Full Paper

Pyrogens Enhance β -Endorphin Release in Hypothalamus and Trigger Fever That Can Be Attenuated by Buprenorphine

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Abstract. At first, we investigated whether both β -endorphin release level in the hypothalamus and body temperature can be altered after intracerebroventricular (i.c.v.) injection of either lipopolysaccharide (LPS), interleukin-1 β (IL-1 β), or prostaglandin E₂ (PGE₂) in rats. It was found that in the rat, i.c.v. administration of either LPS (0.5 μ g in 10 μ l), IL-1 β (10 ng in 10 μ l), or PGE₂ (200 ng in 10 μ l), in addition to producing fever, upregulated the immunoreactivity of β -endorphin in the preoptic anterior hypothalamus of rat brain. Secondly, we assessed whether the fever induced by either LPS, IL-1 β , or PGE₂ can be altered by pretreatment with buprenorphine (an opioid receptor antagonist). The results revealed that i.c.v. administration of buprenorphine (1 – 10 μ g in 10 μ l) alone had an insignificant effect on the body temperature. However, the fever induced by i.c.v. injection of either LPS, IL-1 β , or PGE₂ was significantly attenuated by pretreatment with i.c.v. injection of buprenorphine 1 h before the pyrogen injection in rats. The results suggest that pyrogens enhance β -endorphin release in the hypothalamus and trigger fever which can be attenuated by buprenorphine, an opioid receptor antagonist.

Keywords: buprenorphine, fever, β -endorphin, pyrogen, preoptic anterior hypothalamus

Introduction

The systemic response of fever is the result of pathogenic infection. In particular, the release of lipopolysaccharide (LPS) from Gram-negative bacterial cell wall during most infections triggers a febrile response by stimulating monocytes and macrophages to synthesize and release several pyrogenic cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and interleukin-6 (IL-6) (1). These enter the circulation and reach the endothelial cells of the hypothalamus. From these cells, prostaglandin E₂ (PGE₂) is released into the brain and binds to EP₃ receptors on the cells in the hypothalamic thermoregulatory center (2); this is followed by PGE₂-induced neuronal mechanisms involving cyclic AMP (3) and neurotransmitters (1) to elevate the temperature set-point, resulting in fever. Among the neurotransmitters involved in the fever induced by cytokines are the endogenous opioid

peptide within the preoptic anterior hypothalamus (POAH), a primary central control site for temperature regulation, where opioid receptors are located (4). Because of reports that IL-1 β can induce the secretion of β -endorphin in the POAH and that β -endorphin micro-injected into the POAH of rat brain can induce hyperthermia (5, 6), this raises the possibility that β -endorphin release level in the POAH can also be altered after intracerebroventricular (i.c.v.) injection of pyrogens.

Buprenorphine is an oripavine-derived narcotic with high affinity for both the μ and κ subtypes of the opiate receptors (7). It possesses an antinociceptive action in both animal pain models (8) and humans with moderate-to-severe pain in the clinic (9). In the rat after electrical stimulation of the tail root, buprenorphine displayed a biphasic dose-response curve in the vocalization pain test (8). Acute administration of buprenorphine was also shown to induce typical μ -opioid agonist effects including euphoria, sedation, analgesia, miosis, catalepsy, and decreased gastrointestinal motility (10 – 12). Buprenorphine can act as an opioid receptor antagonist under

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some conditions. For example, acute administration of buprenorphine can attenuate the behavioral effects of μ agonists in a rhesus monkey tail-withdrawal test (13). Clinical studies have shown that chronic treatment with buprenorphine can block the effects of morphine, heroin, and hydromorphone (11, 14). Thus, it appears that buprenorphine has a desirable therapeutic effect to reduce heroin use in opioid-dependent patients (14, 15). Buprenorphine has also been reported to antagonize the diuretic (16) and schedule-controlled effects of κ agonists. These findings suggest that buprenorphine is not only a partial μ agonist and antagonist, but a pure κ -opioid receptor antagonist as well. Recently, buprenorphine has been shown to be a potent μ -opioid receptor antagonist in brain cells in the [35 S]-GTP- γ -S functional binding assay (17). Activation of μ -opioid receptor in brain has also been shown to mediate the fever response in rats (18–21). Furthermore, a more recent report showed that the rectal temperature of dogs was decreased to about 36°C at 2 to 2.5 h after sedation with intra-muscular administration of buprenorphine (22). This raises the additional possibility that buprenorphine has an antipyretic action.

Therefore, in order to deal with the question, in the present study, we first investigated whether the β -endorphin release level in the hypothalamus can be altered following central administration of several pyrogenic substances including LPS, IL-1 β , and PGE₂ in rats. Secondly, we conducted an experiment to explore whether central administration of buprenorphine has a preventive effect in the development of fever in rats.

Materials and Methods

Animals

Male Sprague-Dawley rats, weighing between 250 and 300 g, were used. They had been maintained in a room at 22 $\pm$ 1.0°C and were previously accustomed to being restrained in the rat stocks for several hours at a time. Each animal had freedom to move its limbs and neck and was in an alert condition. Between experiments the animals were individually housed in wire-mesh cages with a 12-h light-dark cycle and were fed with dry powder chow, with tap water available ad libitum. All experiments were started between 09.00 and 10.00 h to minimize the effect of circadian variation in colonic temperature (Tco). All experiments were done in strict compliance with the recommendations for the care and use of laboratory animals and were approved by the Animal Care Committee of Chi-Mei Medical Center (Tainan, Taiwan).

Surgical preparation

For the administration of drugs into the third cerebral ventricle, stainless-steel cannula, consisting of a guide tube (0.81 mm o.d.) with a snug fitting trocar, and a cannula insert, which was introduced into the tube just before injection, were used. The cannula guide tubes with trocars were implanted using the stereotaxic atlas and coordinates of Paxinos and Watson (23): 0.8 mm posterior to the bregma, 1.4 mm from the midline, and 3.6 mm below the dura. The animals were anesthetized with sodium pentobarbitone (6 mg $\cdot$ 100 g⁻¹, i.p.) and placed in a KOPF stereotaxic apparatus. After appropriately located craniotomy holes had been made, two self, tapping screws were attached to the calvarium of the parietal bones, and then the cannula guide tubes were inserted to the desired depth through the craniotomy holes. They were anchored with fast-drying acrylic dental cement to the cranial surface, which had been scraped clean of periosteum. The cannula insert was connected to a 10 μ l Hamilton microsyringe by P.E. 10 polyethylene tubing, and the syringe and tubing were filled with silicone fluid to act as a piston. The volume of injection down each cannula was 10 μ l. A period of 1 week was allowed to permit the animals to recover from the operation before experimentation.

Drug solution

All drug solutions were prepared in pyrogen-free glassware that was heated for 5 h at 180°C before use. All drug solutions were prepared in pyrogen-free sterile 0.9% saline and passed through 0.22- μ m Swinnex bacterial filters. A 10- μ l aliquot containing 0.9% NaCl, PGE₂ (200 ng; Upjohn Laboratories, Kalamazoo, MI, USA), buprenorphine HCl (1–10 μ g; Macfarlan Smith, Edinburgh, UK), *Escherichia coli* lipopolysaccharide (LPS, 0111:B4, 0.5 μ g; Sigma Chemical Co., St. Louis, MO, USA), or IL-1 β (10 ng, Sigma) was injected to each animal.

Pyrogen tests

Each animal was used at an interval of no less than 5 days. Throughout the experiment, Tco was measured every minute with a copper-constantan thermocouple connected to a thermometer (HR1300; Yokogawa, Tokyo). The basal levels of Tco for each animal were allowed to stabilize for at least 90 min before any injection. Microinjection was made into the third cerebral ventricle through an injecting cannula. The volume of fluid injected over about 10 s was 10 μ l. The effects of i.c.v. injection of drugs on Tco were assessed in unanesthetized rats at an ambient temperature of 24°C.

Immunohistochemistry

The rats were deeply anesthetized with a overdose of urethane and perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde. The brains were removed and immersed into 4% paraformaldehyde for 4 h and then 20% sucrose in 0.1 M phosphate buffer for 24 h. For immunocytochemistry, serial cryostat sections (30- μ m-thick) were cut using a dry ice sliding microtome (type HM 400 R; Leica Microsystems Nussloch GmbH, Nussloch, Germany) and air dried onto poly-L-lysine coated slides.

The β -endorphin antiserum (Peninsula Laboratories, San Carlos, CA, USA) at 1:2,000 dilution was used as primary antibodies for overnight at 4°C. Sections were then incubated first with biotinylated goat anti-rabbit secondary antibody (diluted 1:500) followed by a streptavidin-conjugated horseradish peroxidase. The presence of antigen/antibody/enzyme complex can be revealed by addition of diaminobenzidine.

Data presentation and statistical analyses

Area and strength of staining in immunohistochemistry were assessed by two observers independently and blindly at a 400 $\times$ magnification and confirmed by a third observer. There was no significant difference among the assessment by the three. The degrees of positivity of immunoreactivity were semiquantitatively graded as 0 for absence of staining, 1+ for weak, 2+ for moderate, and 3+ for strong staining. The fever index (FI), the area under the curve produced in the 1-h (FI₁) or 4-h (FI₄) period after the injection of LPS, IL-1 β , or PGE₂, in terms of degrees centigrade per 1 or 4 h were

calculated.

Results are expressed as the mean $\pm$ S.E.M. for *n* experiments. One way analysis of variance (ANOVA) was used for the factorial experiment, whereas Student-Newman-Keuls' test was used for *post hoc* multiple comparisons among means. A *P* value less than 0.05 was considered to indicate a statistically significant difference.

Results

Figures 1 and 2 show effects of LPS and IL-1 β on Tco in saline- and buprenorphine-treated rats. Intracerebroventricular injection of LPS (0.5 μ g in 10 μ l) or IL-1 β (10 ng in 10 μ l) resulted in a significant increase in Tco that lasted up to 6 h (end of the observation period). Pretreatment with buprenorphine (1–10 μ g in 10 μ l, i.c.v., 1 h before i.c.v. injection of LPS or IL-1 β) significantly reduced the LPS- or IL-1 β -induced fever (*P*<0.01). Control injection of saline (0.9% NaCl solution) or buprenorphine (1–10 μ g in 10 μ l) produced no significant change in Tco during the 1–4-h period of observation. The data in terms of fever index are summarized in Table 1.

Figure 3 depicts the time course of the effects of i.c.v. injection of buprenorphine on the fever induced by i.c.v. injection of PGE₂ (200 ng in 10 μ l) in rats. Intracerebroventricular administration of PGE₂ produced a significant increase in Tco that lasted up to 60 min. Again, it was found that the PGE₂-induced fever was attenuated by pretreatment with buprenorphine (as shown in both Fig. 3 and Table 1).

Fig. 1. Time course of the effects of buprenorphine in pyrogenic fever induced by intracerebroventricular administration of LPS. Buprenorphine (1 and 10 μ g/10 μ l) was injected intracerebroventricularly 1 h before LPS (0.5 μ g/10 μ l) injected at time 0 h. The values are the mean $\pm$ S.E.M. of 8 rats per group. **P*<0.05, significantly different from the corresponding control values (saline + LPS group) (ANOVA followed by Student-Newman-Keuls' test).

Fig. 2. Time course of the effects of buprenorphine in pyrogenic fever induced by intracerebroventricular administration of IL-1 β . Buprenorphine (1 and 10 μ g/10 μ l) was injected intracerebroventricularly 1 h before IL-1 β (10 ng/10 μ l) injected at time 0 h. The values are the mean $\pm$ S.E.M. of 8 rats per group. * P <0.05, significantly different from the corresponding control values (saline + IL-1 β group) (ANOVA followed by Student-Newman-Keuls' test).

Fig. 3. Time course of the effects of buprenorphine in pyrogenic fever induced by intracerebroventricular administration of PGE₂. Buprenorphine (1 and 10 μ g/10 μ l) was injected intracerebroventricularly 60 min before PGE₂ (200 ng/10 μ l) injected at time 0 min. The values are the mean $\pm$ S.E.M. of 8 rats per group. * P <0.05, significantly different from the corresponding control values (saline + PGE₂ group) (ANOVA followed by Student-Newman-Keuls' test).

Brain sections from normothermic rats pretreated with i.c.v. injection of 0.9% NaCl solution expressed no or weak positive activity of immunoreactive β -endorphin in the POAH of the hypothalamus (Fig. 4A). The hypothalamic regions in hyperthermic rats treated with an i.c.v. injection of either LPS (0.5 μ g), PGE₂ (200 ng), or IL-1 β (10 ng) alone showed stronger immunoreactivity for β -endorphin than those in control sections (Fig. 4: B–D). The immunoreactive data are summarized in Table 2.

Discussion

Our present results demonstrate that β -endorphin release or synthesis in the POAH of rat brain (as demonstrated by immunochemical staining technique) is increased after i.c.v. administration of LPS. The results are in part consistent with the findings of Carr et al. (24). They reported that increases in plasma, cerebrospinal fluid and hypothalamic levels of β -endorphin (which acts on μ -opioid receptors) was observed during systemic administration of LPS. β -Endorphin micro-

Table 1. Effects of intracerebroventricular administration of buprenorphine 1 h before the pyrogen injection on the febrile response to intracerebroventricular injection of LPS, IL-1 β , or PGE₂ in rats

Treatments	Fever index (FI, °C · h)	F values
Saline (10 μ l) + Saline (10 μ l)	FI ₄ = 0.35 $\pm$ 0.13	60.361
Buprenorphine (10 μ g/10 μ l) + Saline	FI ₄ = 0.45 $\pm$ 0.18	
Saline + LPS (0.5 μ g/10 μ l)	FI ₄ = 4.06 $\pm$ 0.26*	
Buprenorphine (1 μ g/10 μ l) + LPS (0.5 μ g/10 μ l)	FI ₄ = 2.95 $\pm$ 0.21 [†]	
Buprenorphine (10 μ g/10 μ l) + LPS (0.5 μ g/10 μ l)	FI ₄ = 0.55 $\pm$ 0.29 [†]	
Saline (10 μ l) + Saline (10 μ l)	FI ₄ = 0.55 $\pm$ 0.15	37.154
Buprenorphine (10 μ g/10 μ l) + Saline	FI ₄ = 0.65 $\pm$ 0.22	
Saline + IL-1 β (10 ng/10 μ l)	FI ₄ = 5.90 $\pm$ 0.32*	
Buprenorphine (1 μ g/10 μ l) + IL-1 β (10 ng/10 μ l)	FI ₄ = 3.61 $\pm$ 0.42 [†]	
Buprenorphine (10 μ g/10 μ l) + IL-1 β (10 ng/10 μ l)	FI ₄ = 2.23 $\pm$ 0.57 [†]	
Saline (10 μ l) + Saline (10 μ l)	FI ₁ = 0.09 $\pm$ 0.04	33.816
Buprenorphine (10 μ g/10 μ l) + Saline	FI ₁ = 0.10 $\pm$ 0.05	
Saline + PGE ₂ (200 ng/10 μ l)	FI ₁ = 0.85 $\pm$ 0.07*	
Buprenorphine (1 μ g/10 μ l) + PGE ₂ (200 ng/10 μ l)	FI ₁ = 0.32 $\pm$ 0.07 [†]	
Buprenorphine (10 μ g/10 μ l) + PGE ₂ (200 ng/10 μ l)	FI ₁ = 0.12 $\pm$ 0.04 [†]	

The values are means $\pm$ S.E.M. of 8 rats per group. FI₄ and FI₁ represent fever index for 4 and 1 h, respectively.

*Significantly different from the corresponding control value (saline plus saline group) ($P < 0.01$, one way analysis of variance of variance followed by Student-Newman-Keuls' test); [†]Significantly different from the corresponding control value (saline plus LPS, saline plus IL-1 β , or saline plus PGE₂ group) ($P < 0.01$, one way analysis of variance of variance followed by Student-Newman-Keuls' test).

Fig. 4. β -Endorphin expression in the preoptic anterior hypothalamus induced by intracerebroventricular injection of saline (A), PGE₂ (B), IL-1 β (C), and LPS (D). Animals were killed 20 min, 2 h, and 4 h after PGE₂, IL-1 β , and LPS injection for immunohistochemistry experiment, respectively. Scale bar, 30 μ m.

Table 2. Expression of β -endorphin in the hypothalamus as well as colonic temperatures from saline-, PGE₂-, IL-1 β -, and LPS-treated rats by 20 min, 2 h, and 4 h after PGE₂, IL-1 β , and LPS injection for immunohistochemical studies, respectively

Number	Rat	Immunohistochemistry ^a	Tco (°C)
1	Saline-treated	1+	36.8
2	Saline-treated	1+	36.7
3	Saline-treated	0	37.0
4	Saline-treated	0	37.1
5	Saline-treated	1+	36.5
6	PEG ₂ -treated	3+	38.8
7	PEG ₂ -treated	3+	39.2
8	PEG ₂ -treated	3+	38.9
9	PEG ₂ -treated	3+	39.4
10	PEG ₂ -treated	3+	39.3
11	IL-1 β -treated	2+	39.5
12	IL-1 β -treated	1+	39.3
13	IL-1 β -treated	1+	39.4
14	IL-1 β -treated	2+	39.5
15	IL-1 β -treated	2+	39.6
16	LPS-treated	3+	38.4
17	LPS-treated	3+	39.0
18	LPS-treated	3+	38.9
19	LPS-treated	3+	39.0
20	LPS-treated	3+	39.2

^aStaining was scored as follows: 0, no staining; 1+, weak staining; 2+, moderate staining; 3+, strong staining.

injected locally into the POAH also produced fever that was blocked by pretreatment with CTAP (the μ -opioid receptor-selective antagonist) (6, 25). These findings are compatible with the contention that the β -endorphin system in the brain is involved in the mediation of febrile response to LPS.

Evidence has accumulated to suggest that increase in several pyrogenic substances including IL-1 β , TNF- α , and macrophage inflammatory protein-1 (MIP-1) accompanied by fever can be induced by LPS (26–28). The fever induced by systemic administration of LPS or central administration of IL-1 β , TNF- α , or MIP-1 can be prevented by pretreatment with intra-POAH injection of CTAP (20, 21, 25, 29). Both the present and previous (5, 25) results further demonstrated that the level of β -endorphin release in the POAH was increased after i.c.v. or intracerebral administration of either IL-1 β , PGE₂, or TNF- α . These observations prompted us to think that the antagonism of LPS-induced fever by CATP may result from the prevention of binding of β -endorphin, produced by these pyrogenic cytokines or PGE₂, to the μ -opioid receptors in the POAH of rat brain.

The aforementioned statement is supported by several

previous findings. For example, intracerebral administration of μ -opioid receptor selective agonist produced hyperthermia which can be blocked by pretreatment with the μ -opioid receptor antagonist (18, 30). On the other hand, central injection of the κ -opioid receptor agonist induces hypothermia that can be blocked by κ -selective opioid receptor antagonist in rats (30). The δ -opioid receptors do not appear to be involved in body temperature regulation (30).

As mentioned before in the introduction section, buprenorphine is not only a partial μ -opioid receptor agonist and antagonist, but a pure κ -opioid receptor antagonist as well. A more recent report has also demonstrated that buprenorphine is a potent and selective μ/κ -opioid receptor antagonist in the [³⁵S]-GTP- γ -S functional binding assay in guinea pig caudate membranes (17). In the present results, the fever induced by i.c.v. administration of LPS, IL-1 β , or PGE₂ was attenuated by pretreatment with i.c.v. injection of buprenorphine in rats. Thus, it appears that the antagonism of pyrogenic fever by buprenorphine may result from the antagonism of the μ -opioid receptors in the POAH of rat brain. This hypothesis is in part supported by the previous results (13, 18, 30). For example, buprenorphine was able to antagonize the antinociceptive effects of several selective μ -opioid receptor agonists in a rhesus monkey tail-withdrawal test (13). In addition, activation of central μ -opioid receptors induced fever reactions (18, 30). However, as shown in Table 2, the release of β -endorphin in rat POAH is less involved in the hyperthermia induced by IL-1 β than those by LPS and PGE₂. This contention is supported by the data presented in Fig. 2 that unlike the fever induced by LPS and PGE₂, the fever induced by IL-1 β was not completely attenuated by buprenorphine. In fact, the κ -selective antagonist nor-BNI failed to block an MIP-1 β -induced fever-like hyperthermia. However, pretreatment with either μ -selective antagonists, β -FNA or CTAP, blocked MIP-1 β -induced fever while having no effect on IL-1 β -induced fever (21). These suggest that in homeotherms, there are multiple pathways for the induction of fever.

Other lines of evidence has accumulated to indicate that the 5HT_{2A} and 5-HT_{1A} receptors activation in the hypothalamus mediate the hyperthermic and hypothermic action, respectively, in rats (31). It was also shown that 5-HT, either endogenous or exogenously delivered, facilitate the release of β -endorphin in the arcuate nucleus and nucleus accumbens (32). According to the findings of Gemma et al. (33), activation of the hypothalamic serotonergic system was produced by central injection of IL-1 β . Systemic administration of 5-HT_{2A}-receptor agonists such as DOI, MK212, or quipazine caused fever in rats (34). The induced fever

was antagonized by pretreatment with 5-HT_{2A}-receptor antagonists (35). Direct injection of DOI into the rat's hypothalamus also caused fever (36). Our unpublished data further showed that the fever induced by LPS, IL-1 β , or PGE₂ in rats could be attenuated by pretreatment with 5-HT_{2A}-receptor antagonists cyproheptadine, ketanserin, or mianserin. The fever induced by central injection of the 5-HT_{2A}-receptor agonist DOI was also reduced by pretreatment with central injection of either 5-HT_{2A}-receptor antagonists or the opioid receptor antagonist buprenorphine. These results suggest that 5-HT_{2A}- μ -opioid receptor mechanisms in the hypothalamus mediate pyrogen fever in rats. In the present results, pyrogenic agents such as LPS, IL-1 β , and PGE₂ may have stimulated 5-HT_{2A} receptors in the POAH and resulted in β -endorphin release in the POAH; excessive accumulation of β -endorphin *in situ* may have activated the μ -opioid receptors in the POAH and resulted in fever.

In summary, the present results showed that central injection of pyrogenic cytokines or PGE₂ stimulated the synthesis or release of β -endorphin in the hypothalamus and induced fever in rats. The fever induced by central injection of either LPS, IL-1 β , or PGE₂ could be attenuated by pretreatment with buprenorphine, probably via the antagonism of μ -opioid receptors in the brain.

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