USA) and St Joseph's Hospital (Phoenix, AZ, USA) Institutional Animal Care and Use Committees.

Surgery

Under ketamine–xylazine–acepromazine (55.6, 5.5, and 1.1 mg kg^{-1}, I.P., respectively) anaesthesia and antibiotic (enrofloxacin, 12 mg kg^{-1}, s.c.) protection, each rat was subjected to chronic catheterization of the jugular vein. A silicone catheter (i.d. 0.5 mm, o.d. 0.9 mm) filled with heparinized (50 U ml^{-1}) pyrogen-free saline was passed into the superior vena cava through the jugular vein and its free end was exteriorized at the nape. On day 1 after surgery, the catheter was flushed with heparinized saline.

Instrumentation

The experiments were performed on day 3. Each rat was placed in a confiner and equipped with two copper–constantan thermocouples, one for recording colonic temperature (T_c) and the other for recording tail skin temperature (T_{sk}). The colonic thermocouple was inserted 10 cm beyond the anal sphincter. The skin thermocouple was positioned on the lateral surface of the tail at the boundary of its middle and distal thirds. The thermocouples were plugged to a data logger (model AI-24; Dianachart, Rockaway, NJ, USA), which was connected to a personal computer. The rat

was transferred to a climatic chamber (Forma Scientific, Marietta, OH, USA). The climatic chamber was equipped with thermocouples for continuous measurement of T_a. The jugular catheter was extended with a 114-cm length of polyethylene-50 tubing (Becton Dickinson, Sparks, MD, USA) filled with saline. The extension was passed through a wall port and connected to a syringe filled with the drug of interest. The syringe was positioned in a pump (Stoelting, Wood Dale, IL, USA) outside the chamber, thus permitting the drug to be infused I.V. without disturbing the rat. After a 1-h stabilization period, T_c, T_{sk}, and T_a were recorded every 2 min for $\geqslant$ 1 h before and $\geqslant$ 4 h after the administration of the drug of interest. All experiments were begun at 9.00 h.

Drugs

PAF. Unless specified otherwise, all drugs and reagents were purchased from Sigma-Aldrich. To prepare a PAF–BSA complex (1:1 molar ratio), solutions of BSA and PAF were used. BSA (essentially fatty acid- and globulin-free) was dissolved in sterile 0.15 M phosphate-buffered saline (pH = 7.2) to a concentration of either 670 μg ml^{-1} (10 μM) or 2.7 mg ml^{-1} (40 μM) and stored at −20 °C. PAF (β-acetyl-γ-*O*-alkyl-L-α-phosphatidylcholine, from bovine heart lecithin) was dissolved extemporaneously in ethanol to a concentration of 550 μg ml^{-1} (1 mM) or 2.2 mg ml^{-1} (4 mM). This solution was diluted (1:100) by either the 10 μM or the 40 μM BSA solution to a final PAF concentration of either 5.5 μg ml^{-1} (10 μM) or 22 μg ml^{-1} (40 μM), respectively. It was then sonicated for 3 min and incubated at 37 °C for 1 h. A solution of free PAF (used as a control) was prepared identically, except that phosphate-buffered saline was used instead of BSA solution. Successful formation of the PAF–BSA complex was verified by establishing the ability of PAF to co-migrate with BSA in a polyacrylamide gel. For this, trace amounts of [H^3]-PAF (60 Ci mmol^{-1}; NEN Life Science Products, Boston, MA, USA) were added to the solutions of both free PAF and the PAF–BSA complex before incubation. Following incubation, both preparations were subjected to native polyacrylamide gel electrophoresis. The gel was either stained with Coomassie brilliant blue to visualize the migrated BSA or dried and exposed to Hyperfilm-MP X-ray film (Amersham Pharmacia Biotech, Piscataway, NJ, USA) at −70 °C for 1 week to visualize the migrated PAF. Free PAF did not move in the gel, whereas PAF incubated with BSA co-migrated with the protein.

BN 52021. A working solution of the PAF receptor antagonist BN 52021 (Biomol, Plymouth, PA, USA) was prepared extemporaneously by first dissolving the drug in ethanol to a concentration of 5 mg ml^{-1} and then diluting the ethanol solution with a 3 mg ml^{-1} BSA solution, alkalinizing it with 2 M sodium hydroxide to a pH of 8.0, and sonicating it for 5 min. The final concentration of BN 52021 in the working solution was 1 mg ml^{-1} (2.4 mM). A vehicle (control for BN 52021) was prepared identically, except that no BN 52021 was added.

LPS. A stock suspension of *Escherichia coli* 0111:B4 LPS (1 mg ml^{-1}) in saline was stored at −20 °C. A working suspension of LPS was prepared extemporaneously by diluting the stock with saline to a final concentration of 10 μg ml^{-1}.

Administration of PAF: a special consideration

Because lipids readily bind to plastic and glass surfaces, the doses of PAF–BSA and free PAF used had to be adjusted based on the loss of PAF during the incubation and infusion procedures. To adjust the doses, equally concentrated (40 μM) solutions of the 1:1 PAF–BSA complex and of free PAF, both containing trace amounts of [H^3]-PAF, were prepared as described above. The solutions were sampled (0.3 ml) immediately before incubation at 37 °C and immediately after perfusion through a 114 cm length of polyethylene tubing (perfusion imitated the I.V. infusion to a rat; see Instrumentation). Each sample was diluted with 5 ml of Sigma-Fluor liquid scintillation cocktail, sonicated for 30 s, and transferred to a Beckman LS 1701 Liquid Scintillation Counter (Beckman Instruments, Irvine, CA, USA) for radioactivity measurement. This experiment was repeated three times. The recovery of PAF from the free-PAF solution following incubation and perfusion through polyethylene tubing was only 14 ± 2 %, whereas the recovery of PAF from the PAF–BSA solution was 56 ± 1 %. Hence, the dose of free PAF had to be 4.0 times higher than the dose of the PAF–BSA complex in order to deliver the same amount of PAF to the animal. This ratio was used in the present study.

Experimental protocols

Experiment 1: thermal effects of PAF in cool and thermoneutral environments. The rats were instrumented as described above and transferred to an environmental chamber set to a T_a of either 20.0 °C (a mild cold exposure) or 30.0 °C (thermal neutrality; see Romanovsky *et al.* 2002). Because it was important to determine whether the thermoregulatory responses to PAF at different T_a values involve changing the vasomotor tone of the tail skin vasculature, T_{sk} was measured. After a 2-h adaptation, the rats were infused I.V. either with saline (45 μl kg^{-1} min^{-1}, 20 min) followed by PAF–BSA (10 μM, 15 μl kg^{-1} min^{-1}, 60 min) or with BSA (10 μM, 45 μl kg^{-1} min^{-1}, 20 min) followed by free PAF (40 μM, 15 μl kg^{-1} min^{-1}, 60 min). Hence, one group was infused with 9 nmol kg^{-1} of BSA-bound PAF, whereas the other was infused with 36 nmol kg^{-1} of free PAF, so that the rats of both groups actually received 5 nmol kg^{-1} (56 % of bound PAF, or 14 % of free PAF; see above) at a rate 83 pmol kg^{-1} min^{-1}. Both groups were infused with the same amount of BSA (9 nmol kg^{-1}) and received the same total amount of fluid (1.8 ml kg^{-1}) over 80 min. In preliminary experiments, this dose of the essentially fatty acid-free preparation of BSA used was found to be ~100 times lower than its minimal pyrogenic dose.

Experiment 2: thermal effects of a PAF antagonist in a neutral environment. PAF may act via two different mechanisms, viz. by signalling through a receptor on the cell surface (Rabinovici *et al.* 1999) and via a non-specific, detergent-like action on cell membranes (Bratton *et al.* 1988). Experiment 2 was performed to determine whether the thermoregulatory action of PAF occurs via the PAF receptor, and whether this receptor is involved in normal thermoregulation and LPS fever. The rats were instrumented as for Expt 1, except that T_a was always set to 30.0 °C, and T_{sk} was not measured. First, the thermal action of the PAF antagonist BN 52021 alone was studied. The rats were infused I.V. (66 μl kg^{-1} min^{-1}, 180 min) with either BN 52021 (29 μmol kg^{-1}) or its vehicle. Second, the effect of BN 52021 on the thermal effect of PAF was studied. Following a pretreatment with I.V. BN 52021 or its vehicle, the rats received I.V. PAF–BSA (5 nmol kg^{-1} over 60 min). Third, the involvement of the PAF receptor in LPS fever was studied. Following a pretreatment with BN 52021 or its vehicle, the rats were injected I.V. with LPS (10 μg kg^{-1}) in saline (1 ml kg^{-1}).

Data processing and analysis

The change in T_c (from its value at time 0) was used to evaluate deep body temperature responses. The heat loss index (HLI) was used to evaluate thermoeffector responses of tail skin vasculature. As justified elsewhere (Romanovsky *et al.* 2002), the HLI was

calculated according to the formula:

$$\mathrm{HLI} = (T_{sk} - T_a) \times (T_c - T_a)^{-1}.$$

The theoretical limits of the HLI are 0 (maximal skin vasoconstriction) and 1 (maximal vasodilation). In practice, however, the upper limit depends on the position of the tail skin thermocouple and can be as low as 0.3. The T_c and HLI responses were compared across treatments and time points by a two-way ANOVA for repeated measures using Statistica AX'99 (StatSoft, Tulsa, OK, USA). The data are reported as means ± S.E.M.

RESULTS

Experiment 1: thermal effects of PAF in cool and thermoneutral environments

At a subneutral T_a, the I.V. infusion of either free PAF or PAF–BSA at a rate of 83 pmol kg^{-1} min^{-1} caused marked hypothermia (Fig. 1*A*). In the case of free PAF, the response started between 40 and 50 min after the onset of the infusion and reached the nadir at 80 min, at which time the mean decrease in T_c was 1.2 ± 0.2 °C. In the case of PAF–BSA, the response was significantly greater ($P < 0.001$). It started with a latency of < 10 min (i.e. when the total amount of PAF infused was < 830 pmol kg^{-1}, or < 250 pmol per rat) and reached the nadir at 90 min, at which time the mean decrease in T_c was 4.5 ± 0.5 °C. Neither the hypothermic response to free PAF nor the response to BSA-bound PAF was accompanied by tail skin vasodilatation: at the T_a used (20 °C), the HLI remained at 0 level throughout the experiment, indicating maximal vasoconstriction.

Under thermoneutral conditions, I.V. infusion of free PAF at a rate of 83 pmol kg^{-1} min^{-1} resulted in a small (~0.5 °C), slow rise in T_c accompanied by transient tail skin vasoconstriction (Fig. 1*B*). The same dose of PAF–BSA caused a vivid, rapidly occurring fever-like response with a latency < 10 min and a peak of 1.0 ± 0.1 °C at 70 min after the onset of the infusion. The response was accompanied by maximal (HLI = 0) tail skin vasoconstriction that lasted throughout the duration of infusion. Both the T_c and HLI responses differed significantly between the treatments ($P < 0.001$ and $P < 0.003$, respectively).

Experiment 2: thermal effects of a PAF receptor antagonist in a thermoneutral environment

At thermoneutrality, neither the I.V. infusion of the PAF receptor antagonist BN 52021 at a dose of 29 μmol kg^{-1} nor the infusion of its vehicle caused any change in T_c (Fig. 2*A*). However, a pre-infusion of the same dose of the antagonist significantly attenuated the T_c rise caused by I.V. PAF–BSA (83 pmol kg^{-1} min^{-1}, 60 min): at 70 min after the onset of PAF–BSA infusion, T_c of the vehicle-pretreated rats increased by 1.8 ± 0.2 °C, whereas T_c of the BN 52021-pretreated rats rose by only 0.8 ± 0.3 °C ($P < 0.009$; Fig. 2*B*). The pre-infusion of BN 52021 was also effective in attenuating the polyphasic febrile response of rats to a bolus I.V. injection of LPS (10 μg kg^{-1}) (Fig. 2*C*). In the vehicle pre-infused rats, the febrile response to LPS had a markedly expressed first phase with a peak at ~40 min after the injection (0.5 ± 0.2 °C), a poorly distinguishable second phase with a peak at ~160 min (0.9 ± 0.2 °C), and a robust third phase with a peak at ~340 min post LPS (1.3 ± 0.1 °C). The BN 52021-pretreated rats exhibited an attenuated response ($P < 0.024$); at the same time points corresponding to the febrile peaks in the controls (40, 160 and 340 min), T_c increased by 0.2 ± 0.1, 0.4 ± 0.1 and 0.8 ± 0.2 °C, respectively.

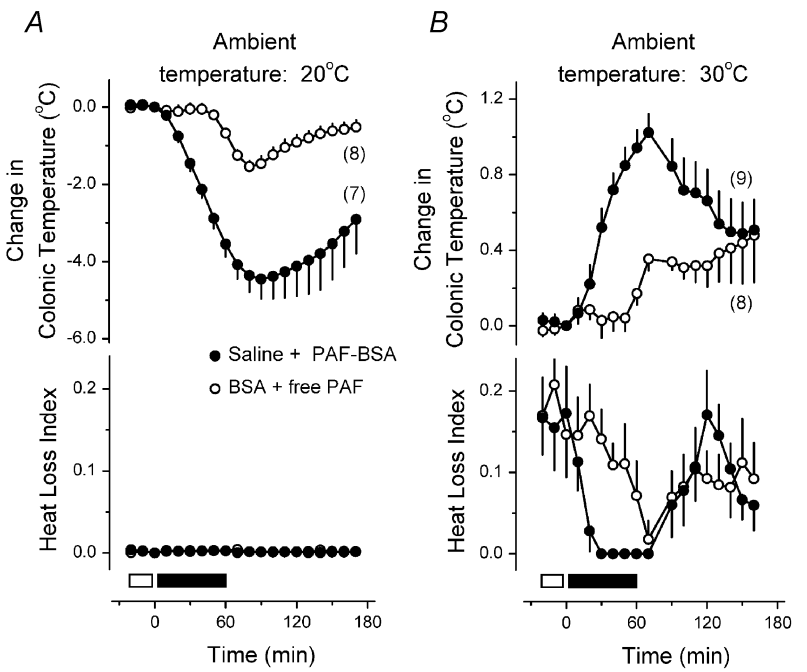

Figure 1. The thermal effects of PAF in rats at different ambient temperatures

The rats were exposed to mild cold (*A*) or thermoneutrality (*B*). During the time period 0–60 min (filled bar along the abscissa axis), the rats were infused I.V. with either the physiologically relevant, monomeric PAF (the PAF–BSA complex; 83 pmol kg^{-1} min^{-1}) or the free (aggregated) form of PAF. This infusion of PAF–BSA or free PAF was preceded by the infusion of saline or BSA, respectively; this 'pre-infusion' was performed over the period −20 to 0 min (open bar along the abscissa axis). Each group received the same total amounts of PAF and BSA in the same total volume (for details, see Methods). At time 0, T_c of the PAF–BSA-infused rats was 38.0 ± 0.1 °C (at 20 °C) and 38.3 ± 0.1 °C (at 30 °C); T_c of the rats infused with free PAF was 38.0 ± 0.2 °C (at 20 °C) and 38.1 ± 0.1 °C (at 30 °C). The number of animals in each group is shown in parentheses.

DISCUSSION

Confirming published data (Ephgrave *et al.* 1997; Million *et al.* 1997), this study shows that systemic administration of PAF to rats in a cool environment causes a hypothermic response. The present study also reports new findings, i.e. that I.V. PAF causes a fever (a rise in deep body temperature occurring due to, at least partly, tail skin vasoconstriction) and that this fever is mediated by the PAF receptor. There may be several reasons why the pyrogenic action of PAF has not been observed previously. First, the present study was conducted at a neutral T_a, whereas most pharmacological studies in small rodents are conducted at 'room temperature', equivalent to a mild to moderate cold exposure (Romanovsky *et al.* 2002). At thermoneutrality, the major effector mechanism of fever is skin vasoconstriction (a low-threshold and low-energetic-cost effector); in the cold, fever is brought about largely by thermogenesis (a high-threshold, high-cost effector) (Székely & Szelényi, 1979). Not only does fever occur more readily and cost less energetically at thermoneutrality, but T_a also modifies the thermoregulatory response to LPS (and possibly to other pyrogens) in such a way that the hypothermic response prevails under subthermoneutral conditions, especially at higher doses (Romanovsky *et al.* 1998; Romanovsky, 2000). Speculatively, T_a may affect the thermoregulatory response to LPS or PAF by affecting the distribution of blood flow (directing more flow to the skin at high T_a values and to the viscera at low T_a values) and, this way, preferentially delivering inflammatory mediators to different tissues.

An insight into the dualism of the thermoregulatory action of PAF can be gained by unwrapping the PAF receptor's intracellular signalling pathways. PAF signals through a canonical G protein-coupled receptor on the cell surface (Ayala & Chaudry, 1996; Prescott *et al.* 2000). Crucial events following the receptor activation are the increase in the intracellular concentration of Ca^{2+} and the consequent stimulation of the Ca^{2+}-dependent cytosolic phospholipase (PL) A_2 (Syrbu *et al.* 1999). The activation of cytosolic PLA_2 by PAF results in an accumulation of arachidonic acid, which is converted by cyclooxygenase (COX)-2, lipoxygenase and terminal synthases into several eicosanoids. Whereas some of these eicosanoids (e.g. PGE_2 and $PGF_2\alpha$) are highly pyrogenic (Milton, 1998), others (e.g. PGD_2 (Ueno *et al.* 1982) and leukotrienes C_4 and D_4 (Brus *et al.* 1986)) can cause hypothermia. The mechanisms determining preferential synthesis of the pyrogenic prostanoids at a neutral T_a and of hypothermic eicosanoids at a subneutral T_a have yet to be identified. At different T_a values, PAF may be preferentially delivered with blood flow to different target cells, in which proximal enzymes of eicosanoid synthesis are coupled with different terminal synthases (for further discussion, see Ivanov *et al.* 2002).

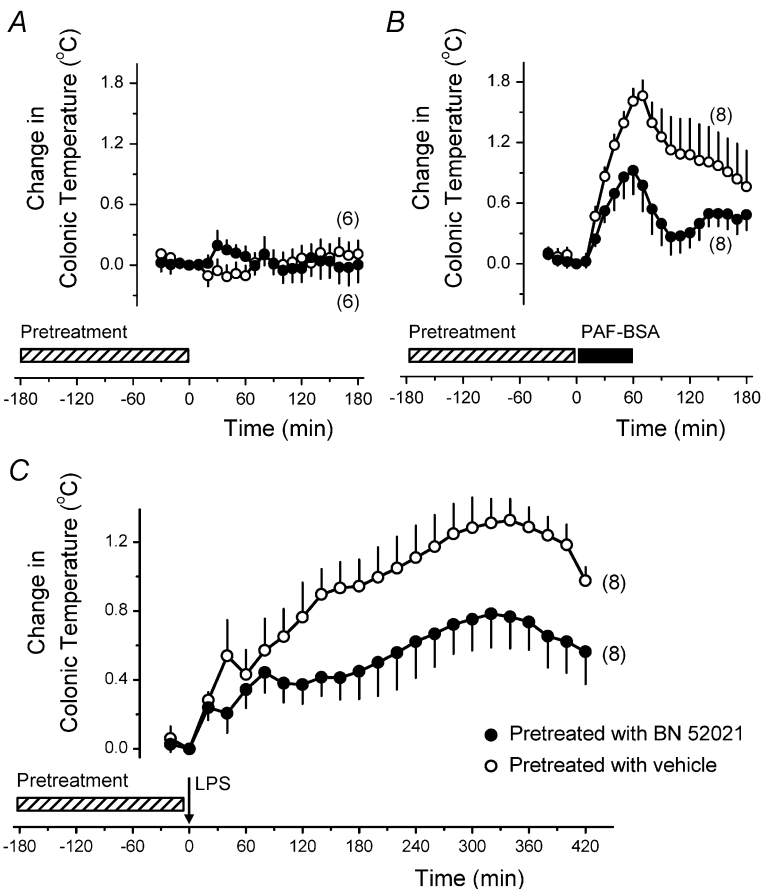

Figure 2. The thermal effects of the PAF receptor antagonist BN 52021

The effects of pretreatment (an I.V. infusion, for the period shown by the hatched bar along the abscissa axis) with BN 52021 (29 μmol kg^{-1}) or its vehicle on the following responses: *A*, the spontaneous dynamic of body temperature; *B*, the pyrogenic effect of I.V. PAF–BSA (83 pmol kg^{-1} min^{-1}, 60 min; filled bar); *C*, the febrile response to I.V. LPS (10 μg kg^{-1}, bolus; arrow). At time 0, the rats had the following T_c values: 37.6 ± 0.1 °C (pretreatment with BN 52021 only); 37.5 ± 0.1 °C (pretreatment with vehicle only); 37.7 ± 0.2 °C (BN 52021 + PAF–BSA); 37.9 ± 0.2 °C (vehicle + PAF–BSA); 38.2 ± 0.1 °C (BN 52021 + LPS); 38.1 ± 0.2 °C (vehicle + LPS).

An important trait of the present study is that very low doses of PAF were employed. Both the hypothermic and febrile responses were registered when PAF was infused I.V. at a rate of 83 pmol kg^{-1} min^{-1}. Both responses started when the total amount of PAF infused was < 830 pmol kg^{-1} (< 250 pmol per rat). Not only does this finding confirm that PAF is a potent hypothermia-inducing agent, but it also makes PAF one of the most potent mediators of fever. Indeed, the I.V. doses of the most accepted downstream mediator of fever, prostaglandin (PG) E_2, required to induce fever in rats and rabbits are ⩾ 140–200 nmol kg^{-1} (Eguchi *et al.* 1988; Romanovsky *et al.* 1999), i.e. at least 170 times higher that the pyrogenic dose of PAF in the present study. Of note is that the pyrogenic dose of PAF used in this study is not simply very low, but also physiologically relevant. The serum concentration of PAF in LPS-treated rats peaks between 5 and 25 nM (Doebber *et al.* 1985; Chang *et al.* 1987; Sun *et al.* 1990). In the present study, the plasma concentration of PAF after its I.V. administration was estimated to peak at 0.6–1.8 nM, provided PAF was evenly distributed in the extracellular compartment (20 % of the body mass), and its half-life was 1–3 min (Ayala & Chaudry, 1996).

The use of such low doses of PAF was possible because it was delivered as a preformed complex with BSA. Indeed, the BSA-bound form of PAF was much more effective than its free, aggregated form (Fig. 1*A* and *B*). The ability of albumin to potentiate *in vivo* effects of PAF (including the formation of oedema in human and guinea-pig skin (Archer *et al.* 1985) and vasodilatation in the rat kidney (Gerkens, 1990)) and of other amphipaths (including the pyrogenic action of PGE_2 (Romanovsky *et al.* 1999)) is well known. In addition to preventing aggregation, albumin is likely to protect PAF from degradation (Ammit & O'Neill, 1997). In the circulation, PAF is inactivated by the extracellular form of its acetylhydrolase, which is associated with blood lipoproteins (Prescott *et al.* 2000). When lipid aggregates are administered I.V., they can be expected to rapidly merge with the blood lipoproteins (Kulikov & Bergel'son, 1984). When free PAF is administered, this merging brings it within reach of PAF acetylhydrolase, whereas PAF bound to albumin does not merge with the lipoproteins and is poorly accessible to PAF acetylhydrolase.

By using BN 52021, a PAF antagonist, the present study shows that the PAF receptor mediates the entire course of the polyphasic LPS fever. This observation fits well with the data showing that the blood level of PAF in rats is increased by LPS in a polyphasic fashion, and that each upsurge in the concentration of PAF corresponds to a distinct wave of NF-κB activation (Han *et al.* 2002). The early phase of the PAF response (peaks at 15–30 min after LPS administration) is thought to be due to a direct action of LPS, whereas the late phase (peaks at ~6 h) is likely to be mediated by pyrogenic cytokines (IL-1β, IL-6, and TNF-α) produced by the first wave of NF-κB activation (Qu *et al.* 1998; Han *et al.* 2002). Both directly and through the NF-κB-dependent cytokines, PAF causes transcriptional and post-translational activation of PGE_2-synthesizing enzymes (including PLA_2 and COX-2) and, consequently, several waves of PGE_2 production (Thivierge & Rola-Pleszynski, 1995; Borman *et al.* 1998; Syrbu *et al.* 1999).

It is difficult to relate this complex cascade of febrigenic mediators to certain tissues, because all its components – from the PAF-synthesizing capacity and the PAF receptor to the PGE_2-synthesizing capacity – are diffusely distributed throughout the body. Although the early production of PAF in response to LPS is localized preferentially in the spleen (Qu *et al.* 1998), LPS also stimulates a massive release of PAF from the circulating polymorphonuclear leukocytes and peripheral tissue macrophages (Ayala & Cahudry, 1996; Rabinovici *et al.* 1999; Prescott *et al.* 2000). Several cell types in the brain tissue, including microglia (Jaranowska *et al.* 1995), are also capable of producing PAF, and the level of PAF in the cerebrospinal fluid is increased by I.V. LPS in rats (Siren *et al.* 1992). Finally, endothelial cells have been shown to readily produce PAF upon stimulation (Camussi *et al.* 1995; Prescott *et al.* 2000). Thus, PAF can be produced outside, inside, or at the blood–brain barrier.

In summary, the present study demonstrates a previously unrecognized physiological effect of PAF – its ability to cause fever when infused I.V. in rats in an albumin-bound form at a dose < 830 pmol kg^{-1}. This study is also a first demonstration of the fact that both the pyrogenic effect of PAF and LPS-induced fever occur via the PAF receptor (rather than being due to a non-specific, detergent-like effect on cell membranes). It is concluded that PAF is a highly potent endogenous pyrogenic substance and a major mediator of LPS fever. Hence, the processes of PAF synthesis, transport, action, and degradation constitute new leads in the search for antipyretic drugs. The numerous PAF receptor antagonists and other anti-PAF agents (e.g. recombinant PAF acetylhydrolases) that are already available and even used clinically should be tested for their effectiveness as antipyretics.

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