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

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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-
genes, such as IL-6, PFPF, PGE_2, PGF_2α, 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 CRF1/CRF2 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 ETB-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°C with a 12:12-light-dark cycle (lights on at 0600) with free access to food, water, 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.** Racial 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 telemethermometer; 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). Briefly, 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 isoelectric 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 ETα 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 pmol), PGF_2α (2.1 nmol), IL-6 (1.46 pmol), PFPF (200 ng of protein), or CRF (1.05 nmol). Some animals were also pretreated with BQ-123 [selective peptidic ETα receptor antagonist (4); 3 pmol icv] or bosen tan [dual nonpeptidic ET_α/ ET_β receptor antagonist (3); 10 mg/kg iv] 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 CRF1/CRF2 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_2α, and rat α-helical CRF_9–41 (Sigma, St. Louis, MO); rat recombinant IL-6, human recombinant IL-1α, 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-dimethylpipеридинокарбо-нл-г-метиллуксил-1-1-этиоксокарбо-нл-норлеукени) and ET-1 (Research Biochemicals International, Natick, MA); bosentan, kindly provided by Dr. M. Clozel (Actelion, Allschwil, Switzerland); murine IL-1β (lot no. BNO24121), rat TNF-α, and murine sTNFRI (R&D Systems, Minneapolis, MN); dexamethasone (Decadronal; Prodome Systems, Minneapolis, MN); dexamethasone (Decadronal; Prodome Laboratories, Campinas, Brazil); and oxytetracycline hydrochloride (Terramicina; Pfizer Laboratorios, 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 ± 0.09°C) and 4.5 h (ΔT of 0.74 ± 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. 1A). Nonetheless, the febrile response induced by PFPF was virtually abolished by BQ-788 pretreatment (Fig. 1B).

As previously described (5), ICV injection of PGE2 (1.42 nmol) induced a short-lived monophasic febrile response, whereas PGF2α (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 PGE2 or PGF2α (Fig. 2).

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 ± 0.14°C; Fig. 3). Prior ICV treatment with the selective ETA receptor antagonist BQ-123 (3 pmol) did not modify CRF-induced fever. In contrast, pretreatment with BQ-788 (3 pmol icv) or the dual ETA/ETB 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 CRF9–41, 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 CRF9–41 (6.5 nmol), a dual CRF1/CRF2 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. 1. Influence of BQ-788, a selective ETb 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 l) 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 ± SE of the changes in rectal temperature (°C) of 4 – 6 animals. Basal temperatures (means ± SE; °C) were as follows: for A, 36.88 ± 0.03 (E), 36.98 ± 0.03 (F), 36.97 ± 0.08 (O), and 37.0 ± 0.07 (■); for B, 36.9 ± 0.08 (*), 37.0 ± 0.06 (O), 36.94 ± 0.1 (O), and 37.05 ± 0.085 (■). BQ-788 failed to significantly modify responses to either PG (P > 0.05, one-way ANOVA test).

Fig. 2. BQ-788, a selective ETb receptor antagonist, failed to modify fever induced by PGE2 and PGF2α in rats. BQ-788 (3 pmol icv) or aCSF (3 l) was administered 15 min before ICV injection of either aCSF, PGE2 (1.42 nmol; A) or PGF2α (2.1 nmol; B). Values represent means ± SE of ΔT of 4 – 10 animals. Basal temperatures (means ± SE; °C) were as follows: for A, 36.88 ± 0.03 (E), 36.98 ± 0.03 (F), 36.97 ± 0.08 (O), and 37.0 ± 0.07 (■); for B, 36.9 ± 0.08 (*), 37.0 ± 0.06 (O), 36.94 ± 0.1 (O), and 37.05 ± 0.085 (■). BQ-788 failed to significantly modify responses to either PG (P > 0.05, one-way ANOVA test).

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nmol, 15 min beforehand intracerebroventricularly), at a dose that markedly reduced fever induced by IL-1/H9252, 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-/H9251 (14.7 pmol icv), did not modify the fever caused by ET-1 (Fig. 6).

Baseline temperature of control animals was unaffected by ICV/H9251-helical CRF9–41, 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 ETB 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, PGE2, and PGF2a.
In our previous study (7), we showed that ICV ET-1, acting through central ETB (but not ETA) 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 ETB receptor antagonist BQ-788, but not the selective ETA 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 ETB receptor-coupled mechanisms. We have already shown that indomethacin-sensitive (i.e., PG-dependent) fever induced by ICV IL-1β/H9252 or TNF-α/H9251 (5) is entirely resistant to selective ETB 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 PGE2, PGF2α, 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 PGF2α are both susceptible to blockade by the dual CRF1/CRF2 receptor antagonist α-helical CRF9–41 (28, 29, 32, 39). Moreover, fever induced by intravenous IL-1β (19) or ICV CRF (M. J. Figueiredo, unpublished observation) is inhibited by selective CRF1 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 ETB receptor antagonist also attenuated the fever...
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 ETB 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 CRF9-41, 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 inhibited fever induced by ET-1 (as well as by PFPF or CRF) implicates IL-1 in the fever mechanisms situated downstream from ETB 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

induced by CRF. In a study (13) investigating the specificity of BQ-788 for the ETB 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 non-specific 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 ETα/ETB receptor antagonist, but not by the ETα-receptor-selective peptidic antagonist BQ-123. Together, these results indicate a specific recruitment of ETB receptors during the fever induced by CRF. In addition, ICV CRF and PFPF also promote an integrated febrile response in rats, in which core
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-1α 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 peptide (VIP) (25). ET-2, which has been found in humans (11), is not present in rats (1). VIP 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 VIP for ETα and ETβ 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 (pro-adrenal axis and to subsequently trigger ACTH release (2, 15).

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GRANTS

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18. R170 ETa RECEPTORS MEDIATE IL-1-DEPENDENT FEVER INDUCED BY PFPF AND CRF

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