Endogenous opioids: role in prostaglandin-dependent and -independent fever

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Fraga D, Machado RR, Fernandes LC, Souza GE, Zampronio AR. Endogenous opioids: role in prostaglandin-dependent and independent fever. Am J Physiol Regul Integr Comp Physiol 294: R411–R420, 2008. First published November 21, 2007; doi:10.1152/ajpregu.00465.2007.—This study evaluated the participation of μ-opioid-receptor activation in body temperature (Tb) during normal and febrile conditions (including activation of heat conservation mechanisms) and in different pathways of LPS-induced fever. The intracerebroventricular treatment of male Wistar rats with the selective opioid μ-receptor-antagonist cyclic D-Phe-Cys-Try-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP; 0.1–1.0 μg) reduced fever induced by LPS (5.0 μg/kg) but did not change Tb at ambient temperatures of either 20°C or 28°C. The subcutaneous, intracerebroventricular, and intrahypothalamic injection of morphine (1.0–10.0 mg/kg, 3.0–30.0 μg, and 1–100 ng, respectively) produced a dose-dependent increase in Tb. Intracerebroventricular morphine also produced a peripheral vasoconstriction. Both effects were abolished by CTAP. CTAP (1.0 μg icv) reduced the fever induced by intracerebroventricular administration of TNF-α (250 ng), IL-6 (300 ng), CRF (2.5 μg), endothelin-1 (1.0 pmol), and macrophage inflammatory protein (500 pg) and the first phase of the fever induced by PGE2 (500 ng) but not the fever induced by IL-1β (3.12 ng) or PGE2 (125.0 ng) or the second phase of the fever induced by PGE2. Morphine-induced fever was not modified by the cyclooxygenase (COX) inhibitor indomethacin (2.0 mg/kg). In addition, morphine injection did not induce the expression of COX-2 in the hypothalamus, and CTAP did not modify PGE2 levels in cerebrospinal fluid or COX-2 expression in the hypothalamus after LPS injection. In conclusion, our results suggest that LPS and endogenous pyrogens (except IL-1β and prostaglandins) recruit the opioid system to cause a μ-receptor-mediated fever.

THE REGULATION OF BODY TEMPERATURE (Tb) is under the control of a hierarchy of neuronal structures that must first integrate afferent and central information before activating appropriate physiological and behavioral responses. In mammals, Tb is regulated with considerable precision, normally varying by only a few degrees Celsius. This is an important adaptation because most biochemical and physiological processes are temperature dependent. In some conditions, adjustments of Tb depend on a number of factors, including species, dosage, route of administration, restraint, ambient temperature, and the type of receptor activated (1). At least three distinct opioid receptors have been identified: μ, κ, and δ (19, 23, 41). All are present in the POA-AH (39). Evidences from studies that used selective agonists in rats indicated that the activation of μ-opioid receptors produces hyperthermia (29, 53), whereas activation of κ-opioid receptor induces hypothermia (17, 53). There are conflicting reports about the participation of δ-opioid receptor in the control of Tb (12, 29, 53), although more recent studies suggest that its activation also causes hypothermia (45, 46). During fever, the endogenous opioid release in the CNS is also unclear. For example, selective opioid antagonists reduced

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the febrile response induced by LPS, but these antagonists were unable to modify the febrile response induced by IL-1β, which is seen as an important contributor to LPS-induced fever (7, 8, 30). IL-1-induced fever was blocked by buprenorphine (55), which has been reported to have high affinities for μ-, κ-, and δ-opioid receptors with \(K_i\) values in the nanomolar range (31). However, other cytokines seem to induce fever through opioid release (5, 8, 30).

In light of these considerations, the present study was undertaken to assess the relationship between the opioid system and known endogenous pyrogens recruited by LPS to establish the relative position of this system in the neural chain mechanism of fever.

**MATERIALS AND METHODS**

**Animals.** Experiments were conducted with male Wistar rats weighing 180–200 g, housed four or five per cage at 24 ± 1°C under a 12:12-h light-dark cycle (lights on 0700) and with free access to food chow and tap water. All experiments were approved by the institution’s ethics committee for research on laboratory animal use and are in accordance with the guidelines set by the National Institutes of Health.

**Intracerebral cannula implantation and microinjection.** Under anesthesia with pentobarbital sodium (40 mg/kg ip; Sigma, St. Louis, MO), a permanent 22-gauge stainless steel guide cannula (0.8 mm outer diameter, 10 mm long) was stereotaxically implanted into the right lateral ventricle at the following coordinates: 1.6 mm lateral to the midline, 1.5 mm posterior to bregma, and 2.5 mm under the brain surface, with the incisor bar lowered 2.5 mm below the horizontal zero (43). In other rats, for intrahypothalamic (ih) injection, a 24-gauge stainless steel guide cannula (0.55 mm outer diameter, 15 mm long) was stereotaxically and unilaterally implanted into the POA-AH. Its stereotaxic coordinates were as follows: 0.6 mm lateral to the midline, 7.7 mm anterior to the interaural line, and 6.5 mm under the brain surface, with the incisor bar lowered 3.0 mm below the horizontal zero (43). Cannulas were fixed to the skull with jeweler’s screws embedded in dental acrylic cement. All procedures were conducted under aseptic conditions. Animals were treated with oxytetracycline hydrochloride (400 mg/kg im) and allowed to recover for 1 wk before experimental use.

After each experiment, each rat was microinjected into the lateral ventricle (5 μl) or into the POA-AH (500 nl) with Evans blue (2.5%). Immediately after dye microinjection, each rat was given an overdose of pentobarbital sodium and perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde. Brains were removed, stored in the same fixative for 6 h, kept in 30% sucrose overnight, and cut at 40 μm on a freezing microtome. From an analysis of the histological material under light microscopy, the positions of the cannulas and micropores were added on the tape. On the day of the experiment, the baseline temperature of each animal was determined by four measurements at 30-min intervals before any injection. Only animals displaying a mean basal rectal temperature between 36.2 and 37.4°C were selected for the study. To access the changes in the peripheral vasomotor tone, the heat loss index (HLI) was calculated to eliminate direct influences of both ambient temperature (\(T_{\text{ambient}}\)) and \(T_b\) on skin temperature (\(T_{\text{skin}}\)), according to the following formula: \(\text{HLI} = \left( T_{\text{skin}} - T_{\text{ambient}} \right) / \left( T_b - T_{\text{ambient}} \right) \). The value of HLI varies from 0 (full vasoconstriction) to 1 (full vasodilation) (47).

**Experimental design.** In the first set of experiments, we evaluated the effects of CTAP, a μ-selective opioid antagonist, on LPS-induced fever to establish a dose of antagonist for the subsequent experiments. First, we validated the opioid involvement in the febrile response induced by LPS by using the nonselective opioid antagonist naloxone. Animals were pretreated with naloxone (0.3 and 1.0 mg/kg sc; Sigma), with CTAP (0.1, 0.3, and 1.0 μg icv; Peninsula Laboratory), or with the appropriate volume of sterile saline 30 min before LPS administration (5.0 μg/kg ip from E. coli 0111:B4; Sigma). The 1.0-μg dose of CTAP was chosen for the remaining experiments. The second goal of these experiments was to verify whether this effective dose of CTAP would induce changes in \(T_b\) either at 20 or 28°C. Control animals received propranolol (20.0 mg/kg ip; Sigma), a nonselective β-blocker, or saline (1.0 ml/200 g ip) to confirm that these ambient temperatures were sufficient to induce changes in the thermoregulatory system.

In the next set of experiments, animals received morphine (1.0, 3.0, and 10.0 mg/kg sc or 3.0, 10.0, and 30.0 μg icv or 1.0, 10.0, and 100.0 ng ih; Merck Sharp & Dome). The animals were also pretreated with 1.0 μg icv CTAP 30 min before the injection of morphine (10.0 μg icv) to evaluate simultaneously the effect of the μ-opioid receptors on body and tail skin temperature.

The next set of experiments was designed to evaluate the participation of opioids on the febrile response induced by peripheral and central mediators. Rats received an intracerebroventricular injection of either CTAP (1.0 μg) or saline 30 min before a similar intracerebroventricular injection of 3.12 ng IL-1β, 250.0 ng TNF-α, 300.0 ng IL-6, 500.0 μg MIP-1α, 2.5 μg CRF, 125.0 ng PGE_2, 500.0 ng PGF_2α, or 1.0 pmol ET-1. The doses of pyrogens were chosen based on dose–response curves determined in previous studies and do not induce supramaximal responses (24, 56, 58).

The following experiments were designed to investigate the interaction between the activation of μ-opioid receptor and of prostaglandin synthesis during the febrile response. First, we evaluated the effect of the nonselective cyclooxygenase COX) inhibitor indomethacin (2.0 mg/kg ip; Merck Sharp & Dome) on the increase of \(T_b\) induced by morphine (3.0 mg/kg sc) or LPS (5.0 μg/kg ip). Control animals received Tris-HCl buffer (pH 8.2) intraperitoneally (1.0 ml/200 g). Afterward, animals were pretreated with indomethacin (2.0 mg/kg ip), CTAP (1.0 μg icv), or the same volume of vehicles (Tris buffer or saline) 30 min before the administration of 5.0 μg/kg ip LPS. Three
hours after administration of LPS, cerebrospinal fluid was collected and frozen at -70°C for subsequent PGE2 level analysis. In the last set of experiments, the animals were pretreated with 1.0 μg of CTAP or the same volume of saline 30 min before LPS (5 μg/kg ip) or morphine (3.0 mg/kg sc) administration. Three hours after the treatment, Tb was evaluated and the hypothalamus removed for Western blot analysis of COX-2 expression.

Cerebrospinal fluid collection and PGE2 ELISA assay. Under anesthesia with pentobarbital sodium (40 mg/kg ip; Sigma), cerebrospinal fluid was directly aspirated through a syringe from the cisterna magna, and the volume of cerebrospinal fluid collected varied from 50 to 100 μl. Samples were transferred to small plastic tubes containing 2 μl of indomethacin (2.5 μg/μl) and centrifuged at 1,000 g, 4°C, for 10 min. The level of PGE2 in the cerebrospinal fluid was analyzed by an ELISA kit for PGE2 from Cayman Chemical.

Western blot analysis. Animals were decapitated, and the hypothalami were quickly removed and homogenized with a cold buffer [0.32 M sucrose, 1.0 mM EDTA, 5.0 mM Tris-HCl, 1.0 mM β-mecaptoethanol, and protease inhibitor (Complete Mini, EDTA-free 1 tablet for 10 ml of solution; Roche)]. Protein content was measured by the Bradford method (11), and 60.0 μg of protein were used in each well for electrophoresis separation in an unidirectional SDS-PAGE gel (12%). The separated proteins were electroblotted onto nitrocellulose membranes (Hybond-ECL; Amersham Biosciences) with a semi-dry blotter (HOEFEF-Mini VE; Amersham). Membranes were then washed with Trizma base (pH 7.4) containing 150 mM NaCl and 0.5% Tween 20 (TBS-T), and unspecific binding sites were blocked for 1 h in TBS-T containing 5% (wt/vol) skimmed milk. After a subsequent washing with TBS-T, antibodies against COX-2 (goat polyclonal IgG anti-COX-2 antibody; Santa Cruz Biotechnology, recommended for detection of rat, mouse, and human COX-2) were added at a dilution of 1:5,000 in TBS-T and incubated overnight. After three washes with TBS-T, membranes were treated for 2 h with horseradish peroxidase-conjugated rabbit anti-goat antibody IgG (Pierce) diluted to 1:5,000 in TBS-T. After six washes, the protein bands were revealed with an enhanced chemiluminescence Western blot analysis system (Super Signal system; Pierce). Blots were scanned with Scion Image software to detect the relative band intensity.

Statistical analysis. For data analyses of changes in body and skin temperature, the baseline temperature before any treatment was calculated for each animal by averaging the last three temperature measurements before any injection, and all subsequent temperatures were expressed as changes from this averaged value (ΔTb). HLI was calculated as described before. All data are reported as means ± SE and were analyzed for statistical significance by two-way ANOVA followed by Bonferroni’s test. The percent reduction was calculated using the area under the curve between control (100%) and treated group. The data were analyzed by Prism software (Graph-Pad, San Diego, CA). Significance was set at P < 0.05.

RESULTS

Our initial goal was to confirm that endogenous opioids acting on μ-opioid receptors participate in LPS-induced fever and to establish effective doses of the antagonist for the subsequent experiments. Administration of LPS at 5.0 μg/kg ip induced an increase in Tb that started 1.5 h after the injection, peaked at 2.5 h, and lasted until 6 h. Treatment of the animals with 1.0 mg/kg sc naloxone did not alter Tb. However, this dose of naloxone administered 30 min before LPS injection abolished the fever (Fig. 1, A and C), whereas a dose of 0.3 mg/kg partially reduced the response (50.1%; Fig. 1C). To assess the role of μ-opioid receptors in this effect, we used CTAP, a selective μ-opioid-receptor antagonist. Pretreatment of the animals with CTAP (0.1, 0.3, and 1.0 μg icv) significantly reduced the febrile response induced by LPS in 49.5, 34.1, and 86.3%, respectively (Fig. 1, B and D). The dose of CTAP that produced maximal reduction on febrile response (1 μg) was chosen for the subsequent experiments. Because some studies showed that the blockage of opioid receptors can cause hypothermia, we evaluated the effect of this dose of CTAP on normal Tb at different ambient temperatures. The administration of 20.0 μg/kg ip propranolol induced a reduction in Tb that was more intense at 20°C than at 28°C, whereas 1.0 μg icv CTAP did not alter Tb at either of the ambient temperatures studied (Fig. 1, E and F).

We then evaluated whether an opioid agonist, morphine, would induce fever. Figure 2 shows that the peripheral, intracerebroventricular, or intrahypothalamic administration of morphine induced a dose-dependent increase in Tb (Fig. 2, A–C, respectively) that started immediately after the administration and peaked between 1 and 2 h. The increase in Tb induced by 10.0 μg icv morphine occurred simultaneously with a reduction in the tail skin temperature (Fig. 3A). Similarly, the HLI dropped from 0.7 to −0.4, indicating the occurrence of vasoconstriction (Fig. 3B). The administration of CTAP (1.0 μg icv) reduced both the rise in Tb and the vasoconstriction (HLI) (Fig. 3).

Once the effect of μ-opioid-receptor blockage was characterized in LPS-induced fever, we decided to evaluate the effect of this blockage on the febrile response induced by the cytokines released after LPS injection. Figure 4A shows that IL-1β induced a significant febrile response, which started 30 min after injection, peaked at 1.5 h, and lasted until hour 6. Pretreatment of the animals with CTAP did not reduce the febrile response induced by this cytokine (Fig. 4A). TNF-α induced a febrile response similar to IL-1, whereas IL-6 induced a more slow-onset response that peaked at 4 h after the injection. Although CTAP (1 μg icv) reduced only partially the febrile response induced by TNF-α (41%, Fig. 4B), IL-6-induced fever was more sensitive to the same treatment with the μ-opioid antagonist (78%; Fig. 4C).

We then evaluated the effect of CTAP on known central mediators of the febrile response induced by these cytokines. Central administration of PGE2 promoted a rapid and intense, but short-lasting, increase in the rectal temperature. The μ-opioid-antagonist CTAP did not modify this response (Fig. 5A). In a different way, PGF2α induced a biphasic response: a rapid and intense increase in rectal temperature similar to that induced by PGE2 followed by a second increase that started 2 h after injection and lasted until hour 6. However, the first peak of fever induced by PGF2α, diversely from PGE2-induced fever, was reduced by CTAP (50%; Fig. 5B), whereas the second phase remained completely unchanged. CRF induced a febrile response that initiated at 30 min, peaked at 4.5 h, and lasted until the end of the experiment. The μ-opioid-antagonist CTAP significantly reduced this febrile response (57%; Fig. 5C).

We then focused our next set of experiment on a better assessment of the interaction between prostaglandin production, probably the most significant event on febrile induction, and its interaction with μ-opioid receptors on fever. The pretreatment of the animals with indomethacin (2.0 mg/kg ip) reduced the febrile response induced by 5.0 μg/kg ip LPS from 2.5 to 6.0 h (data not shown) but did not change the elevation of Tb induced by morphine (3.0 mg/kg sc; Fig. 6A). Indometh-
acine also reduced the increase in prostaglandin levels induced by LPS (51.7%; data not shown). Nevertheless, pretreatment of the animals with 1.0 μg icv CTAP significantly reduced the febrile response (temperature values above the bars on Fig. 6B) but did not change the increased PGE2 levels in the cerebrospinal fluid induced by LPS (Fig. 6B). To confirm these results, we analyzed COX-2 expression, the main prostaglandin-producing enzyme during fever, in the hypothalamus. Figure 6C shows that administration of 3.0 mg/kg morphine alone or in combination with CTAP did not alter significantly the basal COX-2 expression in the hypothalamus. In a different way, administration of LPS increased COX-2 expression in the hypothalamus, but the treatment of the animals with CTAP did not change these levels (Fig. 6D).

The last set of experiments was designed to evaluate the effect of CTAP on stimuli that induce prostaglandin-independent febrile response (24, 40, 56). MIP-1α and ET-1 induced a febrile response that initiated at 30 min to 1 h after injection, peaked at 4.5 h, and lasted until the end of the experiment. The μ-opioid-antagonist CTAP effectively reduced the febrile response induced by MIP-1α (90%; Fig. 7A) and ET-1 (81%; Fig. 7B).

DISCUSSION

This study supports the proposal that, at the thermoneutral zone or lower temperatures, endogenous opioids acting on the μ-opioid receptor do not seem to play an important role in Tb control in rats, although these opioids are essential to the febrile response induced by LPS. During LPS-induced fever, these endogenous opioids are either released simultaneously to prostaglandin synthesis and/or activate a prostaglandin-independent pathway.

We found that CTAP, a μ-selective-receptor antagonist, at a dose that completely blocked the febrile response did not change the Tb of the animals. Similar results after central injection of CTAP or nor-binaltorphimine (nor-BNI), a κ-opioid-receptor antagonist, were also observed by Ghosh et al. (26). Recently, Chen et al. (18) also observed that, at low doses, opioid antagonists did not cause any change in Tb. However, at higher doses, nor-BNI induced hypothermia and CTAP induced hypothermia and these effects could be blocked by CTAP and nor-BNI, respectively. These data suggest that there is a tonic balance between μ- and κ-opioid receptors; e.g., the selective blockade of the κ-opioid receptor would allow endogenous μ-opioid activity to be seen. Although that is a possibility, it is difficult to understand why this effect is observed only at higher doses, although smaller doses of these antagonists can completely block temperature changes induced by several stimuli (4, 26). It should be considered that these drugs, at higher doses, may lose their selectivity and/or have agonistic activity for one type of receptor and antagonistic activity for other type. There is at least one study that shows
that CTAP analogs can have simultaneous δ-agonist and μ-antagonist activities at high doses (9). We show here that, even when we submit the animals to lower ambient temperatures, the μ-opioid antagonist does not cause a hypothermic effect at low doses. Our results suggest that activation of μ-opioid receptors is not crucial for maintaining normal Tb or that the tonic balance between μ- and κ-opioid receptors proposed by Chen et al. (18) is not sensitive to small changes in the activation of opioid receptors.

However, we and others have observed that, when the thermoregulatory system is required to control the Tb at higher levels, such as during LPS fever, the opioid system (through μ-opioid receptors) is highly activated and becomes more sensitive to the effects of blockade with antagonists. In this way, after the injection of LPS, there is an activation of the endogenous opioid system because the resultant fever can be blocked by the nonspecific antagonist naloxone and the μ-specific-receptor antagonist CTAP (7, 8). Also, Carr et al. (16) and Murphy and Lipton (42) showed that plasma and central β-endorphins, respectively, increase immediately after or during the rising temperature phase of fever and that these levels decrease during recovery (42). Our findings are also in agreement with a more recent study that showed the unresponsiveness of μ-opioid-receptor knockout mice to LPS-induced fever (6) and altogether support the idea that the opioid system through the activation of the μ-opioid receptor is involved in LPS-induced febrile response. This statement is confirmed by several studies that show that different μ-opioid agonists given centrally in rats induce an increase in Tb (3, 28, 29, 53). Similarly, in this study, the peripheral, intracerebroventricular, or intrahypothalamic administration of morphine induced a dose-dependent increase in Tb. However, we also show here that the time course of the increase in Tb induced by morphine matched the time course of decrease in tail skin temperature and a peripheral vasoconstriction, suggesting that the effect of morphine in Tb results from central activation (since intracerebroventricular injection causes both effects) of heat-conser-

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**Fig. 2.** Effect of morphine on Tb. Animals received subcutaneous (1.0, 3.0, or 10.0 mg/kg; A), intracerebroventricular (3.0, 10.0, or 30.0 μg/2 μl; B), or intrahypothalamic (1.0, 10.0, or 100.0 ng/0.5 μl; C) morphine (Morph) or the same volume of sterile Sal (1.0 ml/kg sc, 2.0 μl icv, or 0.5 μl ih). Values are means ± SE of the change in rectal temperature (ΔTb) in °C observed in 5–9 rats. Basal temperatures (means ± SE; °C) were as follows: in A: 37.1 ± 0.1 (●), 37.0 ± 0.0 (●), 37.0 ± 0.1 (●), and 36.9 ± 0.1 (●); in B: 37.2 ± 0.1 (●), 37.0 ± 0.2 (●), 37.1 ± 0.1 (●), 37.2 ± 0.0 (●); in C: 37.1 ± 0.0 (●), 37.2 ± 0.1 (●), 37.0 ± 0.1 (●), and 37.0 ± 0.2 (●). *P < 0.05 compared with saline group.

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**Fig. 3.** Effect of morphine on Tb and skin temperature and the involvement of μ-opioid receptor is involved in LPS-induced fever (6) and altogether support the idea that the opioid system through the activation of the μ-opioid receptor is involved in LPS-induced febrile response. This statement is confirmed by several studies that show that different μ-opioid agonists given centrally in rats induce an increase in Tb (3, 28, 29, 53). Similarly, in this study, the peripheral, intracerebroventricular, or intrahypothalamic administration of morphine induced a dose-dependent increase in Tb. However, we also show here that the time course of the increase in Tb induced by morphine matched the time course of decrease in tail skin temperature and a peripheral vasoconstriction, suggesting that the effect of morphine in Tb results from central activation (since intracerebroventricular injection causes both effects) of heat-conser-
receptors consistent with the observed data. Altogether, these results suggest that morphine activates coordinated mechanisms of heat gain and conservation through the activation of the central μ-opioid receptors present in temperature-controlling neurons. Mahinda et al. (37) showed that morphine injected peripherally, at similar doses, causes a hypotension (vasodilation) that would lead to an increase in tail skin temperature (increased heat loss). Therefore, our results could not be explained by a peripheral action of morphine and are in agreement with previous studies (20, 36).

We and others showed recently that different pathways are activated during LPS-induced febrile response (24, 54). We then evaluated the participation of μ-opioid-receptor activation...
in the febrile response induced through these pathways, initially focusing on prostaglandin-dependent pathways. We demonstrate that CTAP did not modify the febrile response induced by IL-1β, reduced the fever induced by TNF-α, and almost abolished the fever induced by IL-6. Some of these results confirm previous results. Handler et al. (30), using the same antagonist, also did not observe a reduction in the febrile response induced by IL-1β. This is surprising because IL-1β is considered to be one of the most important endogenous pyrogens during LPS-induced fever and the LPS-induced fever is almost abolished by CTAP (5). In an opposite direction, Tsai et al. (55) showed that antagonizing opioid receptors can reduce the febrile response induced by IL-1β. However, in their study, they used buprenorphine, a partial μ-agonist and κ-antagonist. In addition, other studies showed that the febrile response induced by intravenous administration of TNF-α and IL-6 in guinea pigs were reduced by subcutaneous administration of naloxone (8) or, in the case of IL-6, the specific μ-opioid-antagonist CTAP in rats (5). This is the first study that shows that a selective μ-opioid-receptor antagonist reduced TNF-α-induced fever. TNF-α-induced fever and the effectiveness of the antagonists (naloxone vs. CTAP) are different in both studies (Ref. 8 and the present study), and this probably results from different routes of administration and animal species used, making comparisons between these studies difficult. Also, Blatteis et al. (8) could not infer whether naloxone was acting either within or outside the blood-brain barrier. Our results suggest that, at least in part, this action was in the CNS.

These cytokines induce fever through COX-2 induction (14, 15, 35) and consequently prostaglandin synthesis (21, 50). At first, it seems unreasonable that cytokines that induce fever through prostaglandin synthesis may have opioid-dependent and -independent pathways to induce fever. However, different prostaglandins and other central mediators seem to be involved in each cytokine-induced pathway. It seems that the most important mediator involved in the febrile response induced by TNF-α is PGE2, whereas for IL-1β- and IL-6-induced fever, PGE2, PGF2α, and CRF seem to be contributing (21, 24, 50, 58). The most reasonable explanation for these differences is that each cytokine, when administered separately, would activate different pathways maybe in different cell types. If some of these mediators were causing endogenous opioid release, one would expect that the febrile response induced by these mediators would be blocked by CTAP. The febrile response induced by CRF was significantly reduced by CTAP, whereas PGE2-induced fever remained unchanged. The first phase of PGF2α-induced fever was partially reduced; however, after some hours, the response seemed to reach normal levels.

These data may explain the reduction on IL-6-induced fever because it is dependent on CRF release but not the ineffectiveness of CTAP on IL-1β-induced fever and its effectiveness in TNF-α-induced fever. A possible explanation for this apparent contradiction in the case of IL-1β is the demonstration that this cytokine promotes a significant reduction in the binding of all opioid agonists (μ, κ, and δ) in several brain areas of guinea pigs, particularly of μ-opioid agonists in the hypothalamus (2).
induced by LPS, did not change the PGE2 levels in cerebrospinal fluid or COX-2 expression in the hypothalamus. Altogether, these data suggest to us that, during the febrile response induced by macrophage inflammatory protein-1α (MIP-1α) and endothelin-1 (ET-1), Animals received CTAP (1.0 μg/2 μl icv) or sterile Sal (2.0 μl icv) 30 min before the injection of MIP-1α (500 pg icv; A) or ET-1 (1 pg icv; B) or the same volume of sterile Sal (2.0 μl icv). Values are means ± SE of the change in the rectal temperature observed in 5–8 rats. Basal temperatures (means ± SE; °C) were as follows: for A: 37.1 ± 0.1 (□) and 37.2 ± 0.0 (●); for B: 37.1 ± 0.1 (□) and 37.2 ± 0.1 (●). *Values different from Sal/stimulus, \( P < 0.05 \).

Also, Ruzicka and Akil (51) demonstrated no evidence of expression of the \( \mu \)-opioid-receptor mRNA in nonstimulated or IL-1β-stimulated astrocytes from hypothalamus but an increased expression in cells from other brain regions such as cerebellum and hippocampus. Considering that the febrile response induced by TNF-α, but not by PGE2, is reduced by CTAP, there are two possible explanations. First, opioid release may precede prostaglandin release. Second, TNF-α-induced fever may depend on the concomitant release of prostaglandins and endogenous opioids. An additional evidence for this second hypothesis is that TNF-α-induced fever is only partially reduced by CTAP.

To better understand the interaction between prostaglandin synthesis and endogenous opioid release, we evaluated the effect of indomethacin, a nonspecific COX inhibitor, on morphine-induced fever, PGE2 levels in cerebrospinal fluid during LPS-induced fever after treatment with CTAP, and the effect of CTAP on COX-2 expression after LPS and morphine treatment. Indomethacin did not change the febrile response induced by morphine, suggesting that prostaglandins are not involved in this response. The blockade of \( \mu \)-receptors by CTAP, although it completely blocked the febrile response induced by LPS, did not change the PGE2 levels in cerebrospinal fluid or COX-2 expression in the hypothalamus. Also, morphine injection did not increase COX-2 expression in the hypothalamus. Altogether, these data suggest to us that, during fever, the release of endogenous opioids occurs after or concomitantly and not before prostaglandin synthesis.

There is convincing evidence that the ventromedial preoptic area of the hypothalamus is an important site of fever induction. Systemic injection of LPS or the injection of PGE2 in this area induced a selective Fos expression (22, 52), and both warm-sensitive and temperature-insensitive neurons in this area change their firing activity when treated with PGE2 (44). These warm-sensitive neurons may also provide excitatory input to the paraventricular nucleus (44). It is interesting to notice that \( \mu \)-opioid receptors are also found in the ventromedial preoptic area (39).

If this is true, then it is possible that other mediators that induce fever independently from prostaglandin synthesis may use the opioidergic system to do so. We evaluated two of these mediators: MIP-1α and ET-1. It was observed that pretreatment with CTAP abolished the fever induced by MIP-1α, suggesting that the synthesis and release of endogenous opioids are essential for this response. Other authors, using the same antagonist, have observed similar results for another member of the chemokine family (MIP-1β) (30). In our laboratory, it was shown that \( \alpha \)-helical CRF9–41 antagonist (dual CRF1/CRF2 receptor antagonist) blocks the fever induced by MIP-1α (Soares DM and Souza GE, unpublished observations), suggesting that the fever induced by this chemokine depends on CRF, which possibly recruits the opioidergic system.

Fabricio et al. (25) demonstrated that intracerebroventricular administration of ET-1 or intravenous administration of LPS induced an ETB receptor, but not ETA receptor-dependent increase in \( T_b \). We demonstrated in this study that the fever induced by ET-1 was completely abolished by this antagonist, suggesting that ET-1 subsequently would induce endogenous opioid release. Interestingly, Khodorova et al. (32) demonstrated that the activation of the ETB receptor activates an antinociceptive mechanism that develops unexpectedly with a pronociceptive mechanism induced by ET-1 binding to the ETA receptor. The antinociceptive action initiated by ETB receptor stimulation is blocked by the opioid-receptor antagonist naloxone, suggesting an involvement of opioid receptors. One year later, the same group demonstrated that the ETB receptor-induced analgesia resulted from the release of \( \beta \)-endorphin and its action on \( \mu \)-opioid receptors (33). It is possible that similar mechanisms are activated during LPS-induced fever.

**Perspectives and Significance**

Evidences for the participation of endogenous opioids in the febrile response have been shown previously (6, 8). In this study, we show that LPS and endogenous pyrogens (except IL-1β and prostaglandins) recruit the opioid system to cause a \( \mu \)-receptor-mediated fever independent of prostaglandins. Of particular interest is that the opioidergic system seems to be activated simultaneously or after prostaglandin synthesis. Further studies are necessary to answer this question. However, the opioidergic system may exert a major function when prostaglandins are not necessary for fever induction such as during the febrile response elicited by MIP-1α or ET-1. It is possible that, in some clinical situations such as in infection (34) and sepsis, these mediators are more promptly released to induce fever than those that induce prostaglandin synthesis. In this case, the blockade of \( \mu \)-opioid receptors would represent a...
more effective therapeutic approach. This hypothesis needs further experiments to be confirmed.

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REFERENCES