Deletion of \(\mu\)-Opioid Receptor in Mice Alters the Development of Acute Neuroinflammation

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Received August 10, 2007; accepted September 25, 2007

ABSTRACT

The realization that the \(\mu\)-opioid system plays a key role in the control of the process of neuroinflammation is a new concept that may lead to identification of novel therapies for this extremely widespread and intractable syndrome. Fever is the hallmark among the defense mechanisms evoked by the entry into the body of pathogens to initiate the innate immune responses. In an attempt to determine the possible involvement of \(\mu\)-opioid receptors in the control of brain inflammation, we examined the effect of their deletion on the fever induced by i.c.v. injection of lipopolysaccharide (LPS). The first series of experiments examined the thermal consequence of the absence of \(\mu\)-opioid receptors on circadian body temperature rhythm and basal body temperature. \(\mu\)-Opioid receptor knockout mice (MOP-KO) showed a normal circadian body temperature rhythm and basal body temperature compared with the wild type (WT). The second series of experiments investigated i.c.v. administration of LPS on body temperature in WT and MOP-KO. In the WT, i.c.v. injection of 100 ng of LPS induced fever, but there was no increase in body temperature in the MOP-KO mice. Saline, given i.c.v., did not alter the body temperature, either in WT or MOP-KO. These results show that the \(\mu\)-opioid system participates in the control of acute neuroinflammation, further reinforcing our earlier finding that the opioid system is involved in the pathogenesis of fever induced by bacterial LPS, and that \(\mu\)-opioid receptors are the target for morphine-induced hyperthermia.

Because it is now known that opioids have many diverse effects on the immune system, there is continuing interest in elucidating their actions. Morphine, given in vivo either peripherally or centrally, has been shown to suppress a variety of immune responses, including natural killer cell activity (Lysle et al., 1993; Carr et al., 1994), antibody responses (Eisenstein and Hilburger, 1998), spleen and peripheral blood cell responses to mitogens (Bayer et al., 1990), and macrophage function (Bussiere et al., 1992; Rojavin et al., 1993). In addition, exogenous opioids have been shown to modify release of the cytokines interleukin (IL)-\(\beta\) and tumor necrosis factor (TNF)-\(\alpha\) (Pacifici et al., 2000). Studies examining the involvement of opioids in LPS-induced fever have reported an elevation in plasma, cerebrospinal fluid, and hypothalamic levels of \(\beta\)-endorphin during lipopolysaccharide (LPS) and endogenous pyrogen-induced fever (Carr et al., 1982, Leshin and Malven, 1984), and the \(\mu\)-opioid receptors within the preoptic anterior hypothalamus mediate, at least in the early phases, LPS-induced fever (Benamar et al., 2000).

Because of the discovery that the opioid system has many diverse effects on the immune system and that it is involved in the pathogenesis of fever, targeting this system has represented a promising therapeutic approach. A prominent component of the acute phase reaction to immune and inflammatory stimuli is the development of fever. Although fever is an important indicator for the severity of the inflammation, no one has investigated and/or linked this parameter with the opioid system during neuroinflammation. Using a model of acute neuroinflammation, we sought to determine the effects of the genetic deletion of the \(\mu\)-opioid receptor on the fever by i.c.v. injection of LPS.

Materials and Methods

Animals. \(\mu\)-Opioid receptor knockout mice (MOP-KO) mice were developed by disruption of exon-1 of the MOP-1 gene through homologous recombination as described previously (Schuller et al., 1999). The 129S6\(\times\)C57BL/6J chimeras were directly crossed with 129S6 mice to produce the inbred 129S6 MOP-1 mutant strain, whereas the 129S6\(\times\)C57BL/6J F1 mutants were produced by directly crossing F10 C57BL/6J MOP-1 KO mice with the 129S6 MOP-1-deficient strain.

ABBREVIATIONS: IL, interleukin; LPS, lipopolysaccharide; MOP-KO, \(\mu\)-opioid receptor knockout mice; WT, wild type; DAMGO, \([d-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin\); TNF, tumor necrosis factor.
Mice (WT C57BL/6J mice as well as MOP-KO) weighing 20 to 30 g were used in this study. They were housed five per cage for at least 1 week before surgery and they were fed laboratory chow and water ad libitum. Mice were housed on a 12-h light/dark cycle (on/off; lights on at 6:00 AM) at an ambient temperature of 22 ± 0.3°C. All experiments were started between 9:00 AM and 10:00 AM to minimize the effect of circadian variation in body temperature. All animal use procedures were conducted in strict accordance with the Institute of Laboratory Animal Resources (1996), and they were approved by the Institutional Animal Care and Use Committee.

**Surgery Procedures.** Mice were anesthetized with an i.p. injection of a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). An incision 0.5 cm in length was made along the linea alba, and the underlying tissue was dissected and retracted. A transmitter (model E-4000; Mini-Mitter Co. Inc., Sunriver, OR) was then implanted in the i.p. space. After the transmitter was passed through the incision, the abdominal musculature and dermis were sutured independently (Benamar et al., 2005). On the same day as the surgery, each animal was placed into a stereotaxic instrument (Cunningham Mouse and Neonatal Rat Adaptor; Stoelting, Wood Dale, IL). The position of the head was adjusted so that the height of skull surface at bregma and lambda was the same. A sterilized stainless steel C315-GS-4 cannula guide (26-gauge; Plastics One, Roanoke, VA) was implanted i.c.v. Stereotaxic coordinates were as follows: −0.5 mm anterior to bregma, 1 mm from midline, and 3 mm ventral to the dura mater (Paxinos and Franklin, 2001). A C315DCS cannula dummy (Plastics One) of identical length was inserted into the guide tube to prevent its occlusion. The animals were returned to individual cages in the environmental room.

**Microinjection and Measurement of Body Temperature.** At 1 week post-surgery, the mice were tested in an environmental room (22 ± 0.3°C ambient temperature and 52 ± 2 relative humidity). After 1 h of adaptation, two readings were averaged to determine the baseline. During the recording period (pre- and postinjection), the body temperature was measured at 15-min intervals. The body temperature and circadian body temperature were measured by a biotelemetry system using calibrated transmitters. Signals from the transmitter were delivered through a computer-linked receiver. This method minimized stress to animals during the body temperature reading. Thus, the body temperature was monitored continuously and recorded without restraint or any disturbance to the animal. Either saline or drug was microinjected i.c.v. in a volume of 3 μl. With aseptic procedures, the C315IS-4 internal cannula (33-gauge; Plastics One) was connected by polyethylene tubing to a 1-μl Hamilton syringe. The mice were placed into individual plastic cages in an environmental room kept at 21 ± 0.3°C with 52 ± 2% relative humidity.

**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 followed by Dunnett’s 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 in our laboratory (Xin et al., 1997a).

**Drugs.** Morphine sulfate and the selective μ-opioid receptor agonist [d-Ala²,N-Me-Phe³,Gly⁵-ol]-enkephalin (DAMGO) (supplied by the National Institute on Drug Abuse, Bethesda, MD) were dissolved in sterile pyrogen-free saline. LPS was a phenol-extracted preparation of *Escherichia coli* (0111:B4), and it was obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in pyrogen-free saline.

**Results**

**Baseline Body Temperature and Circadian Changes in Body Temperature on MOP-KO and WT.** To examine whether the absence of μ-opioid receptors influenced the normal circadian body temperature rhythm, we compared the daily change in body temperature in the MOP-KO with WT. The recordings of the diurnal body temperature changes displayed no significant differences between the two groups (Fig. 1; P > 0.05). The basal body temperature measured before any treatment was comparable in the MOP-KO and WT, indicating that basal body temperature is the same in both groups (Fig. 1; P > 0.05).

To prove that these MOP-KO and WT responded properly to the action of morphine on body temperature and to define the role of the μ-opioid receptors in morphine-induced hyperthermia, we injected a hyperthermic dose of morphine (1 mg/kg i.p.) and examined the thermal response in WT and MOP-KO. In WT, i.p. injection of morphine (1 mg/kg) produced an elevation in body temperature that peaked at 60 min (1.41 ± 0.19°C), and it remained approximately 1°C above baseline for 120 min. The administration of morphine (1 mg/kg i.p.) to MOP-KO did not evoke any elevation in body temperature during the 210-min recording period (Fig. 2; F₃,₂₈ = 2.95, P < 0.005). Mean body temperature before injection was 36.68 ± 0.13°C for the WT group and 36.79 ± 0.12°C for MOP-KO. A similar effect was found using the selective μ-opioid receptor agonist DAMGO given i.c.v. (Fig. 3).

**Fever Evoked by Intracerebroventricular Injection of LPS.** Animals were injected i.c.v. with either LPS or vehicle (sterile, pyrogen-free saline), and body temperature was monitored 360 min after injection. The i.c.v. injection of LPS (50 ng) did not significantly affect the body temperature relative to vehicle (Fig. 4; P > 0.05). However, LPS in doses of 75 or 100 ng caused a fever that reached a peak of 1.15 ± 0.2°C and 1.47 ± 0.23°C at 240 min, respectively (Fig. 4; F₃,₄₀ = 2.84, P < 0.05). Mean body temperature before injection was 36.67 ± 0.15°C for the saline group, 36.76 ± 0.12°C for LPS (50 ng) group, 36.84 ± 0.19°C for LPS (75 ng) group, and 36.75 ± 0.07°C for LPS (100 ng) group. There was no significant difference in baseline body temperature among these groups.

**Effect of LPS on Body Temperature in MOP-KO and WT.** The administration of LPS (100 ng; i.c.v.) to WT caused an increase in body temperature of approximately 1.56 ± 0.3°C, which remained elevated throughout the 360-min recording period. However, LPS (100 ng; i.c.v.) administration to MOP-KO did not show any increase in body temperature compared with WT (Fig. 5; F₃,₄₀ = 2.84; P < 0.05).

![Fig. 1. Diurnal temperature changes in WT and MOP-KO. WT and KO mice displayed similar diurnal temperature variations. Data are expressed as the mean ± S.E.M. of body temperature. N, number of mice. Horizontal bar indicates dark phase.](image-url)
To verify that the transfer of LPS from the central nervous system into the periphery is not responsible for the fever after i.c.v. administration, we administered LPS (0.1–4 µg/kg i.p.) at doses ranging from 0.1 to 4 µg/kg to WT. As can be seen in Table 1, these doses did not alter WT body temperature compared with saline controls.

**Discussion**

In the first series of experiments, we determined the thermoregulatory characteristics of these MOP-KO mice. We examined the consequence of the absence of µ-opioid receptors on the basal body temperature and circadian body temperature rhythm. These receptors do not seem to be critical for the normal body temperature and circadian body temperature rhythm, because both the WT and MOP-KO showed similar baseline body temperature and diurnal/nocturnal fluctuations.

Interestingly, although µ-opioid receptors play an important physiologic role, the knockout mice did not demonstrate any apparent developmental or physiologic abnormalities during the time of observation. Other studies have also reported that size, development, fertility, and locomotor activity of knockout animals did not differ significantly from WT (Tian et al., 1997; Schuller et al., 1999). A previous study has shown that naloxone blocks morphine-induced hyperthermia (Geller et al., 1983). In using a genetic approach to correlate functional activity of the MOP gene with the known pharmacology of morphine on body temperature.
temperature, and to show that MOP-KO respond properly to the effect of μ-opioid agonist, we administered a hyperthermic dose of morphine to MOP-KO and WT. Morphine (1 mg/kg) produced hyperthermia in WT mice, but it did not evoke the same response in MOP-KO during the recording period. Because morphine is not a selective μ-opioid agonist, it could be argued that the effect of morphine on body temperature may involve actions on receptors other than μ. In the present study we tested this possibility. The selective μ-opioid agonist DAMGO was administered to WT and MOP-KO, and body temperature was monitored. As expected, a normally hyperthermic dose of DAMGO produced an increase in body temperature in WT mice, but it did not evoke the same response in MOP-KO. These findings clearly indicate that the μ-opioid receptor is required for the action of morphine on body temperature in mice, confirming the pharmacological effects of the selective μ-opioid-receptor antagonists (Spencer et al., 1988; Handler et al., 1992; Adler and Geller, 1993).

In the second series of experiments, we used an experimental model of fever associated with brain inflammation, in which mice received an i.c.v. injection of LPS, to investigate the role of μ-opioid receptors in LPS-induced fever. Using pharmacological approaches, it has been shown that μ-opioid antagonists (naloxone or n-Phe-Cys-Tyr-d-Trp-Arg-Thr-Pen-Thr-NH₂) block the LPS-induced fever (Blatteis et al., 1991; Benamar et al., 2000), indicating that the opioid system is involved in the pathogenesis of fever. With gene deletion of μ-opioid receptors, we have recently confirmed that the μ-opioid receptor mediates the fever induced by systemic administration of LPS (Benamar et al., 2005). The purpose of the present study was to determine whether such an effect occurs when LPS is given centrally. The injection of LPS, an endotoxin derived from the cell wall of Gram-negative bacteria, directly into the brain has been used as an animal model for the study of neuroinflammation. The present studies show that the i.c.v. injection of LPS (50–100 ng) produced a dose-dependent, significant elevation in body temperature in WT during the 360-min recording period. However, the administration of LPS (100 ng i.c.v.) to MOP-KO did not evoke any increase in body temperature during the same recording period, indicating that the μ-opioid receptors are critical for the development of fever induced by central administration of LPS in mice. One study has suggested that transfer of LPS from the central nervous system into the periphery in significant amounts is what accounts for the observed effects of i.c.v. LPS (Cunningham et al., 2005). In our study, it is highly unlikely that the small amount of LPS (100 ng) injected via the i.c.v. route evoked fever through its leakage into the system compartment, because the same amount of LPS, when injected peripherally, did not evoke fever, even at a dose 10 times higher. Our results suggest that an inflammatory response occurs in the brain following the administration of LPS i.c.v., manifested by increases in body temperature.

It has been shown that i.c.v. administration of LPS caused a rapid and prolonged elevation of IL-1 throughout the brain (Quan et al., 1997). Microglial cells readily produce detectable IL-1 (Cunningham et al., 2005) and intense immunoreactivity to IL-1β in hypothalamic microglial cells (Gonzalez et al., 2004) after i.c.v. stimulation. The in vivo stereotaxic injection of LPS into the brain has been reported to lead to a rapid production by microglial cells of proinflammatory factors, such as TNF-α (Kalehua et al., 2000). Previous experimental data strongly suggest the important roles of IL-1β, IL-6, TNF-α, and macrophage inflammatory protein-1 in fever induced by LPS (Blatteis, 2006), and recent results showed that microinjection of a selective μ-opioid-receptor antagonist centrally prevents the fever produced by interleukin-1β, TNF-α, MIP-1β, IL-6, and LPS (Xin et al., 1997b; Handler et al., 1998; Benamar et al., 2000, 2002), indicating that μ-opioid receptors are involved in the pathogenesis of fever induced by these endogenous and exogenous pyrogens. μ-Opioids have also been shown to alter the release of cytokines important for both host defense and the inflammatory response (Chao et al., 1993; Lysle et al., 1993). Cells involved in neuroinflammation, astrocytes and microglia, as well as neurons, express μ-opioid receptors (Ruzicka et al., 1995). Furthermore, μ-opioid-receptor mRNA has been observed in various regions in the brain, including the preoptic anterior hypothalamus (Mansour et al., 1995), the main area involved in fever and thermoregulation. In view of these findings, an interaction between the cytokine/chemokine and the μ-opioid systems could take place under neuroinflammatory conditions.

Although a large part of the response to LPS-induced fever has been attributed to the action of cytokines, NO, a proinflammatory mediator in the immune system with both antiviral (Lowenstein et al., 1996) and antibacterial (Nathan and Hibbs, 1991) actions, is one of the mediators produced following brain inflammation (Zamora et al., 2000). It is also considered to be an important mediator of LPS-induced fever (Roth et al., 1998). In addition, we have shown that NO produced by neuronal nitric-oxide synthase mediates morphine-induced hyperthermia (Benamar et al., 2001, 2003). Another explanation of our data is that by deleting the μ-opioid receptor, it is possible that the NO release decreases, leading to a decline in cumulative NO levels and therefore absence of LPS-induced fever mediated by endogenous NO.

The current report demonstrates a role of μ-opioid receptors in an animal model of acute neuroinflammation, pointing out their critical role in the fever induced by central administration of LPS. The realization that the μ-opioid system plays a key role in the control of the process of neuroinflammation is a new concept and may well lead to a fruitful approach to identify novel therapies for neuroinflammatory conditions. For example, it may be possible to use a μ-opioid antagonist as a therapeutic strategy to prevent and treat brain diseases associated with neuroinflammation (e.g., multiple sclerosis, Alzheimer’s disease). In addition, these studies confirm that the μ-opioid system is involved in bacterial LPS-induced fever. In uncontrolled conditions, fever can threaten cellular homeostasis and survival. Treating such dysregulation of body temperature would be aided by an understanding of the role of μ-opioid system in the pathogenesis of fever. In addition, these results provide direct genetic evidence that μ-opioid receptors play a predominant role in morphine-induced hyperthermia and reinforce our earlier pharmacological findings.

Acknowledgments

We thank Drs. Keith Latham and Ellen Unterwald for help in breeding the knockout mice.
References


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