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Essential role for endothelin ET_B receptors in fever induced by LPS (*E. coli*) in rats

[1]Aline S.C. Fabricio, [1]Carlos A.A. Silva, [2]Giles A. Rae, [3]Pedro D'Orléans-Juste & [1,4]Glória E.P. Souza

[1]Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Universidade de São Paulo, 14040-903 - Ribeirão Preto, SP, Brazil; [2]Department of Pharmacology, Biological Sciences Center, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil and [3]Department of Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Canada

1 The influence of endothelin receptor antagonists on febrile responses to *E. coli* lipopolysaccharide (LPS), interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and endothelin-1 (ET-1) was assessed in conscious rats.

2 Intravenous (i.v.) LPS (5.0 μg kg^{-1}) markedly increased rectal temperature to a peak of 1.30°C over baseline at 2.5 h. Pretreatment with the mixed endothelin ET_A/ET_B receptor antagonist bosentan (10 mg kg^{-1}, i.v.) or the selective endothelin ET_B receptor antagonist BQ-788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-D-norleucine; 3 pmol, into a lateral cerebral ventricle–i.c.v.) reduced the peak response to LPS to 0.90 and 0.75°C, respectively. The selective endothelin ET_A receptor antagonist BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]; 3 pmol, i.c.v.) was ineffective.

3 Increases in temperature caused by IL-1β (180 fmol, i.c.v.), TNF-α (14.4 pmol, i.c.v.) or IL-1β (150 pmol kg^{-1}, i.v.) were unaffected by BQ-788 (3 pmol, i.c.v.).

4 Central injection of endothelin-1 (0.1 to 3 fmol, i.c.v.) caused slowly-developing and long-lasting increases in rectal temperature (starting 2 h after administration and peaking at 4–6 h between 0.90 and 1.15°C) which were not clearly dose-dependent. The response to endothelin-1 (1 fmol, i.c.v.) was prevented by BQ-788, but not by BQ-123 (each at 3 pmol, i.c.v.). Intraperitoneal pretreatment with the cyclo-oxygenase inhibitor indomethacin (2 mg kg^{-1}), which partially reduced LPS-induced fever, did not modify the hyperthermic response to endothelin-1 (3 fmol, i.c.v.).

5 Therefore, central endothelin(s) participates importantly in the development of LPS-induced fever, *via* activation of a prostanoid-independent endothelin ET_B receptor-mediated mechanism possibly not situated downstream from IL-1β or TNF-α in the fever cascade.

Keywords: Fever (rat); LPS; TNF-α; IL-1β; endothelins; ET_A and ET_B receptors; bosentan; BQ-123; BQ-788; indomethacin

Introduction

Fever, one of the most outstanding signs of the acute phase response to infection in mammals, is a multimediated process triggered by exogenous pyrogenic substances such as lipopolysaccharides (LPS) from Gram-negative bacteria. This defense reaction to aggression is mediated via the release of several endogenous pyrogens from host cells, among which are interleukin (IL)-1α, IL-1β, IL-6, IL-8, tumour necrosis factor-α (TNF-α), macrophage inflammatory protein-1 (MIP-1) α and β, and interferon-β and γ (Zampronio *et al*., 1994; Miñano *et al*., 1996; for review see Kluger, 1991). Many of these pyrogenic cytokines, as well as LPS, can stimulate the release of endothelin-1 from different cell types *in vitro* (Ehrenreich *et al*., 1990; Zeballos *et al*., 1991; Lamas *et al*., 1992; Marsden & Brenner, 1992; Corder *et al*., 1995). In addition, raised plasma endothelin-1 levels have been detected following injection of LPS, IL-1 or TNF-α in various animal species (Nambi *et al*., 1994; Vemulapalli *et al*., 1994; Klemm *et al*., 1995), and in humans with septicaemia, in which levels of the peptide are strongly correlated with plasmatic levels of TNF-α and illness severity (Pittet *et al*., 1991; Takakuwa *et al*., 1994a,b).

Endothelin-1, which causes its widespread actions via endothelin ET_A and ET_B receptors (for reviews see Masaki *et al*., 1994; Rae & Henriques, 1998; Webb & Meek, 1997) may well be an important inflammatory mediator. It enhances vascular permeability (Filep *et al*., 1995), activates leukocytes (Ishida *et al*., 1990), upregulates adhesion molecule expression on the endothelium (McCarron *et al*., 1993) and induces mast cell degranulation (Yamamura *et al*., 1994). The peptide also triggers production of the endogenous pyrogens IL-1β, IL-6, IL-8, TNF-α and MIP-1 in human cultured monocytes (Cunningham *et al*., 1993; Helset *et al*., 1993, 1994) and of IL-6 from rat and human endothelial cells (Xin *et al*., 1995; Stankova *et al*., 1996). Furthermore, it has been shown that endothelin-1 can cause fever when injected intravenously to rabbits, an effect which is dose-dependently inhibited by the cyclo-oxygenase blocker indomethacin (Koshi *et al*., 1992).

In view of such considerations, the present study aimed to investigate, by use of mixed and selective endothelin ET_A and ET_B receptor antagonists, if endothelins participate, alongside IL-1β and TNF-α, in the febrile response induced by LPS in conscious rats. In addition, we have assessed the effects of centrally-administered endothelin-1 on body temperature in this species and how these can be modified by treatment with antagonists of its receptors or a blocker of cyclo-oxygenase.

Methods

Animals

Experiments were conducted using male Wistar rats weighing 180–200 g, housed at 24±1°C under a 12:12 h light-dark

[4]Author for correspondence at: Lab Pharmacology, Fac. Ciências Farmacêuticas de Ribeirão Preto, USP, 14040-903, Ribeirão Preto, SP, Brazil.

cycle (lights on at 06.00 h) and which had free access to food chow and tap water.

Intracerebral cannula implantation

Animals were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, intraperitoneally) and a stainless steel guide cannula (0.7 mm OD, 10 mm long) was stereotaxically placed into the right lateral ventricle (Paxinos & Watson, 1986) and fixed to the skull with jeweller's screws embedded in dental acrylic cement. All animals were then promptly treated with oxytetracycline hydrochloride (400 mg kg^{-1}) and the experiments were performed 1 week later. After each experiment, the animal was anaesthetized as before, and the location of the cannula track was histologically verified. Animals which showed cannula misplacement or blockage upon injection, or displayed abnormal weight gain patterns were excluded from the study.

Temperature measurements

Rectal temperature was measured in conscious and unrestrained rats by gently inserting a small thermistor probe (model 402 coupled to a model 46 telethermometer, Yellow Springs Instruments, U.S.A.) 4 cm into the rectum, without removing them from their home cages, for 1 min every 30 min for up to 6 h. Experimental measurements were conducted at the thermoneutral zone for rats (Gordon, 1990) in a temperature-controlled room ($28 \pm 1^{\circ}C$), following adaptation of the animals to this environment for at least 1 h. After this period, baseline temperature was determined four times at 30 min intervals before any injections, and only animals displaying mean basal rectal temperatures between 36.8 and 37.4°C were selected for the study. To minimize core temperature changes due to handling, animals were habituated to this environment and procedure twice on the preceding day.

Experimental protocols

In a first set of experiments, rats were treated intravenously (i.v., into a tail vein) with either the mixed endothelin ET_A/ET_B receptor antagonist bosentan (Clozel *et al*., 1994; 10 mg kg^{-1}, dissolved in sterile double-distilled water) or saline (1 ml kg^{-1}, control) 15 min prior to injection of *E. coli* LPS (5 μg kg^{-1}, i.v.), IL-1β (150 pmol kg^{-1}, i.v.) or sterile saline (1 ml kg^{-1}, i.v., control). Other animals received intracerebroventricular (i.c.v., 3 μl over 1 min) injection of either BQ-123 (selective endothelin ET_A receptor antagonist; Ihara *et al*., 1992; 3 or 30 pmol), BQ-788 (selective endothelin ET_B receptor antagonist; Ishikawa *et al*., 1994; 3 or 9 pmol) or vehicle (artificial cerebro-spinal fluid, aCSF; composition mmol L^{-1} NaCl 138.6, KCl 3.35, $CaCl_2$ 1.26, $NaHCO_3$ 11.9) 15 min prior to injection of LPS (5 μg kg^{-1}, i.v.), endothelin-1 (0.1 to 3 fmol, i.c.v.), IL-1β (180 fmol, i.c.v.; or 150 pmol kg^{-1}, i.v.) or TNF-α (14.4 pmol, i.c.v.). In these later experiments, control animals were similarly treated with the vehicles (saline i.v. or aCSF i.c.v., as appropriate). In a final set of experiments, rats were treated with indomethacin (a non-steroidal cyclo-oxygenase inhibitor, Vane, 1971; 2 mg kg^{-1}) or vehicle (tris[hydroxymethyl]aminomethane. HCl, pH 8.2) 30 min before injecting either LPS (5 μg kg^{-1}, i.v.), endothelin-1 (3 fmol, i.c.v.) or sterile saline. Pyrogenic stimuli were always injected between 10.00 and 11.00 h.

Drugs

The following drugs were employed: LPS from *E. coli* 0111 : B4 (Sigma Chem Co., St. Louis, U.S.A.), endothelin-1 (Peptide Institute, Inc., Osaka, Japan), BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]; American Peptide Co., U.S.A.), BQ-788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-D-norleucine; Research Biochemicals International, Natick, U.S.A.), bosentan (kindly provided by Dr M. Clozel, Hoffmann La-Roche, Basel, Switzerland), indomethacin (a gift from Merck, Sharp & Dohme, São Paulo, Brazil), murine IL-1β (lot No. BN024121, R&D Systems, Inc., Minneapolis, U.S.A.), rat TNF-α (Lot No. 050297 - III, kindly provided by Dr S. Poole, NIBSC, Hertfordshire, U.K.), oxytetracycline hydrochloride (Terramicina®, Pfizer, São Paulo, Brazil).

Statistical analysis

All variations in rectal temperature were expressed as changes from the mean basal value (i.e. ΔT in °C). All values are presented as means ± s.e.mean. Statistical comparisons were performed by means of one-way ANOVA analysis followed by Tukey's test by use of SPSS statistical software. Significance was set at $P < 0.05$.

Results

Fever induced by LPS

Control animals treated only with the vehicles showed no significant variations in rectal temperature over baseline values up to 6 h after administration. In sharp contrast, those given LPS (5 μg kg^{-1}, i.v.) displayed an increase in rectal temperature which reached significance at 1.5 h, peaked at 2.5 h and remained elevated for the remaining of the observation period. Prior injection of the mixed endothelin ET_A/ET_B receptor antagonist bosentan (10 mg kg^{-1}, i.v.) failed to modify basal temperature values significantly in saline-treated animals, but reduced the development of LPS-induced fever at all time points beyond 2.0 h, by about 30–50%. These results are depicted in Figure 1.

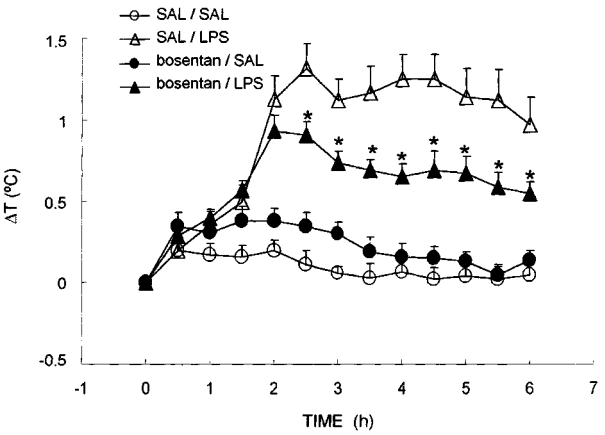

Figure 1 Effect of mixed endothelin ET_A/ET_B receptor antagonist bosentan on fever induced by LPS in rats. Bosentan (10 mg kg^{-1}) or saline (SAL, 1 ml kg^{-1}) were given i.v., 15 min before the i.v. injection of LPS (5 μg kg^{-1}) or SAL. Values represent the means ± s.e.mean of the changes in rectal temperature (ΔT) of 8–12 animals. *$P < 0.05$ when compared to corresponding value of vehicle-pretreated LPS-treated group. Basal rectal temperatures before treatment ranged between 37.0 and 37.3°C.

Prior i.c.v. treatment with the selective peptidic endothelin ET_A receptor antagonist BQ-123 (3 pmol) did not change significantly the basal temperature of saline-treated rats or the fever response to LPS (Figure 2a). Likewise, treatment with a tenfold higher dose of BQ-123 (30 pmol, i.c.v.) also failed to affect the responsiveness of either control or LPS-injected rats ($n=4$; results not shown). However, identical i.c.v. treatment with 3 pmol of BQ-788, a selective peptidic endothelin ET_B receptor antagonist, inhibited the LPS-induced fever response in a similar fashion to that seen following i.v. bosentan, without altering *per se* the baseline temperature of control animals (Figure 2b). No additional inhibition of LPS-induced fever was observed by treating the rats with a higher dose of BQ-788 (9 pmol, i.c.v.; $n=5$; results not shown).

Fever induced by IL-1β or TNF-α

Intravenous injection of IL-1β (150 pmol kg^{-1}, Figure 3a) clearly induced significant fever, which was not altered by prior treatment with bosentan (10 mg kg^{-1}, i.v.) or with BQ-788 (3 pmol, i.c.v.). Moreover, as shown in Figure 3b, i.c.v. treatment with BQ-788 did not change fever induced by i.c.v. injection of IL-1β (180 fmol) or TNF-α (14.4 pmol).

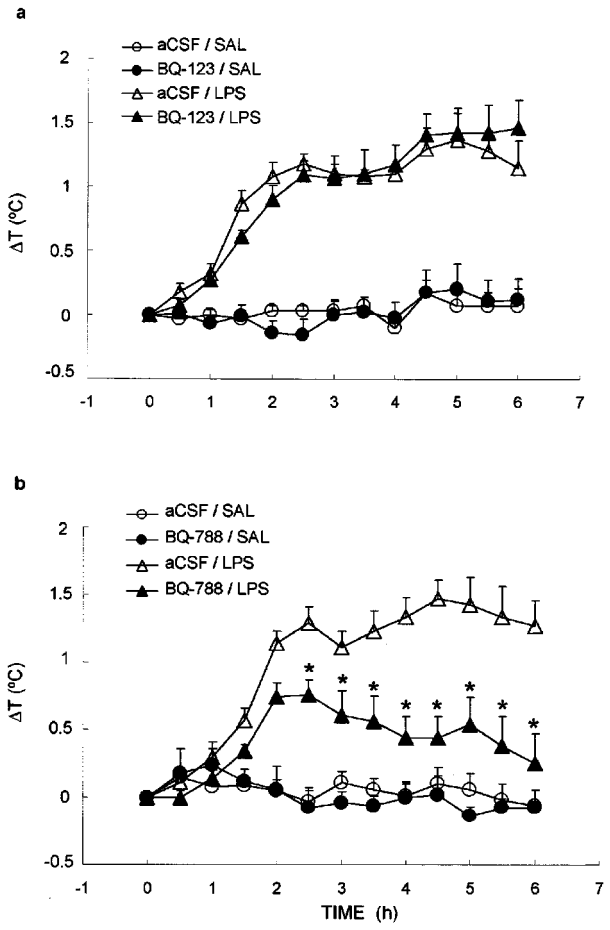

Figure 2 Effect of central administration of BQ-123 and BQ-788, selective antagonists of endothelin ET_A and ET_B receptors respectively, on fever induced by LPS in rats. BQ-123 (a), BQ-788 (b), each at 3 pmol, or artificial cerebro-spinal fluid (aCSF, 3 μl) were administered i.c.v., 15 min before i.v. injection of saline (SAL, 1 ml kg^{-1}) or LPS (5 μg kg^{-1}). Values represent the means$\pm$s.e.mean of the changes in rectal temperature (ΔT) of six to ten animals. *$P<0.05$ when compared to corresponding value of aCSF-pretreated LPS-treated group. Basal rectal temperatures before treatment ranged between 37.0 and 37.3°C.

Temperature changes induced by endothelin-1

Central injection of endothelin-1 induced a slowly-developing and long-lasting increase in rectal temperature, which began 2 h after administration, peaking at 4–6 h between 0.9 and 1.15°C. However, this effect, which was seen with a dose as low as 0.1 fmol of the peptide, was not clearly dose-dependent as doses in excess of 0.3 fmol (up to 3 fmol) did not cause further increases in temperature (Figure 4).

Prior i.c.v. treatment with BQ-123 (3 pmol) did not modify significantly the basal temperature of aCSF-treated rats or the raise in body temperature induced by 1 fmol of endothelin-1 (Figure 5a), but in rats treated with BQ-788 the response to this dose of endothelin-1 was fully abolished (Figure 5b). No overt behavioural changes were observed in rats receiving i.c.v.

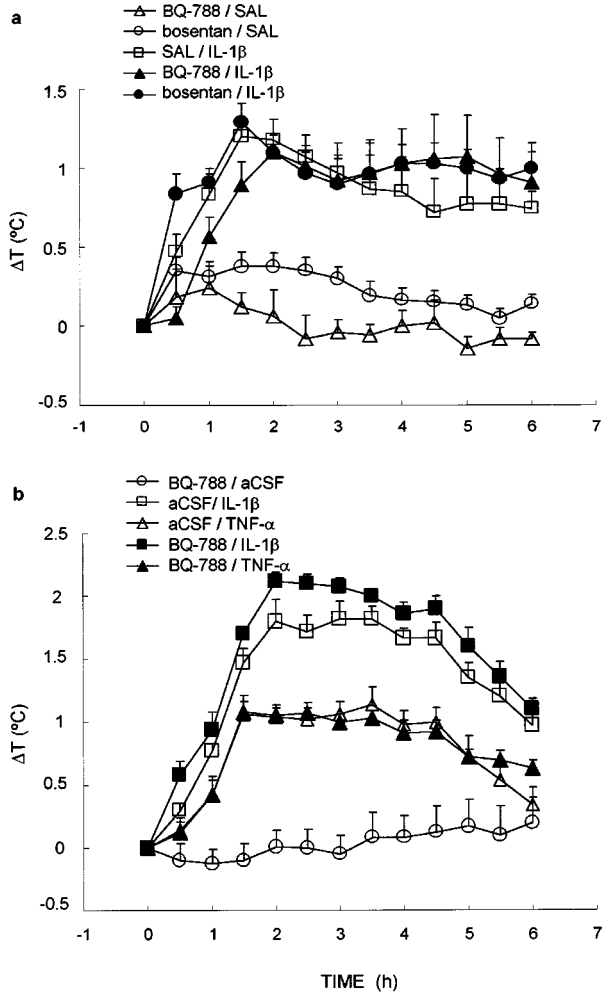

Figure 3 Effect of the mixed endothelin ET_A/ET_B receptor antagonist bosentan or the selective ET_B receptor antagonist BQ-788 on fever induced by IL-1β and TNF-α in rats. (a) BQ-788 (3 pmol, i.c.v.) or bosentan (10 mg kg^{-1}, i.v.) were injected 15 min before IL-1β (150 pmol kg^{-1}, i.v.). Three groups of control animals received either saline (SAL, 1 ml kg^{-1}, i.v.) before IL-1β, or bosentan or BQ-788 prior to SAL. (b) IL-1β (180 fmol) or TNF-α (14.4 pmol) were injected i.c.v. following injection of BQ-788 (3 pmol, i.c.v.). Three groups of control animals received either artificial cerebro-spinal fluid (aCSF, 3 μl) before IL-1β or TNF-α, or BQ-788 prior to aCSF. Values represent the means$\pm$s.e.mean of the changes in rectal temperature (ΔT) of 8–12 animals in (a) and six animals in (b). No values display $P<0.05$ when compared to corresponding value of vehicle-pretreated same cytokine-treated group. Basal rectal temperatures before treatment ranged between 37.0 and 37.3°C in (a) and between 36.8 and 37.3°C. in (b).

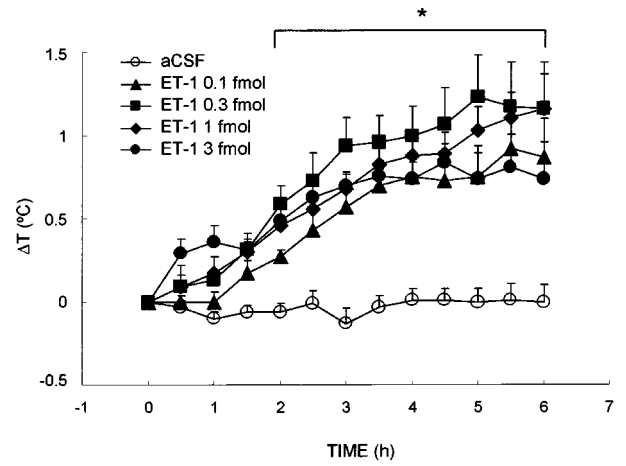

Figure 4 Temperature changes induced by central injection of endothelin-1 in rats. Endothelin-1 (ET-1) was given i.c.v. (3 μl) at the doses indicated and control rats received a similar injection of artificial cerebro-spinal fluid (aCSF). Values represent the means ± s.e.mean of the changes in rectal temperature (ΔT) of six to ten animals. $^*P<0.05$: statistical difference between ET-1 and aCSF treated groups. Basal rectal temperatures before treatment ranged between 37.1 and 37.4°C.

injections of endothelin-1 at any of the doses used up to 3 fmol.

Effect of indomethacin on fever induced by LPS or endothelin-1

The pretreatment of rats with indomethacin (2 mg kg^{-1}, i.p.) significantly reduced, but by no means abolished, the pyrogenic response to i.v. injection of LPS (5 μg kg^{-1}, Figure 6a). In sharp contrast, indomethacin did not affect the fever response to endothelin-1 (3 fmol, i.c.v.; Figure 6b), or the basal temperature of saline-treated animals (Figure 6a).

Discussion

The results of the present study demonstrate, to our knowledge for the first time, that fever induced by LPS in rats is substantially reduced by endothelin receptor antagonists, and also that centrally-injected endothelin-1 raises rectal temperature. As the development of LPS-induced fever was attenuated by the mixed endothelin ET_A/ET_B receptor antagonist bosentan and by selective blockade of endothelin ET_B

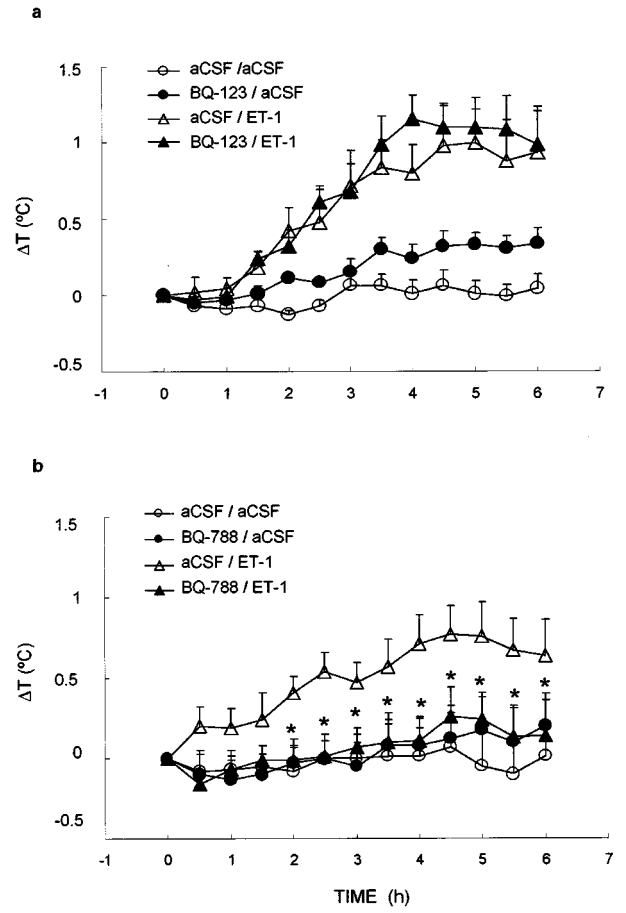

Figure 5 Effect of BQ-123 and BQ-788, selective antagonists of endothelin ET_A and ET_B receptors, respectively, on rectal temperature changes caused by i.c.v. injection of endothelin-1 in rats. BQ-123 (a), BQ-788 (b), each at 3 pmol, or artificial cerebro-spinal fluid (aCSF, 3 μl) were administered i.c.v. 15 min before aCSF or endothelin-1 (ET-1, 1 fmol). Values represent the means ± s.e.mean of the changes in rectal temperature (ΔT) of six to ten animals. $^*P<0.05$ when compared to corresponding value of aCSF-pretreated ET-1-treated group. Basal rectal temperatures before treatment ranged between 37.0 and 37.4°C.

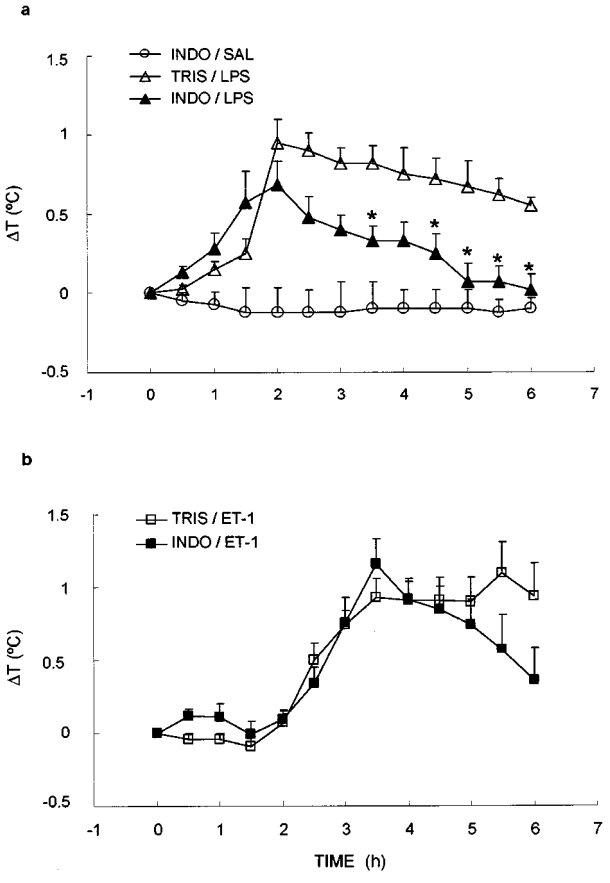

Figure 6 Effect of indomethacin on rectal temperature changes caused by i.v. injection of LPS or i.c.v. injection of endothelin-1 in rats. Indomethacin (INDO, 2 mg kg^{-1}, i.p.) or tris[hydroxymethyl]aminomethane. HCI (TRIS, pH 8.2) were injected 30 min before LPS (5 μg kg^{-1}, i.v.) or saline (SAL, 1 ml kg^{-1}, i.v.) in (a), or endothelin-1 (ET-1, 3 fmol, i.c.v.) in (b). Values represent the means ± s.e.mean of the changes in rectal temperature (ΔT) of four to six animals in (a) and 8–11 animals in (b). $^*P<0.05$ when compared to corresponding value of TRIS-pretreated LPS-treated group. Basal rectal temperatures before treatment ranged between 37.1 and 37.4°C in (a) and between 36.8 and 37.1°C in (b).

receptors with BQ-788, but not BQ-123 (an endothelin ET_A receptor antagonist), only ET_B receptors appear to be involved in this phenomenon. This view is strengthened by the finding that the enhancement of rectal temperature by i.c.v. endothelin-1, a potent agonist at both endothelin ET_A and ET_B receptors (Masaki *et al*., 1994), was also blocked by BQ-788, but not by BQ-123. On the other hand, as none of the endothelin receptor antagonist treatments affected basal rectal temperature, it seems that endothelins are not involved in the thermoregulatory mechanisms under normal conditions.

The efficacy of centrally injected BQ-788 to reduce LPS-induced fever would suggest that this pyrogen raises central endothelin levels, especially in view of the pyrexic effect of locally administered endothelin-1. Indeed, there is ample evidence for the presence of particularly high expression of endothelins in cells of the rat and human hypothalamus (Takahashi *et al*., 1991; Yoshimi *et al*., 1991), a region critically involved in temperature control and fever responses (for review see Blatteis & Sehic, 1997). Although the main isoform present in human hypothalamus appears to be endothelin-1 (Takahashi *et al*., 1991), endothelin-3 is the most abundant in that of the rat (Samson *et al*., 1991). Moreover, this brain region also displays high endothelin-converting enzyme activity (Warner *et al*., 1992), as well as both endothelin ET_A and ET_B receptors (Stojilkovic & Catt, 1996). Therefore, the hypothalamic region seems to be endowed with all the functional requirements needed for local formation and action of endothelins. However, one cannot discard the possibility that endothelins formed in the periphery may reach the hypothalamus via the *organum vasculosum laminae terminalis* (OVLT, as indeed do several cytokines; Stitt & Shimada, 1989), or that cytokines acting on endothelial cells of this structure stimulate endothelin formation. Whatever the source of endothelins mobilized by LPS to cause fever, it is noteworthy that bosentan and BQ-788 only inhibited this response significantly from 2.5 h onwards, a finding consistent with the slow onset of temperature changes induced by i.c.v. endothelin-1 injection. Thus, though LPS may promptly mobilize endothelins, their participation in fever induced by this agent appears to be restricted to later stages of the process.

As already pointed out, LPS is a potent stimulus of endothelin-1 production and secretion both *in vitro* and *in vivo* (see Introduction for references). Furthermore, several of the cytokines released by cells in response to LPS, such as IL-1β, IL-6, IL-8 and TNF-α, are well known pyrogens (Kluger, 1991; Zampronio *et al*., 1994) which can also stimulate endothelin release (Nambi *et al*., 1994; Vemulapalli *et al*., 1994; Klemm *et al*., 1995). Thus, given that endothelin-1 increases body temperature in the rabbit (Koshi *et al*., 1992) and the rat (present study), the ability of LPS to trigger endothelin-mediated fever is not entirely unexpected. However, it appears that none of the *in vivo* studies conducted to date have assessed the influence of LPS on endothelin levels in the CSF or on their expression in the brain.

Recent studies showed that systemic injection of LPS enhances quite rapidly the expression of mRNA for IL-1β, IL-6 and TNF-α in the mouse hypothalamus (Goujon *et al*., 1996; Pitossi *et al*., 1997). Considering that IL-1β and TNF-α can release endothelin-1 from endothelial cells (Corder *et al*., 1995), we examined if IL-1β and TNF-α-induced fever would be mediated via endothelin production. However, as neither bosentan nor BQ-788 modified fever induced by IL-1β injected intravenously, nor BQ-788 affected fever induced by i.c.v. injections of IL-1β or TNF-α, it is unlikely that these cytokines cause these responses via endothelin formation in the brain or systemically. On the other hand, the lack of effects of endothelin receptor antagonists on fever elicited by IL-1β and TNF-α suggest that their efficacy against increases in temperature triggered by LPS or endothelin-1 is related to specific actions of these compounds on endothelin receptors.

The potency of endothelin-1 in eliciting a pyrexic response upon i.c.v. administration is remarkably high, and the effective doses possibly lead to alterations of the peptide's levels in the CSF within the higher physiological range (0.4–4 pM; Pluta *et al*., 1997; for review see Battistini *et al*., 1993). Indeed, these doses are much lower than those usually employed to evoke central effects. Nevertheless, confirming previous reports (Lecci *et al*., 1990; Gross & Weaver, 1993), we also observed that endothelin-1 induces barrel rotation convulsions when injected i.c.v. at doses of 9 pmol or above (results not shown). Moreover, the fact that BQ-788, but not BQ-123, blocked the pyrexic response to central endothelin-1 (or i.v. LPS), whereas BQ-788 failed to modify those induced by IL-1β and TNF-α, argues in favour of a true receptor-mediated action of endothelin-1 at such low i.c.v. doses. This view is further substantiated by reports showing the existence of super-high affinity ET_B receptors, with K_D for endothelin-1 in the pM range, in some peripheral tissues and in several brain regions, including the hypothalamus (Sokolovsky *et al*., 1992; Migas *et al*., 1993).

We were unable to demonstrate a clear dose-response relationship for endothelin-1-induced fever. Although this could be taken as an argument against a receptor-mediated action of the peptide, other explanations for this intriguing fact would seem more plausible. For example, endothelin-1 may simultaneously activate an antipyretic (or hypothermic) mechanism, such as those involving arginine-vasopressin (AVP), α-melanocyte stimulating hormone (α-MSH) or glucocorticoid, which would progressively counteract its hyperthermic effect. It is well known that such mechanisms can limit the pyrexic effects of several fever-inducing agents (for reviews see Lipton & Catania, 1997; Roth & Zeisberger, 1992; Kluger, 1994). In this regard, central administration of endothelin-1 enhances plasma levels of AVP in the rat (Mosqueda-Garcia *et al*., 1995; Rossi *et al*., 1997), whereas i.v. endothelin-3 enhances (*via* CRH release) plasma levels of adrenocorticotropic hormone and corticosterone in the rat (Hirai *et al*., 1991). This possibility remains to be adequately investigated in future studies.

On the other hand, although the cerebral vasculature is very sensitive to the constrictor effects of endothelin-1 (for review see Willete *et al*., 1995), it would appear unlikely that cerebral vasoconstriction underlies the changes in rectal temperature induced by i.c.v. injection of the peptide. First, because of the low doses involved. Secondly, because endothelin-1-induced vasoconstriction of the rat cerebral microvasculature is essentially mediated *via* BQ-123-sensitive endothelin ET_A receptors (Gulati *et al*., 1996), whereas the fever it causes is unaffected by a selective antagonist of this type of receptor, but blocked by an ET_B receptor antagonist. Indeed, at least in rabbits, LPS induces marked dilation, rather than constriction, of cerebral arterioles, which is associated with local production of nitric oxide and prostanoids (Brian *et al*., 1995). Finally, it appears even less likely that the changes in rectal temperature result from a peripheral vascular effect since, given i.c.v. in a similar range of doses (1–10 pmol kg^{-1}), endothelin-1 has been reported to cause no overt effects on the cardiovascular system (Siren & Feuerstein, 1989).

Fever induced by LPS in rats is known to involve prostanoid-dependent (probably prostaglandins E_2 and $F_{2\alpha}$) and -independent mechanisms (Strijbos *et al*., 1992). In addition, although the pyrexic effects of centrally administered

IL-1β, TNF-α and IL-6 are effectively supressed by cyclo-oxygenase blockers, those of IL-8 are resistant to this treatment but are susceptible to inhibition by glucocorticoids or antagonists of corticotropin-releasing hormone receptors (Zampronio *et al*., 1994; Rothwell, 1990). In line with these reports, we have confirmed that the cyclo-oxygenase blocker indomethacin attenuates LPS-induced fever, but does not abolish it. However, as indomethacin failed to affect endothelin-1-induced fever, endothelins seem to participate in the prostanoid-independent pathways of fever triggered by LPS in rats. Though it is tempting to speculate that central endothelins are somehow linked in the IL-8-mediated and corticotropin-releasing hormone-dependent arm of the LPS-induced fever cascade (Strijbos *et al*., 1992), this remains to be adequately addressed. The absence of effect of indomethacin showed here was not observed in rabbits, since in this specie the fever induced by intravenously injected endothelin-1 was dose-dependently inhibited by this antipyretic (Koshi *et al*., 1992). This discrepancy may be species-related since in rabbits IL-8 also induces fever dependent on prostaglandin synthesis (Zampronio *et al*., 1995).

In conclusion, we have shown that the fever induced by LPS in the rat is partially mediated through stimulation of central ET_B receptors by endothelin. As the febrile response is the result of a complex interplay between various cytokines, acting both *via* prostanoid-dependent and -independent pathways in the central nervous system (for review see Luheshi & Rothwell, 1996), the exact position of endothelins in this fever cascade remains to be elucidated, yet they do not appear to be downstream from IL-1β or TNF-α or to depend on prostaglandin synthesis.

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