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Blockade of lipopolysaccharide-induced fever by a μ-opioid receptor-selective antagonist in rats

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Abstract

The endogenous opioid system has been found to be involved in fever caused by pyrogens. In the present study, we have investigated the role of the μ-opioid receptor in the brain in fever induced by lipopolysaccharide. Rats were microinjected with 1 μg of the μ-opioid receptor-selective antagonist, cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP), into the preoptic anterior hypothalamus. Thirty minutes later, lipopolysaccharide (50 μg/kg) was injected intraperitoneally (i.p.). CTAP reduced by 1°C the fever induced by lipopolysaccharide. However, it did not affect lipopolysaccharide fever when it was given 3 h after lipopolysaccharide injection. These data indicate that μ-opioid receptors within the preoptic anterior hypothalamus mediate the initiation of lipopolysaccharide fever and suggest that the opioid system is involved in the pathogenesis of fever in rats.

Keywords: Lipopolysaccharide; Fever; μ-Opioid receptor antagonist; Preoptic anterior hypothalamus

1. Introduction

It is currently believed that fever due to lipopolysaccharide and other exogenous pyrogens is caused by the synthesis and release from monocytes and macrophages of a number of well-characterized pyrogenic factors, including interleukin-1, interleukin-6, tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-1 (MIP-1) (Blatteis, 1992; Davidson et al., 1990; Long et al., 1990; Myers et al., 1994). Each endogenous pyrogen apparently acts as a signal to the brain which activates thermosensitive neurons in the preoptic anterior hypothalamus to evoke a rise in body temperature (Blatteis, 1992). Prostaglandin was originally proposed to be the intermediary messenger at the interface between the blood and brain in a febrile response (Blatteis, 1992). It has also been demonstrated that endogenous glucocorticoids are involved in lipopolysaccharide-fever and in the modulation of cytokine-induced fever through the inhibition of prostaglandin. Thus, it has been shown that subcutaneous (s.c.) dexamethasone pretreatment abolished the fever induced by lipopolysaccharide, interleukin-1β, interleukin-6 and TNF-α (Coelho et al., 1995). However, much experimental evidence has failed to support the need for prostaglandin and glucocorticoid in the pathogenesis of fever. In fact, a fever produced by interleukin-8 and MIP-1 is unaffected by indomethacin (Coelho et al., 1995; Miñano et al., 1991) and fever induced by MIP-1β is not modified by prior treatment with dexamethasone (Tavares and Miñano, 1998).

The possible involvement of the μ-opioid receptors in fever production has been suggested by various experiments (Zhao et al., 1995). μ-Opioid receptor mRNA has been observed in various regions in the brain, including preoptic anterior hypothalamus (Mansour et al., 1995). The μ-opioid receptor-selective agonists given into the preoptic anterior hypothalamus inhibit the activity of the warm-sensitive neurons which regulate heat-loss and activate cold-sensitive neurons which regulate heat-gain (Baldino et al., 1980; Yakimova et al., 1996), resulting in body temperature increase. Both peripheral and central injections of β-endorphin and the prototypic μ-opioid agonist morphine cause fever-like hyperthermia. The elevation of plasma, cerebrospinal fluid, and hypothalamic levels of β-endorphin has been found during lipopolysaccharide and endogenous pyrogen-induced fever (Carr et al., 1982; Leshin and Malven, 1984; Murphy et al., 1983). Interleukin-1, which is generally thought to be the primary

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endogenous pyrogen, has been shown to induce β-endorphin secretion by pituitary cells (Fagarasan et al., 1990) and modulate opioid receptor binding in the brain (Ahmed et al., 1985). Naloxone, a general opioid receptor antagonist, prevents the effects of the pyrogenic cytokines interleukin-6 (Xin and Blatteis, 1992) and IFN-α (Hori et al., 1991) on the activity of hypothalamic thermosensitive neurons in slice preparations. It has been found that the febrile response of guinea pigs to both exogenous *Escherichia coli* and endogenous (Interleukin-6, TNF-α and INF-α) pyrogens was significantly attenuated by the prior s.c. injection of naloxone (Blatteis et al., 1991; Romanovsky et al., 1994; Ahokas et al., 1985; Zawada et al., 1997). Recently, it has been demonstrated that cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP), a somatostatin analog which acts as μ-opioid receptor-selective antagonist (Kramer et al., 1989; Pelton et al., 1986), administered intracerebroventricularly (i.c.v.) blocks the fever induced by MIP-1β given i.c.v. (Handler et al., 1998), and previous results from our laboratory demonstrated that microinjection of CTAP into the preoptic anterior hypothalamus prevents the fever produced by central administration of TNF-α (Zhao et al., 1995).

The present experiments using a μ-opioid receptor-selective antagonist were conducted to investigate whether μ-opioid receptors in the brain play an important role in the development of lipopolysaccharide fever.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Zivic-Miller) weighing 250–300 g were used in this study. They were housed two per cage for at least 1 week before surgery and were fed laboratory chow and water ad libitum. The ambient temperature was 22 ± 2°C and a 12-h light/12-h dark cycle was used. All experiments were started between 09:00 and 10:00 h to minimize the effect of circadian variation in body temperature.

Table 1
Maximum change (mean ± S.E.M) in body temperature induced by 10, 25, 50 and 100 μg/kg of lipopolysaccharide given i.p., ΔTb is the mean change in body temperature, Tb is body temperature and *n* is number of rats

Dose (μg/kg i.p.)	*n*	Baseline Tb (°C)	Peak ΔTb (°C)	Time to peak (min)
10	3	37.37 ± 0.1	0.6 ± 0.2	330
25	3	37.40 ± 0.09	0.83 ± 0.2	330
50	6	37.76 ± 0.09	1.34 ± 0.1	300
100	6	37.30 ± 0.1	1.34 ± 0.2	300

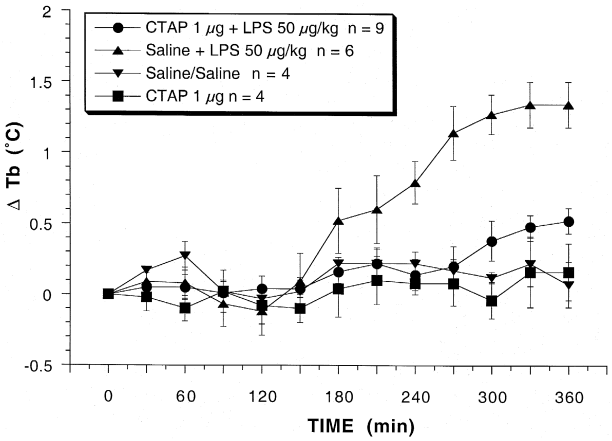

Fig. 1. Effect of intrahypothalamic pretreatment with CTAP (1 μg, −30 min) or saline (1 μl, −30 min) on the fever response induced by lipopolysaccharide (50 μg/kg i.p), given at time 0, and effect of CTAP or saline on body temperature. Values represent mean ± S.E.M from baseline body temperature. ΔTb is the mean change in body temperature, LPS is lipopolysaccharide and *n* is the number of rats.

2.2. Implantation of cannula

Rats were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). Each animal was placed in a stereotaxic instrument with the upper incisor bar set at 3.0 mm below the interaural line. A sterilized stainless-steel 21-gauge cannula guide was placed just above the right or left preoptic anterior hypothalamus 0.3 mm anterior to bregma, 0.5 mm from midline and 6 mm ventral to the dura mater (Paxinos and Watson, 1986). A 26-gauge stylet of identical length was inserted into the guide tube to prevent its occlusion. The animals were returned to individual cages in the environmental room.

2.3. Microinjection and body temperature

One week after surgery, either saline or drug was microinjected into the preoptic anterior hypothalamus in a volume of 1 μl. With aseptic procedures, the injection cannula (26 gauge) was connected by polyethylene tubing to a 10-μl Hamilton syringe. The rats were placed into individual plastic cages in an environmental room kept at 21 ± 0.3°C and 52 ± 2% relative humidity. After a 1-h acclimatization period, a thermistor probe (YSI series 400, Yellow Springs Instrument, Yellow Springs, OH) was lubricated and inserted approximately 7 cm into the rectum. Body temperature was read from a digital thermometer (Model 49 TA, YSI). During the reading, the tail of the rat was held gently between two fingers and the animal was otherwise free to move within the cage during the experiment. The first three readings were taken at 30-min intervals. To allow for adaptation to the procedure, the first

reading was discarded and the subsequent two averaged to establish a baseline.

2.4. Drugs

Lipopolysaccharide was the phenol-extracted preparation of *E. coli* (0111: B4), (List Biological Laboratories) and was dissolved in pyrogen-free saline and injected i.p. in a dose of 10, 25, 50 or 100 μg/kg. CTAP (Multiple Peptide Systems) was dissolved in pyrogen-free saline and microinjected into the preoptic anterior hypothalamus (1 μg). The dose of CTAP used was based on a previous study (Xin et al., 1997).

2.5. Statistical and histology analysis

All results were expressed as mean ± S.E.M. Statistical analysis of differences between groups was determined by analysis of variance (ANOVA) followed by a *t*-test. A value of P less than 0.05 was considered statistically significant.

Cannula placement was confirmed by checking the location of the tip by 1% Evans blue injection after the experiment according to standard procedures (Xin et al., 1997).

3. Results

Administration of lipopolysaccharide in low doses (10–25 μg/kg) i.p. produced a small increase in body temperature (0.5–0.83°C), which peaked at 330 min. However, lipopolysaccharide in doses of 50 μg/kg or 100 μg/kg caused a fever which reached a peak of 1.34 ± 0.1°C at 300 min (Table 1). Fig. 1 shows the effect of injection into preoptic anterior hypothalamus of saline and CTAP (1 μg) on the febrile response induced by i.p. administration of lipopolysaccharide (50 μg/kg) 30 min later. In the group pretreated with saline, i.p. administration of 50 μg/kg lipopolysaccharide induced a prolonged fever that started to increase ~3 h after the injection and peaked at ~5 h. This fever is significantly reduced by CTAP (1 μg) given into preoptic anterior hypothalamus 30 min later at each 30 min time point during period of 3 to 6 h after lipopolysaccharide injection ($P < 0.05$). Saline or CTAP (1 μg) given alone into preoptic anterior hypothalamus did not significantly alter body temperature. As demonstrated in Fig. 2, CTAP (1 μg) injected into the preoptic anterior hypothalamus 3 h after i.p. lipopolysaccharide injection did not alter the lipopolysaccharide fever ($P > 0.05$).

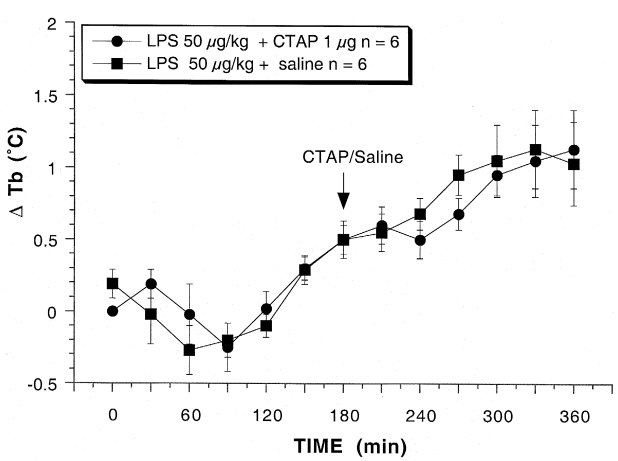

Fig. 2. Effect of intrahypothalamic treatment of CTAP (1 μg, +3 h) or saline (1 μl, +3 h) on febrile response induced by lipopolysaccharide (50 μg/kg i.p.), given at time 0. Arrow indicates time of injection of CTAP or saline. Values represent mean ± S.E.M. from baseline body temperature. ΔTb the mean is change in body temperature, LPS is lipopolysaccharide and *n* is the number of rats.

4. Discussion

Previous research demonstrated that lipopolysaccharide activates endogenous opioid systems. Endogenous opioid levels are higher in the plasma of lipopolysaccharide-treated animals (Carr et al., 1982). Mononuclear cells synthesize large quantities of opioids upon exposure to lipopolysaccharide (Behar et al., 1994; Harbour et al., 1987). Lipopolysaccharide-induced elevation of endogenous opioids may be mediated by the secretion of interleukin-1α, as i.c.v. administration of interleukin-1α increases immunocyte concentrations of β-endorphin (Sacerdote et al., 1992), induces β-endorphin secretion by pituitary cells (Fagarasan et al., 1990) and modulates opioid receptor binding (Ahmed et al., 1985) in the brain. Furthermore, endogenous opioids appear to mediate at least some of the physiological effects of lipopolysaccharide; naloxone attenuates the physiological effects of lipopolysaccharide and increases the overall survival rate of lipopolysaccharide-treated rats (Holaday, 1984). Other findings in the literature have demonstrated that naloxone prevented the effects of the pyrogenic cytokine interleukin-6 (Xin and Blatteis, 1992) on the activity of hypothalamic thermosensitive neurons in slice preparations, and naloxone given i.p. antagonized the interleukin-6 response induced by i.c.v. or i.p. interleukin-1β (Bertolucci et al., 1996).

The i.p. injection of lipopolysaccharide 50 μg/kg, a dose commonly used to induce fever by various authors (Kozak et al., 1997; Miñano et al., 1996), produced a significant elevation in body temperature with the maximum increase 5 h after injection. This febrile response was significantly reduced by pretreatment with CTAP into preoptic anterior hypothalamus (central control site for body temperature regulation). This result is in agreement with previous studies that demonstrated that the opioid antagonist naloxone, given s.c., reduced the first phase and

suppressed the second phase of the characteristically bimodal febrile response to lipopolysaccharide (Blatteis et al., 1991).

Although naloxone induced changes in the febrile response, it is a general antagonist opioid acting on all types of opioid receptors (μ, κ and δ). Our results demonstrated that the febrile effect of lipopolysaccharide is mediated via central μ-opioid receptors, as it is blocked by the μ-opioid receptor-selective antagonist, CTAP. Our findings are compatible with the hypothesis that the opioid system is involved in the mediation of the febrile response to lipopolysaccharide. Indeed, increases in plasma, cerebrospinal fluid and hypothalamic levels of β-endorphin (which acts on μ-opioid receptors) have been reported during lipopolysaccharide administration (Carr et al., 1982). β-Endorphin and various other opioid peptides are hyperthermic when microinjected in low doses (Huidobro-Toro and Way, 1980; Adler et al., 1988). β-Endorphin microinjected directly into the preoptic anterior hypothalamus induced a body temperature increase which was blocked by pretreatment with CTAP in the same side (Xin et al., 1997).

Previous experimental data strongly suggest the important roles of interleukin-1β, TNF-α and MIP-1β in the fever induced by lipopolysaccharide (Long et al., 1990; Nakamori et al., 1994; Miñano et al., 1996) and recent results showed that microinjection of CTAP into the preoptic anterior hypothalamus prevents the fever produced by central administration of interleukin-1β, TNF-α and MIP-1β (Blatteis et al., 1991; Xin et al., 1997; Handler et al., 1998). β-Endorphin also is involved in the fever induced by the same cytokines, as the level of β-endorphin release in the preoptic anterior hypothalamus was increased after interleukin-1β or TNF-α injection (Xin et al., 1997; Zhao et al., 1995). Taken together, these data suggest that the antagonism of lipopolysaccharide-fever by CTAP may result from the prevention of binding of β-endorphin induced by endogenous cytokines to the μ-opioid receptor.

CTAP given into the preoptic anterior hypothalamus 3 h after lipopolysaccharide did not affect the fever induced by lipopolysaccharide. Our results confirm the hypothesis that the central opioid system may act in the first part of lipopolysaccharide fever. These data suggest that an additional or chemical pathway could be involved in the second phase.

Because previous data suggest that fever can be induced by mechanisms independent of prostaglandin and glucocorticoids (Coelho et al., 1995; Miñano et al., 1991; Tavares and Miñano, 1998), and our results show that the central opioid system is involved in lipopolysaccharide fever, our present findings support and extend the idea that fever is a complex mechanism in which multiple pathways may be involved.

In summary, these data indicate that μ-opioid receptors within the preoptic anterior hypothalamus mediate at least the early phase of lipopolysaccharide-induced fever and that the opioid system is involved in the pathogenesis of fever in rats. It also suggests that there may be multiple pathways for induction of fever.

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