Am J Physiol Regul Integr Comp Physiol 290: R164–R171, 2006.
First published August 25, 2005; doi:10.1152/ajpregu.00337.2005.

CALL FOR PAPERS | *Physiology and Pharmacology of Temperature Regulation*

Central endothelin ET_B receptors mediate IL-1-dependent fever induced by preformed pyrogenic factor and corticotropin-releasing factor in the rat

Aline S. C. Fabricio,[1] Giles A. Rae,[2] Aleksander R. Zampronio,[3] Pedro D'Orléans-Juste,[4] and Glória E. P. Souza[1]

[1]*Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil;* [2]*Department of Pharmacology, Biological Sciences Center, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil;* [3]*Department of Pharmacology, Biological Sciences Section, Universidade Federal do Paraná, Curitiba, Paraná, Brazil;* [4]*Department of Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Canada*

Submitted 11 May 2005; accepted in final form 19 August 2005

Fabricio, Aline S. C., Giles A. Rae, Aleksander R. Zampronio, Pedro D'Orléans-Juste, and Glória E. P. Souza. Central endothelin ET_B receptors mediate IL-1-dependent fever induced by preformed pyrogenic factor and corticotropin-releasing factor in the rat. *Am J Physiol Regul Integr Comp Physiol* 290: R164–R171, 2006. First published August 25, 2005; doi:10.1152/ajpregu.00337.2005.— Blockade of central endothelin ET_B receptors inhibits fever induced by LPS in conscious rats. The contribution of ET_B receptor-mediated mechanisms to fever triggered by intracerebroventricular IL-6, PGE_2, $PGF_{2\alpha}$, corticotropin-releasing factor (CRF), and preformed pyrogenic factor derived from LPS-stimulated macrophages (PFPF) was examined. The influence of natural IL-1 receptor antagonist or soluble TNF receptor I on endothelin (ET)-1-induced fever was also assessed. The selective ET_B receptor antagonist BQ-788 (3 pmol icv) abolished fever induced by intracerebroventricular ET-1 (1 pmol) or PFPF (200 ng) and reduced that caused by ICV CRF (1 nmol) but not by IL-6 (14.6 pmol), PGE_2 (1.4 nmol), or $PGF_{2\alpha}$ (2 nmol). CRF-induced fever was also attenuated by bosentan (dual ET_A/ET_B receptor antagonist; 10 mg/kg iv) but unaffected by BQ-123 (selective ET_A receptor antagonist; 3 pmol icv). α-Helical CRF_{9-41} (dual CRF_1/CRF_2 receptor antagonist; 6.5 nmol icv) attenuated fever induced by CRF but not by ET-1. Human IL-1 receptor antagonist (9.1 pmol) markedly reduced fever to IL-1β (180 fmol) or ET-1 and attenuated that caused by PFPF or CRF. Murine soluble TNF receptor I (23.8 pmol) reduced fever to TNF-α (14.7 pmol) but not to ET-1. The results of the present study suggest that PFPF and CRF recruit the brain ET system to cause ET_B receptor-mediated IL-1-dependent fever.

prostaglandins; cytokines; interleukin-1 receptor antagonist

FEVER IS COMMONLY ASSOCIATED with microbial or parasitic infection and is part of the acute phase response to injury. The febrile response per se depends critically on secretion, from peripheral and/or central leukocytes and other cell types, of various endogenous pyrogens, including IL-1, IL-6, IL-8, TNF-α, macrophage inflammatory protein 1, and preformed pyrogenic factor derived from LPS-stimulated macrophages (PFPF; molecular mass >30 kDa, isoelectric point: 4.7–5.8) (16, 26, 39, 42). Directly or indirectly, such pyrogens alter the activity of hypothalamic thermoregulatory neurons, through actions that depend, to varying extents, on two key types of mediator: prostaglandins (PGs; particularly PGE_2 and $PGF_{2\alpha}$) and corticotropin-releasing factor (CRF) (26). At least in the rat, the fever induced by intracerebroventricular injection of IL-1α, IL-1β, IL-6, or TNF-α, but not that caused by IL-8, macrophage inflammatory protein 1, or PFPF, is reduced subsequently to the inhibition of PG synthesis by indomethacin, a well-known nonselective COX-1/COX-2 blocker (22, 29, 30, 40, 41). The dual CRF_1/CRF_2 receptor antagonist α-helical CRF_{9-41}, on the other hand, blocks fever induced by intracerebroventricular $PGF_{2\alpha}$, IL-1β, IL-6, IL-8, or PFPF but not by PGE_2, TNF-α, or IL-1α (28, 29, 32, 39). Worthy of notice, including IL-8 in the cascade of fever mediators in the rat should be considered cautiously as this species does not express that particular cytokine but rather a functionally related yet distinct chemokine called cytokine-induced neutrophil chemoattractant (35). The pyrogenic activity of cytokine-induced neutrophil chemoattractant is thus far unknown.

We have proposed that endothelins (ETs), a family of peptides causing potent and widespread biological actions mediated via stimulation of specific ET_A and ET_B G protein-coupled receptors (14, 20), also act as endogenous pyrogens in LPS-induced fever in rats (7). The proposal was supported by the fact that ICV administration of the selective ET_B receptor antagonist BQ-788 substantially attenuates the fever induced by intravenous *E. coli* LPS, indicating that endogenous ETs contribute significantly to this response (7). Moreover, ICV ET-1 also causes a potent pyrogenic effect of ET-1 in the rat, which is fully prevented by prior ICV injection of the selective ET_B receptor antagonist BQ-788 but is unaffected by the selective ET_A receptor antagonist BQ-123 or prior systemic treatment with indomethacin (7, 8). In contrast, fever induced by either ICV IL-1β or TNF-α is sensitive to inhibition by indomethacin but resistant to pretreatment with the ET_B receptor antagonist (5, 7).

In light of the above considerations, the present study attempts to detect possible interactions of the ET system with those of other mediators involved in the febrile response. We examined the participation of ET_B receptor-mediated mechanisms in fever triggered by important centrally acting pyro-

Address for reprint requests and other correspondence: G. E. P. Souza, Laboratório de Farmacologia, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n-Campus USP 14040-903 Ribeirão Preto, SP, Brazil (E-mail: gepsouza@usp.br).

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.

gens, such as IL-6, PFPF, PGE_2, $PGF_{2\alpha}$, and CRF. In addition, because mechanisms and mediators subserving the fever induced by ICV ET-1 remained uncharacterized, we assessed the susceptibility of this response to inhibition by the dual CRF_1/CRF_2 receptor antagonist α-helical CRF_{9-41}, human recombinant IL-1 receptor antagonist (IL-1ra), or murine soluble TNF receptor I (sTNFRI). We also examined the effect of IL-1ra on ET_B-dependent fever induced by CRF or PFPF. Both IL-1ra and sTNFRI are expressed in vivo and act as an antagonist of IL-1 receptors and as a scavenger of TNF-α, respectively (10, 27).

METHODS

Animals. Experiments were conducted using male Wistar rats weighing 180–200 g, housed individually at 24 ± 1°C under a 12:12-h light-dark cycle (lights on at 0600) with free access to food chow and tap water until the day of the experiment, when only water was made available. The experimental procedures and protocols were previously approved by the committee on ethical use of laboratory animals of the University of São Paulo and were performed at this institution in accordance with Brazilian legislation, as well as in accordance with the *Guide for the Care and Use of Laboratory Animals* (12).

Intracerebral cannula implantation. Under anesthesia with pentobarbital sodium (40 mg/kg ip), a stainless steel guide cannula (0.7 mm OD, 10 mm long) was stereotaxically implanted into the right lateral ventricle (8) and fixed to the skull with jeweler's screws embedded in dental acrylic cement. Animals were then treated with oxytetracycline hydrochloride (400 mg/kg im) and allowed to recover for 1 wk before the experiments. After each experiment, the animal was anesthetized (as before) and the location of the cannula track was verified histologically. Animals showing cannula misplacement or blockage on injection or abnormal weight gain patterns during the postimplantation period were excluded from the study.

Temperature measurements. Rectal temperature was measured in conscious and unrestrained rats for 1 min every 30 min for up to 6 h, in most cases by gently inserting a small Vaseline-coated thermistor probe (model 402 coupled to a model 46 telethermometer; Yellow Springs Instruments, Yellow Springs, OH) 4 cm into the rectum, without removing them from their home cages. Experimental measurements were conducted at the thermoneutral zone for rats (9) in a temperature-controlled room (28 ± 1°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 injection, 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 conditioned to this environment and procedure twice on the preceding day. The experiments involving dexamethasone, IL-1ra, and sTNFRI administration were conducted essentially as described above, except that core body temperature was measured by using battery-operated biotelemetry transmitters (Data Science, St. Paul, MN) implanted in the peritoneal cavity at the same time as ICV cannula implantation. ET-1-induced fever recorded using the radiotelemetry system was indistinguishable from that assessed by the rectal probe method (8).

Production of the PFPF from LPS-stimulated macrophage monolayers. PFPF was prepared as described before (39). Briefly, rats received a 10-ml intraperitoneal injection of 3% thioglycolate. Peritoneal macrophages were harvested 4 days later, using 10 ml of RPMI 1640 medium (pH 7.4) containing 5 U/ml heparin, and incubated in culture dishes for 1 h at 37°C, 5% CO_2. Monolayers of adherent cells (1.95×10^6 viable cells per dish) were then washed with PBS and incubated with fresh medium containing dexamethasone (2.3 μM) for another 1 h under the same conditions. Cells were then washed again with PBS and incubated with medium containing dexamethasone plus LPS (10 μg/ml) for another 30 min. After a final wash with PBS, the macrophages were incubated with 5 ml of LPS-free RPMI 1640 medium containing dexamethasone for 1 h. The supernatant was collected and concentrated on an Amicon YM30 membrane, and the retained portion was resuspended in water. After its protein content was inspected with a spectrophotometer at 280 nm, the material was then lyophilized and stored at −70°C until use. We have previously shown, by preparative isoelectric focusing, that the ability of the concentrated supernatant to cause fever on ICV administration or release of IL-6 from cultured macrophages is due to a semipurified protein with an isoeletric point between 4.7 and 5.8 and a molecular mass above 30 kDa, which we named PFPF (39).

Experimental protocols. Rats received an ICV injection (3 μl over 1 min) of either BQ-788 [selective ET_B receptor antagonist (4); 3 pmol] or artificial cerebrospinal fluid (aCSF; composition, in mmol/l: 138.6 NaCl, 3.35 KCl, 1.26 $CaCl_2$, and 11.9 $NaHCO_3$) 15 min before a similar ICV injection of either ET-1 (1 pmol), PGE_2 (1.42 nmol), $PGF_{2\alpha}$ (2.1 nmol), IL-6 (14.6 pmol), PFPF (200 ng of protein), or CRF (1.05 nmol). Some animals were also pretreated with BQ-123 [selective peptidic ET_A receptor antagonist (4); 3 pmol icv] or bosentan [dual nonpeptidic ET_A/ET_B receptor antagonist (3); 10 mg/kg iv into tail vein] before they received ICV injection of CRF.

In another set of experiments, rats were given ICV injections of CRF (1.05 nmol), IL-1β (180 fmol), TNF-α (14.7 pmol), or ET-1 (1 pmol) 15 min after the treatment with ICV α-helical CRF_{9-41} [dual CRF_1/CRF_2 receptor antagonist (29); 6.5 nmol], IL-1ra (9.1 nmol), or sTNFRI (23.8 pmol). The doses of the antagonists were selected on the basis of preliminary dose-response studies performed in our own laboratory (data not shown). In all experiments, the respective control groups were similarly treated with the corresponding vehicles, as appropriate (subcutaneous saline or ICV aCSF). For ICV injections, a 31-gauge needle, connected by polyethylene tubing to a 5-μl Hamilton gas-tight syringe (Hamilton, Birmingham, UK), was lowered into the guide cannula so that it protruded 1.5 mm beyond its tip into the ventricle. Pyrogenic stimuli were always given between 10:00 and 11:00 AM (to minimize possible diurnal variability) at the threshold dose for the induction of fever (5, 7, 8, 39).

Drugs. The following drugs were employed: PGE_2, $PGF_{2\alpha}$, and rat α-helical CRF_{9-41} (Sigma, St. Louis, MO); rat recombinant IL-6, human recombinant IL-1ra, and CRF_{1-41} (NIBSC, Hertfordshire, UK); BQ-123 (cyclo-[D-Trp-D-Asp-Pro-D-Val-Leu]; American Peptide, Sunnyvale, CA); BQ-788 (*N*-cis-2,6-dimethylpiperidinocarbonyl-L-g-methylleucyl-D-1-ethoxycarbonyl-D-norleucine) and ET-1 (Research Biochemicals International, Natick, MA); bosentan, kindly provided by Dr. M. Clozel (Actelion, Allschwil, Switzerland); murine IL-1β (lot no. BN024121), rat TNF-α, and murine sTNFRI (R&D Systems, Minneapolis, MN); dexamethasone (Decadronal; Prodome Laboratories, Campinas, Brazil); and oxytetracycline hydrochloride (Terramicina; Pfizer Laboratories, São Paulo, Brazil).

Statistical analysis. All variations in body temperature were expressed as changes from the mean basal value (i.e., ΔT, in °C), and baseline temperatures were not statistically different between groups included in any particular set of experiments. All values are presented as means ± SE, and statistical comparisons were performed by means of one-way ANOVA followed by Tukey's test, by use of SPSS statistical software (SPSS, Chicago, IL). Significance was set at $P < 0.05$.

RESULTS

Influence of ET receptor antagonists on fever induced by different stimuli. Confirming our previous report (7), ICV injection of ET-1 (1 pmol) caused a slowly developing and long-lasting increase in rectal temperature, which peaked at 3.5 h (ΔT of 0.76 ± 0.13°C, $P < 0.05$, $n = 5$) after administration and was fully prevented by ICV pretreatment with BQ-788 (3 pmol, ΔT of 0.15 ± 0.04°C, $n = 9$).

Intraventricular administration of IL-6 (14.6 pmol) or PFPF (200 ng) also induced fever, which peaked at 2.5 h (ΔT of 0.72 $\pm$ 0.09°C) and 4.5 h (ΔT of 0.74 $\pm$ 0.12°C) after injection, respectively (Fig. 1). Prior ICV treatment with BQ-788 (3 pmol) did not modify the baseline temperature of control animals or the fever induced by IL-6-injected rats (Fig. 1*A*). Nonetheless, the febrile response induced by PFPF was virtually abolished by BQ-788 pretreatment (Fig. 1*B*).

As previously described (5), ICV injection of PGE_2 (1.42 nmol) induced a short-lived monophasic febrile response, whereas $PGF_{2\alpha}$ (2.1 nmol) elicited a biphasic response, composed of an initial transient peak followed by a second more sustained component. Prior injection of BQ-788 (3 pmol) failed to affect the hyperthermic responses to either PGE_2 or $PGF_{2\alpha}$ (Fig. 2).

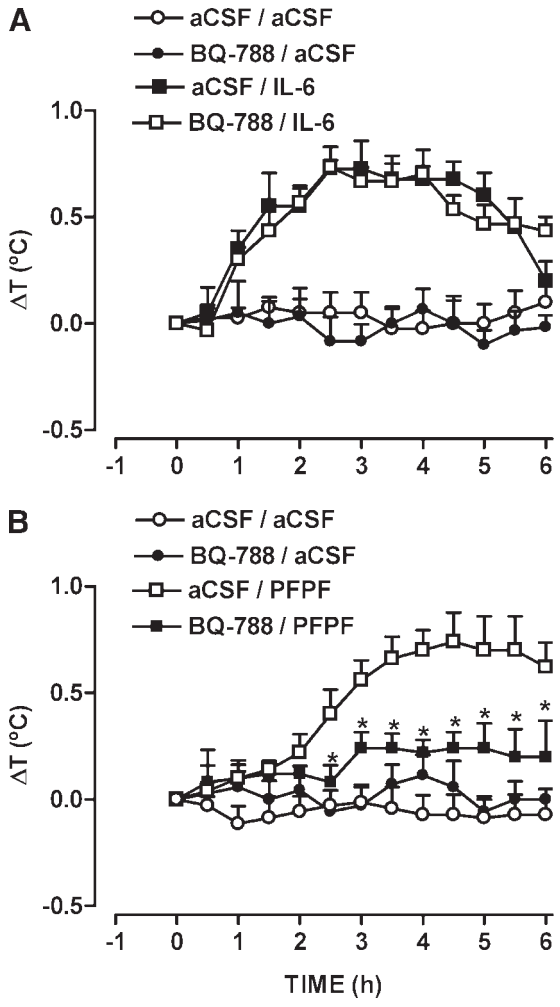

Fig. 1. Influence of BQ-788, a selective ET_B receptor antagonist, on fever induced by IL-6 or preformed pyrogenic factor derived from LPS-stimulated macrophages (PFPF) in rats. BQ-788 (3 pmol icv) or artificial cerebrospinal fluid (aCSF; 3 μl) was administered 15 min before intracerebroventricular (ICV) injection of either aCSF, IL-6 (14.6 pmol; *A*) or PFPF (200 ng of purified protein; *B*). Values represent means $\pm$ SE of the changes in rectal temperature (ΔT) of 4–6 animals. Basal temperatures (means $\pm$ SE; °C) were as follows: for *A*, 36.9 $\pm$ 0.058 (○), 37.0 $\pm$ 0.06 (●), 36.97 $\pm$ 0.07 (□), and 36.98 $\pm$ 0.07 (■); for *B*, 37.3 $\pm$ 0.04 (○), 37.1 $\pm$ 0.08 (●), 37.0 $\pm$ 0.05 (□), and 37.0 $\pm$ 0.06 (■). *$P < 0.05$ compared with corresponding value of aCSF-pretreated/PFPF-treated group.

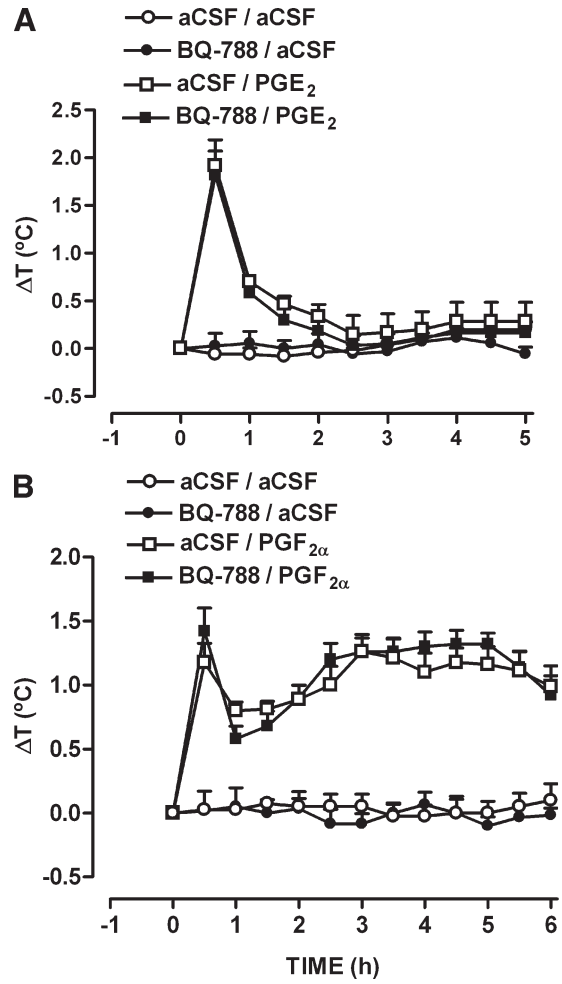

Fig. 2. BQ-788, a selective ET_B receptor antagonist, failed to modify fever induced by PGE_2 and $PGF_{2\alpha}$ in rats. BQ-788 (3 pmol icv) or aCSF (3 μl) was administered 15 min before ICV injection of either aCSF, PGE_2 (1.42 nmol; *A*) or $PGF_{2\alpha}$ (2.1 nmol; *B*). Values represent means $\pm$ SE of ΔT of 4–10 animals. Basal temperatures (means $\pm$ SE; °C) were as follows: for *A*, 36.88 $\pm$ 0.03 (○), 36.98 $\pm$ 0.03 (●), 36.97 $\pm$ 0.08 (□), and 37.0 $\pm$ 0.07 (■); for *B*, 36.9 $\pm$ 0.08 (○), 37.0 $\pm$ 0.06 (●), 36.94 $\pm$ 0.1 (□), and 37.05 $\pm$ 0.085 (■). BQ-788 failed to significantly modify responses to either PG ($P > 0.05$, one-way ANOVA test).

Central injection of CRF (1.05 nmol icv) induced a slowly developing and long-lasting increase in body temperature, which was maximal 4 h after administration (ΔT of 0.93 $\pm$ 0.14°C; Fig. 3). Prior ICV treatment with the selective ET_A receptor antagonist BQ-123 (3 pmol) did not modify CRF-induced fever. In contrast, pretreatment with BQ-788 (3 pmol icv) or the dual ET_A/ET_B receptor antagonist bosentan (10 mg/kg iv) significantly reduced (but did not abolish) the response to CRF. Like BQ-788, neither BQ-123 nor bosentan significantly changed basal temperature values in aCSF-treated control animals.

Influence of α-helical CRF_{9-41}, IL-1ra, or sTNFRI on fever induced by PFPF, CRF, or ET-1. As shown in Fig. 4, ICV pretreatment of rats with α-helical CRF_{9-41} (6.5 nmol), a dual CRF_1/CRF_2 receptor antagonist that blocks PFPF-induced fever (39), significantly attenuated the pyrogenic response to centrally injected CRF but did not affect ET-1-induced fever. In contrast, treatment of rats with rat recombinant IL-1ra (9.1

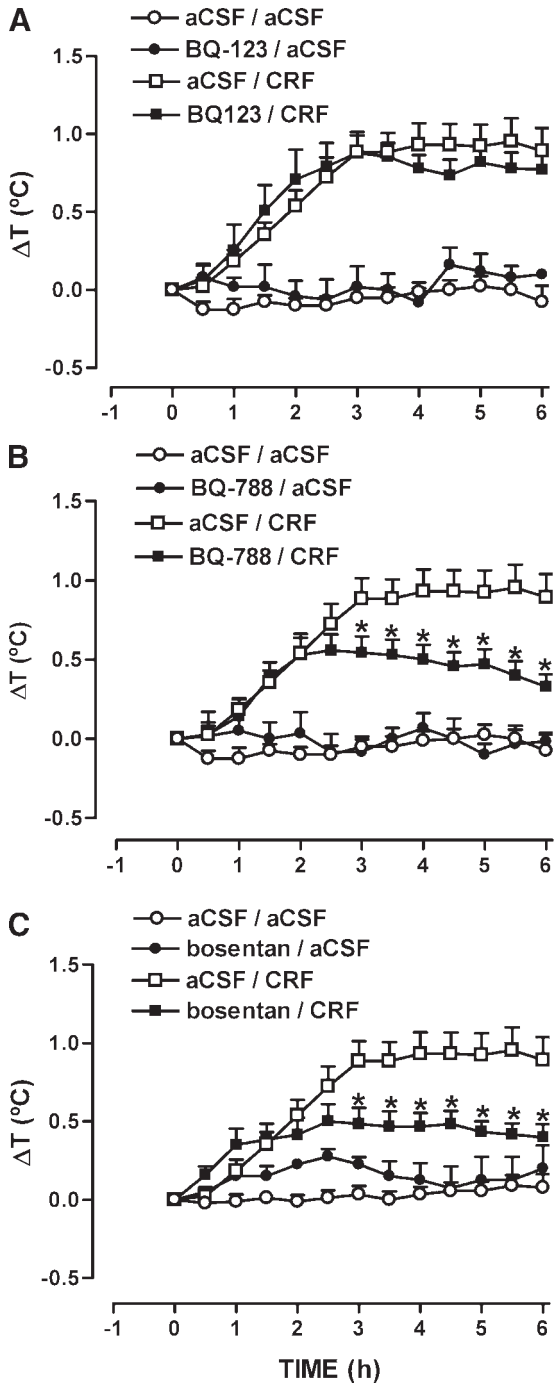

Fig. 3. Influence of BQ-123, BQ-788, and bosentan (selective ET_A, selective ET_B, and dual ET_A/ET_B receptor antagonists, respectively) on fever induced by corticotropin-releasing factor (CRF) in rats. BQ-123 (3 pmol icv; *A*), BQ-788 (3 pmol icv; *B*), or bosentan (10 mg/kg iv; *C*) were injected 15 min before CRF (1.05 nmol icv). The control groups received aCSF (3 μl) either before CRF or 15 min after the ET_A and/or ET_B receptor antagonists. Values represent means ± SE of ΔT of 6–10 animals. Basal temperatures (means ± SE; °C) were as follows: for *A*, 37.02 ± 0.095 (○), 37.0 ± 0.03 (●), 37.1 ± 0.041 (□), and 36.9 ± 0.06 (■); for *B*, 36.97 ± 0.06 (○), 37.0 ± 0.03 (●), 37.0 ± 0.06 (□), and 37.0 ± 0.07 (■); for *C*, 37.1 ± 0.09 (○), 37.17 ± 0.048 (●), 36.9 ± 0.036 (□), and 36.86 ± 0.03 (■). $*P < 0.05$ compared with corresponding value of aCSF-pretreated/CRF-treated group.

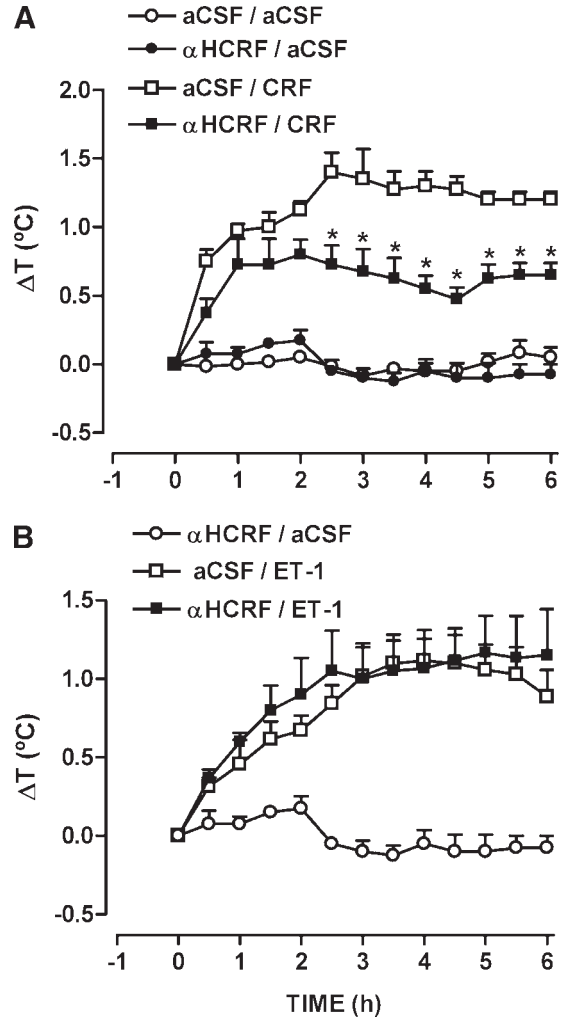

Fig. 4. Influence of α-helical CRF_{9-41}, a nonselective CRF_1/CRF_2 receptor antagonist, on fever induced by CRF or ET-1 in rats. α-Helical CRF_{9-41} (αHCRF, 6.5 nmol icv) or aCSF (3 μl) was given 15 min before ICV injection of CRF (1.05 nmol; *A*) or ET-1 (1 pmol; *B*). Values represent means ± SE of ΔT of 4–7 animals. Basal temperatures (mean ± SE; °C) were as follows: for *A*, 36.9 ± 0.06 (○), 36.98 ± 0.05 (●), 37.2 ± 0.09 (□), and 37.0 ± 0.1 (■); for *B*, 36.98 ± 0.05 (●), 37.1 ± 0.087 (□), and 36.95 ± 0.1 (■). $*P < 0.05$ compared with corresponding value of aCSF-pretreated/CRF-treated group.

nmol, 15 min beforehand intracerebroventricularly), at a dose that markedly reduced fever induced by IL-1β, attenuated the febrile responses to either CRF or PFPF, and almost abolished that caused by ET-1 (Fig. 5). Conversely, prior treatment of rats with murine recombinant sTNFRI (23.8 pmol icv), at a dose that markedly reduced fever induced by TNF-α (14.7 pmol icv), did not modify the fever caused by ET-1 (Fig. 6). Baseline temperature of control animals was unaffected by ICV α-helical CRF_{9-41}, IL-1ra, or sTNFRI.

DISCUSSION

The present results shed new light on the role played by endogenous ETs in fever generation in the rat. We have shown herein that ETs, acting via ET_B receptors, and to a significant extent via IL-1 receptor-mediated mechanisms, are engaged in the fever induced by CRF and PFPF. Furthermore, ETs do not seem to participate in the fever induced by other important endogenous pyrogens, such as IL-6, PGE_2, and $PGF_{2\alpha}$.

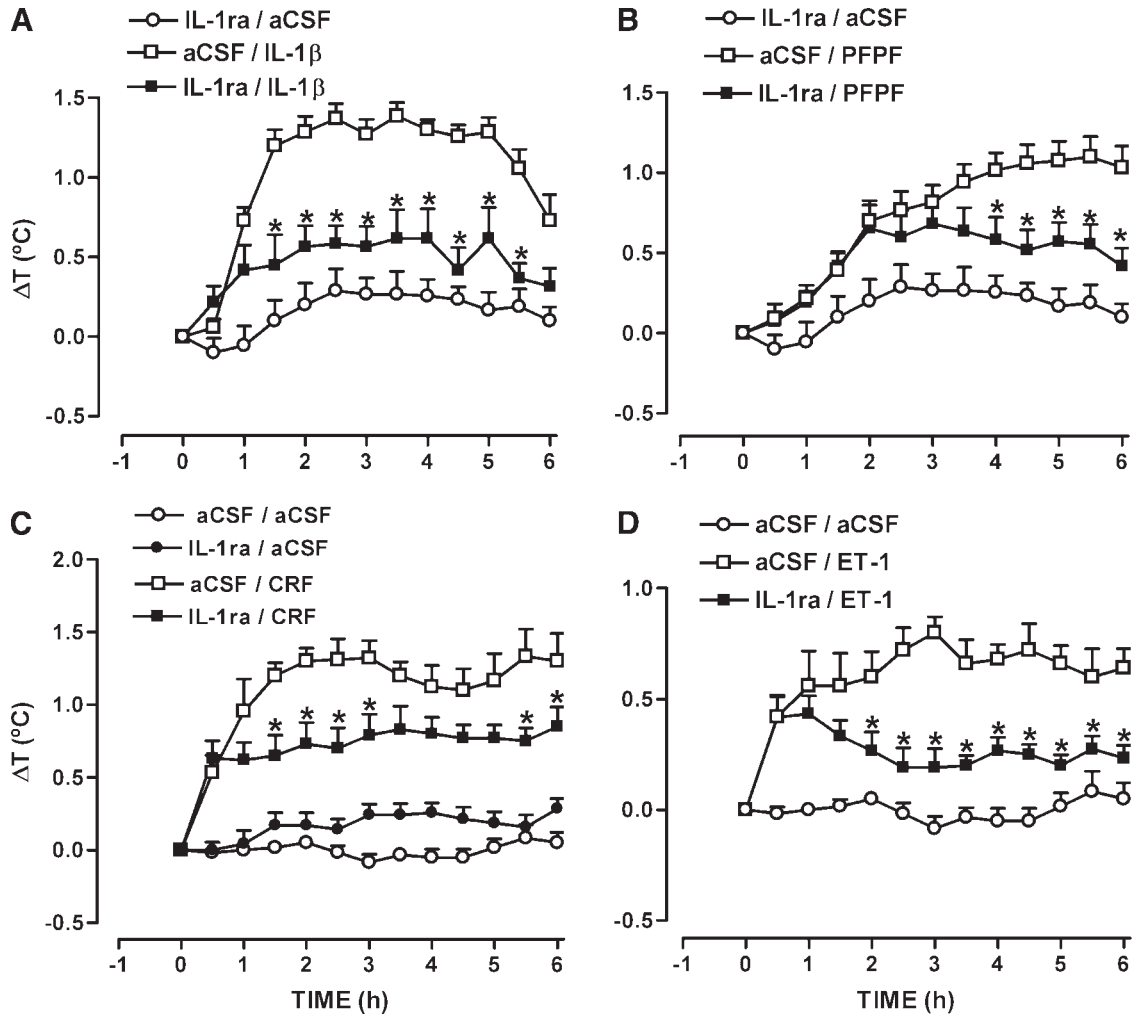

Fig. 5. Influence of IL-1 receptor antagonist (IL-1ra) on fever induced by IL-1β, PFPF, CRF or ET-1 in rats. IL-1ra (9.1 pmol icv) was given 15 min before ICV injection of IL-1β (180 fmol; *A*), PFPF (200 ng; *B*), CRF (1.05 nmol; *C*), or ET-1 (1 pmol; *D*). The control groups received aCSF (3 μl) either 15 min before the pyrogenic stimuli or 15 min after IL-1ra. Values represent means ± SE of ΔT of 5–12 animals. Basal temperatures (means ± SE; °C) were as follows: for *A*, 36.98 ± 0.08 (●), 36.9 ± 0.05 (□), and 37.0 ± 0.06 (■); for *B*, 37.02 ± 0.04 (●), 37.1 ± 0.04 (□), and 36.9 ± 0.06 (■); for *C*, 37.2 ± 0.17 (○), 37.13 ± 0.03 (●), 37.06 ± 0.051 (□), and 37.18 ± 0.08 (■); for *D*, 37.07 ± 0.09 (○), 37.0 ± 0.08 (□), and 36.98 ± 0.04 (■). *$P < 0.05$ compared with corresponding value of aCSF-pretreated/pyrogen-treated group.

In our previous study (7), we showed that ICV ET-1, acting through central ET_B (but not ET_A) receptors, closely mimicked the ability of intravenous LPS to raise core temperature of rats. Moreover, ET-1 appears to cause a true fever response involving readjustments of thermoeffector mechanisms, since the rise in core temperature induced by ICV ET-1 was accompanied by vasoconstriction of the cutaneous vessels of the tail (our unpublished observation). We also previously showed that the selective ET_B receptor antagonist BQ-788, but not the selective ET_A receptor antagonist BQ-123, markedly reduced LPS-induced fever.

In the present study, considerable effort was expended to identify which endogenous pyrogens could trigger fever via ET_B receptor-coupled mechanisms. We have already shown that indomethacin-sensitive (i.e., PG-dependent) fever induced by ICV IL-1β or TNF-α (5) is entirely resistant to selective ET_B receptor blockade by ICV BQ-788 (7). Likewise, we now have demonstrated that ICV BQ-788, at a dose that abolishes ICV ET-1-induced fever, failed to affect the febrile response triggered by PGE_2, $PGF_{2\alpha}$, or IL-6 [another PG-dependent pyretic cytokine (5)]. These findings, added to the fact that indomethacin does not inhibit fever induced by ICV ET-1 (7, 8), strongly support the possibility that the brain ET system is recruited by PG-independent pathways to induce fever.

On the other hand, both PG-dependent and independent pathways can converge at the level of synthesis and/or release of CRF, another prominent pyrogen, to promote fever. Thus the indomethacin-sensitive fever induced by IL-1β and IL-6 and the indomethacin-resistant fever induced by PFPF and $PGF_{2\alpha}$ are both susceptible to blockade by the dual CRF_1/CRF_2 receptor antagonist α-helical CRF_{9-41} (28, 29, 32, 39). Moreover, fever induced by intravenous IL-1β (19) or ICV CRF (M. J. Figueiredo, unpublished observation) is inhibited by selective CRF_1 receptor blockade, whereas IL-1β and PFPF trigger pronounced CRF release from rat hypothalamic explants (24, 39). Here, we found that indomethacin-insensitive fever caused by PFPF was abolished by ICV BQ-788. This same peptidic ET_B receptor antagonist also attenuated the fever

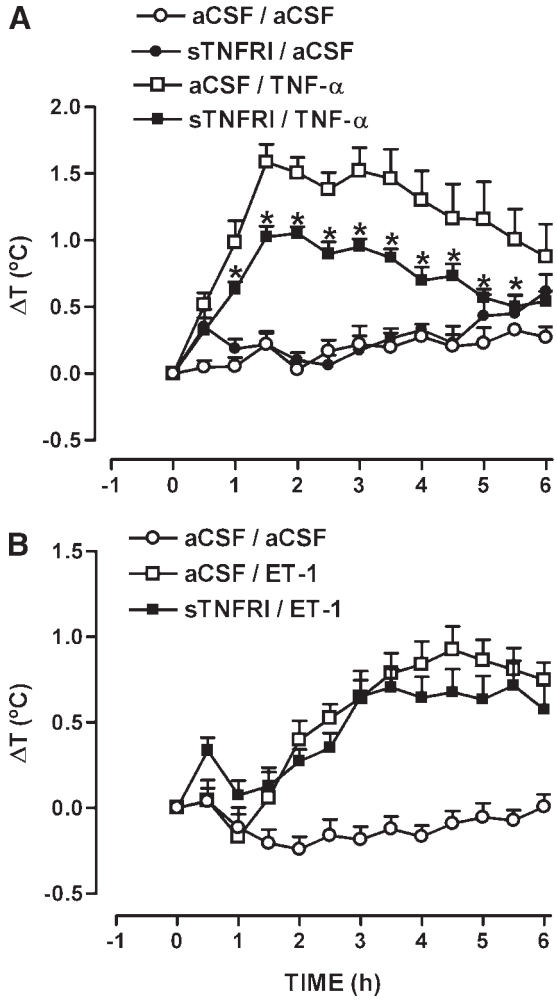

Fig. 6. Influence of soluble TNF receptor I (sTNFRI) on fever induced by TNF-α or ET-1 in rats. Mouse recombinant sTNFRI (23.8 pmol icv) was given 15 min before the ICV injection of TNF-α (14.7 pmol; *A*) or ET-1 (1 pmol; *B*). Control groups received aCSF (3 μl) either 15 min before TNF-α (in *A*) or ET-1 (in *B*) or 15 min after sTNFRI. Values represent means ± SE of ΔT of 6–12 animals. Basal temperatures (means ± SE; °C) were as follows: for *A*, 37.01 ± 0.06 (○), 36.98 ± 0.03 (●), 37.0 ± 0.08 (□), and 36.97 ± 0.07 (■); for *B*, 37.2 ± 0.13 (○), 37.05 ± 0.05 (□), and 37.18 ± 0.08 (■). *$P < 0.05$ compared with corresponding value of aCSF-pretreated/TNF-α-treated group.

induced by CRF. In a study (13) investigating the specificity of BQ-788 for the ET_B receptor relative to other hormone receptors, it was showed that BQ-788 did not inhibit or facilitate the specific binding of several peptides unrelated to ET-1. However, CRF was not investigated in this paradigm (13). Therefore, one could argue that the reason for the effect of BQ-788 on CRF-induced fever might be solely attributable to a nonspecific interaction of the peptidic antagonist with CRF receptors. To exclude this possibility, we tested the effect of two additional ET antagonists on the response induced by CRF. Similarly to BQ-788, CRF-induced fever was reduced by systemic injection of bosentan, a nonpeptidic brain permeating dual ET_A/ET_B receptor antagonist, but not by the ET_A-receptor-selective peptidic antagonist BQ-123. Together, these results indicate a specific recruitment of ET_B receptors during the fever induced by CRF. In addition, ICV CRF and PFPF also promote an integrated febrile response in rats, in which core body temperature increases coincide with decreases in tail skin temperature (M. J. Figueiredo and F. H. Veiga-Souza, unpublished observations). Thus there seem to exist various parallel CRF-dependent pathways for induction of fever in the rat, but only those activated by PFPF appear to recruit the ET system in the brain.

Another potentially important issue addressed by the present study relates to the fever mechanisms occurring downstream from activation of ET_B receptors in response to PFPF and CRF. In this regard, we examined whether ET-1-induced fever required the participation of CRF, TNF-α, or IL-1. The fever caused by ICV ET-1 was not influenced by prior ICV treatment with α-helical CRF_{9-41}, a result in line with the finding that ET-1 does not trigger CRF release from explants of rat hypothalamus (38). In addition, our finding that ICV sTNFRI, a TNF-α scavenger that effectively attenuates fever induced by TNF-α (Ref. 33 and present study), muramyl dipeptide, or LPS (27), did not modify ICV ET-1-induced fever seems to rule out this cytokine as an effector of the response. In contrast, IL-1ra attenuated the febrile responses to ICV ET-1, as well as to PFPF or CRF, thus demonstrating that IL-1 mediates their pyrogenic actions. LPS not only triggers fever sensitive to substantial inhibition by IL-1ra (18) and BQ-788 (7) but it also triggers the release of PFPF from peritoneal macrophages (40). Thus IL-1 may well constitute a pivotal mediator in the pathway through which LPS, PFPF, CRF, and ET-1 generate fever. Moreover, the finding that ICV IL-1ra markedly inhibits fever induced by ET-1 (as well as by PFPF or CRF) implicates IL-1 in the fever mechanisms situated downstream from ET_B receptors. It also fits well with reports that CRF triggers the release of a preformed hypothalamic pool of IL-1β (34) and that IL-1 enhances central sympathetic outflow when given intracerebroventricularly (23) and causes fever when microinjected into the preoptic area (POA) (17). Furthermore, ET-1 stimulates IL-1 release from different cell types, including macrophages, microglia, and astrocytes (6, 31). The increase in

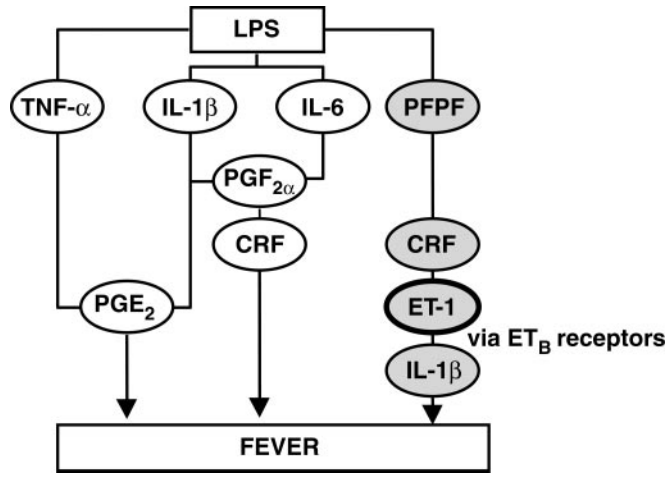

Fig. 7. Schematic representation of pathways involved in the LPS-induced fever in rats. Intravenous LPS triggers release of the pyrogenic cytokines IL-1β, TNF-α, IL-6, and PFPF by different host defense cells to activate 2 parallel fever pathways. One of them clearly depends on cyclooxygenase-derived PGE_2 and $PGF_{2\alpha}$ (open ellipses). The other pathway is indomethacin-insensitive and requires central stimulation of ET-1 production and activation of ET_B receptors coupled to IL-1 release (gray ellipses). CRF is implicated in both pathways.

cytokine synthesis induced by ET-1 seems to depend on NF-κB-mediated signaling pathways (36, 43).

Recently, we observed that 1 and 2.5 mg/kg celecoxib, a selective inhibitor of COX-2, did not significantly alter the fever produced by ET-1 but did inhibit the production of PGs in the CSF induced by the peptide (8). Higher doses (5 and 10 mg/kg) blocked both responses. Thus, despite the fact that ICV injection of ET-1 increases PGs production in the CSF, we suggest that these eicosanoids are not essential for the development of ET-1-induced fever in rats. On the other hand, the observation that IL-1ra inhibits ET-1-induced fever suggests that this response is strongly mediated by IL-1. Moreover, IL-1 was shown to directly interact with thermosensitive neurons in the POA (37). Therefore, it is suggested that ET-1 can act through PG- (and COX-2) independent mechanism(s) and that IL-1 produced in response to ET-1 directly interacts with thermosensitive neurons in the POA. It remains to be investigated whether the stimulation of IL-1 receptors could further stimulate the production of PGs.

Although the specific isoform of ET produced in response to CRF was not characterized in the present study, recent data from our laboratory, along with other experimental evidence indicate that ET-1 is the isoform preferentially involved in the mechanisms of fever. In fact, the ET family consists of four isoforms of the ET peptide, ET-1, ET-2, ET-3, and vasoactive intestinal contractor (VIC) (25). ET-2, which has been found in humans (11), is not present in rats (1). VIC is thought to be the mouse or rat counterpart of human ET-2 (1). However, the expression of this isoform of ET in the hypothalamus is at most twofold lower than that reported for ET-1 (21) and the selectivity of VIC for ET_A and ET_B receptors remains unclear. We observed that pyrogenic doses of LPS induce an increase of ET-1 levels in the CSF of rats and that a dose 30-fold higher of ET-3 (30 pmol) is needed to induce an increase in the rectal temperature similar to that induced by 1 pmol ET-1 injected intracerebroventricularly (Fabricio, unpublished observations). Moreover, in vitro and in vivo studies have shown that ET-1, but not ET-3, is able to activate the hypothalamic-pituitary-adrenal axis and to subsequently trigger ACTH release (2, 15).

Previous data from our group (7, 8) and the results of the present study lead us to propose that the activation of the central ET system occurs in a PG-independent pathway for induction of fever, at a position downstream from PFPF (produced by macrophages) and CRF (released from hypothalamic neurons), which in turn stimulates ET_B receptors coupled to local IL-1β release (Fig. 7). This pathway runs in parallel to a second, indomethacin-sensitive, fever pathway that requires peripheral TNF-α, IL-1β, and IL-6, which then trigger release of PGE_2, $PGF_{2\alpha}$, and CRF in the brain. Interestingly, CRF seems to contribute to both pathways. This novel ET_B receptor-mediated pathway presented here, which seems to contribute toward LPS-induced fever (7), might constitute a target for novel antipyretic therapy. The fact that bosentan, a systemically active blood-brain barrier permeating dual ET_A/ET_B receptor antagonist, is already clinically used to treat other disease states should encourage and facilitate studies on its antipyretic potential in humans.

ACKNOWLEDGMENTS

We are most grateful to Dr. Stephen Poole (National Institute of Biological Standards and Control, England) for the kind gifts of rat recombinant IL-6, human recombinant IL-1ra, and CRF_{1-41}. We also thank Dr. M. Clozel from Actelion (Allschwil, Switzerland) who kindly provided bosentan, as well as to Miriam Cristina Contin de Melo, Juliana Aparecida Vercesi, and Rodrigo Cesar Penatti for their expert technical assistance.

Present address of A. S. C. Fabricio: Institute of Pharmacology, Catholic University Medical School, 00168 Rome, Italy (E-mail: alinefabricio@hotmail.com).

GRANTS

We thank Fundação de Amparo à Pesquisa do Estado de São Paulo for financial support (FAPESP Grants 97/09837-6 and 98/09738-0).

REFERENCES

1. **Bloch KD, Hong CC, Eddy RL, Shows TB, and Querternous T.** cDNA cloning and chromosomal assignment of the endothelin 2 gene: vasoactive intestinal contractor peptide is rat endothelin 2. *Genomics* 10: 236–242, 1991.
2. **Calogero AE, Raiti F, Nicolosi G, Burrello N, D'Agata R, and Mantero F.** Effects of endothelin-1 and endothelin-3 on rat hypothalamic corticotrophin-releasing hormone and pituitary ACTH release in vitro. *J Endocrinol* 140:419–424, 1994.
3. **Clozel M, Breu V, Gray AG, Kalina B, Löffler BM, Burri K, Cassal JM, Hirth G, Müller M, Neidhart W, and Ramuz H.** Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J Pharmacol Exp Ther* 270: 228–235, 1994.
4. **Davenport AP.** International Union of Pharmacology. XXIX. Update on endothelin receptor nomenclature. *Pharmacol Rev* 54: 219–226, 2002.
5. **De Souza GE, Cardoso RA, Melo MC, Fabricio ASC, Silva VM, Lora M, De Brum-Fernandes AJ, Rae GA, Ferreira SH, and Zampronio AR.** A comparative study of the antipyretic effects of indomethacin and dipyrone in rats. *Inflamm Res* 51: 24–32, 2002.
6. **Didier N, Romero IA, Creminon C, Wijkhuisen A, Grassi J, and Mabondzo A.** Secretion of interleukin-1β by astrocytes mediates endothelin-1 and tumour necrosis factor-α effects on human brain microvascular endothelial cell permeability. *J Neurochem* 86: 246–254, 2003.
7. **Fabricio ASC, Silva CAA, Rae GA, D'Orléans-Juste P, and Souza GEP.** Essential role for endothelin ET_B receptors in fever induced by LPS (*E. coli*) in rats. *Br J Pharmacol* 125: 542–548, 1998.
8. **Fabricio ASC, Veiga FH, Cristofoletti R, Navarra P, and Souza GEP.** The effects of selective and nonselective cyclooxygenase inhibitors on endothelin-1-induced fever in rats. *Am J Physiol Regul Integr Comp Physiol* 288: R671–R677, 2005.
9. **Gordon CJ.** Thermal biology of the laboratory rat. *Physiol Behav* 47: 963–991, 1990.
10. **Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, and Thompson RC.** Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 343: 336–340, 1990.
11. **Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, and Masaki T.** The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA* 86: 2863–2867, 1989.
12. **Institute for Laboratory Animal Research. National Research Council.** *Guide for the Care and Use of Laboratory Animals.* Washington, DC: National Academy Press, 1996.
13. **Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuruda T, Fukami T, Ozaki S, Nagase T, Nishikibe M, and Yano M.** Biochemical and pharmacological profile or newly-developed, potent and selective endothelin B receptor antagonist, BQ-788. *Proc Natl Acad Sci USA* 91: 4892–4896, 1994.
14. **Kedzierski RM and Yanagisawa M.** Endothelin system: the double-edged sword in health and disease. *Annu Rev Pharmacol Toxicol* 41: 851–876, 2001.
15. **Kiefer F, Kellner M, Jahn H, and Wiedemann K.** Comparison of the effects of endothelin-1 and -3 on secretion of pituitary hormones in healthy male volunteers. *Exp Clin Endocrinol Diabetes* 108: 378–381, 2000.
16. **Kluger MJ.** Fever: role of pyrogens and cryogens. *Physiol Rev* 71: 93–127, 1991.
17. **Long NC, Morimoto A, Nakamori T, and Murakami N.** Systemic injection of TNF-α attenuates fever due to IL-1β and LPS in rats. *Am J Physiol Regul Integr Comp Physiol* 263: R987–R991, 1992.
18. **Luheshi G, Miller AJ, Brouwer S, Dascombe MJ, Rothwell NJ, and Hopkins SJ.** Interleukin-1 receptor antagonist inhibits endotoxin fever

and systemic interleukin-6 induction in the rat. *Am J Physiol Endocrinol Metab* 270: E91–E95, 1996.

19. **Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, and Moreau JL.** A nonpeptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur J Pharmacol* 309: 195–200, 1996.
20. **Masaki T.** Historical review: endothelin. *Trends Pharmacol Sci* 25: 219–224, 2004.
21. **Masuo Y, Ishikawa Y, Kozakai T, Uchide T, Komatsu Y, and Saida K.** Vasoactive intestinal contractor/endothelin-2 gene expression in the murine central nervous system. *Biochem Biophys Res Commun* 300: 661–668, 2003.
22. **Miñano FJ, Sancibrian M, Viszcaino M, Paez X, Davatelis G, Fahey T, Sherry B, Cerami A, and Myers RD.** Macrophage inflammatory protein-1: unique action on the hypothalamus to evoke fever. *Brain Res Bull* 24: 849–852, 1990.
23. **Murakami Y, Okada S, and Yokotani K.** Brain inducible nitric oxide synthase is involved in interleukin-1β-induced activation of the central sympathetic outflow in rats. *Eur J Pharmacol* 455: 73–78, 2002.
24. **Navarra P, Tsagarakis S, Faria MS, Rees LH, Besser GM, and Grossman AB.** Interleukins-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. *Endocrinology* 128: 37–44, 1991.
25. **Nayler WG.** Endothelin: isoforms, binding sites and possible implications in pathology. *Trends Pharmacol Sci* 11: 96–99, 1990.
26. **Roth J and De Souza GE.** Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz J Med Biol Res* 34: 301–314, 2001.
27. **Roth J, Martin D, Storr B, and Zeisberger E.** Neutralisation of pyrogen-induced tumour necrosis factor by its type 1 soluble receptor in guinea-pigs: effects on fever and interleukin-6 release. *J Physiol* 509: 267–275, 1998.
28. **Rothwell NJ.** CRF is involved in the pyrogenic and thermogenic effects of interleukin 1β in the rat. *Am J Physiol Endocrinol Metab* 256: E111–E115, 1989.
29. **Rothwell NJ.** Central activation of thermogenesis by prostaglandins: dependence on CRF. *Horm Metab Res* 22: 616–618, 1990.
30. **Rothwell NJ, Busbridge NJ, Lefeuvre RA, Hardwick AJ, Gauldie J, and Hopkins SJ.** Interleukin-6 is a centrally acting endogenous pyrogen in the rat. *Can J Physiol Pharmacol* 69: 1465–1470, 1991.
31. **Speciale L, Roda K, Saresella M, Taramelli D, and Ferrante P.** Different endothelins stimulate cytokine production by peritoneal macrophages and microglial cell line. *Immunology* 93: 109–114, 1998.
32. **Strijbos PJ, Hardwick AJ, Relton JK, Carey F, and Rothwell NJ.** Inhibition of central actions of cytokines on fever and thermogenesis by lipocortin-1 involves CRF. *Am J Physiol Endocrinol Metab* 263: E632–E636, 1992.
33. **Takahashi S, Kapas L, and Krueger JM.** A tumor necrosis factor (TNF) receptor fragment attenuates TNF-α- and muramyl dipeptide-induced sleep and fever in rabbits. *J Sleep Res* 5: 106–114, 1996.
34. **Tringali G, Mirtella A, Mancuso C, Guerriero G, Preziosi P, and Navarra P.** The release of immunoreactive interleukin-1β from rat hypothalamic explants is modulated by neurotransmitters and corticotropin-releasing hormone. *Pharmacol Res* 36: 269–273, 1997.
35. **Watanabe K, Kinoshita S, and Nakagawa H.** Purification and characterization of cytokine-induced neutrophil chemoattractant produced by epithelioid cell line of normal rat kidney (NRK-52E cell). *Biochem Biophys Res Commun* 161: 1093–1099, 1989.
36. **Wilson SH, Simari RD, and Lerman A.** The effect of endothelin-1 on nuclear factor κB in macrophages. *Biochem Biophys Res Commun* 286: 968–972, 2001.
37. **Xin L and Blatteis CM.** Blockade by interleukin-1 receptor antagonist of IL-1 beta-induced neuronal activity in guinea pig preoptic area slices. *Brain Res* 569: 348–352, 1992.
38. **Yasin SA, Costa A, Navarra P, Pozzoli G, Kostoglou-Athanassiou I, Forsling ML, and Grossman A.** Endothelin stimulates the in vitro release of neurohypophyseal hormones, but not corticotrophin-releasing hormone, via ET_A receptors. *Neuroendocrinology* 60: 553–558, 1994.
39. **Zampronio AR, Melo MCC, Hopkins SJ, and Souza GEP.** Involvement of CRH in fever induced by a distinct preformed pyrogenic factor (PFPF). *Inflamm Res* 49: 473–479, 2000.
40. **Zampronio AR, Melo MCC, Silva CAA, Pelá IR, Hopkins SJ, and Souza GEP.** A preformed pyrogenic factor released by lipopolysaccharide-stimulated macrophages. *Mediat Inflam* 3: 365–373, 1994.
41. **Zampronio AR, Souza GEP, Silva CAA, Cunha FQ, and Ferreira SH.** Interleukin-8 induces fever by a prostaglandin-independent mechanism. *Am J Physiol Regul Integr Comp Physiol* 266: R1670–R1674, 1994.
42. **Zeisberger E.** From humoral fever to neuroimmunological control of fever. *J Therm Biol* 24: 287–326, 1999.
43. **Zidovetzki R, Chen P, Chen M, and Hofman FM.** Endothelin-1-induced interleukin-8 production in human brain-derived endothelial cells is mediated by the protein kinase C and protein tyrosine kinase pathways. *Blood* 94: 1291–1299, 1999.