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Effects of neuropeptide FF on intestinal motility and temperature changes induced by endotoxin and platelet-activating factor

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Abstract

Several effects of bacterial endotoxins involve an opioid pathway and neuropeptide FF is an endogenous peptide known to modulate opioid activity, mainly in the central nervous system. The aim of this study was to investigate in rats the role of central neuropeptide FF receptors in intestinal motor disturbances and body temperature changes induced by endotoxins and platelet-activating factor (PAF), a major endotoxin mediator. Rats were fitted with intestinal electrodes, an intraperitoneal thermistor probe and an intracerebroventricular (i.c.v.) cannula for long-term use. *E. coli* endotoxin (100 μg/kg, i.v.) disrupted the cyclic pattern of intestinal migrating myoelectric complexes and induced a biphasic increase in body temperature while PAF (25 μg/kg, i.p.) disrupted the migrating myoelectric complexes and induced hypothermia for about 2 h. The neuropeptide FF analog, (1 DME)Y8Fa (D-Tyr-D-Leu[N–Me]–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) administered i.c.v. 40 and 100 μg/kg reduced the duration of migrating myoelectric complex disruption induced by endotoxin and PAF and abolished the PAF-induced hypothermia. Only at the dose of 100 μg/kg did (1 DME)Y8Fa change the biphasic endotoxin-induced hyperthermia into a monophasic increase. Naloxone (1 mg/kg, s.c.) reduced only the duration of migrating myoelectric complex disruption induced by endotoxin. These results indicate that central neuropeptide FF modulates the intestinal motor disturbances and changes in body temperature induced by endotoxin and PAF. Its action against endotoxin may involve an anti-opioid pathway whereas its action against PAF does not. © 1997 Elsevier Science B.V.

Keywords: Neuropeptide FF; Intestinal motility; Fever; Endotoxin; PAF (platelet-activating factor)

1. Introduction

Neuropeptide FF, Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2, is a mammalian peptide considered to be an endogenous modulator of opioid activity (Yang et al., 1985; Raffa, 1988). Based on nociceptive studies, neuropeptide FF possesses several naloxone-like properties. It antagonizes opioid-induced analgesia (Raffa, 1988) and precipitates the withdrawal syndrome in morphine-dependent rats (Malin et al., 1990a) while immunoglobulin G from neuropeptide FF antiserum attenuates naloxone-induced withdrawal syndrome (Malin et al., 1990b). In the gastrointestinal tract, neuropeptide FF displays both opioid agonist and antagonist properties. In the guinea pig ileum in vitro, it antagonizes the inhibition by morphine of electrically induced contractions (Demichel et al., 1993), whereas in mice it delays, as does morphine, intestinal (Gicquel et al., 1993) and colonic transit (Raffa and Jacoby, 1989). In rats also, both neuropeptide FF and an enkephalin analog were found to restore a fasting pattern of intestinal motility after a meal (Million et al., 1993). Despite growing evidence for its anti-opioid and pro-opioid properties, and the presence of neuropeptide FF specific receptors in the central nervous system (Allard et al., 1989) and in peripheral tissues (Allard et al., 1995), the mechanisms involved in the gastrointestinal actions of neuropeptide FF remain poorly understood. Moreover, the digestive effects of neuropeptide FF have been evaluated under physiological conditions but not in pathological disturbances where endogenous opioids are involved.

Diarrhoea is a commonly observed effect of endotoxins (Thomas, 1954) and can be fatal in endotoxin shock in young animals (Lohuis et al., 1988). On the other hand, endotoxins induce the release of endogenous opioids (Holaday and Faden, 1978) that have been found responsible for cardiovascular effects by acting at μ- and δ-opioid

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receptors (D'Amato and Holaday, 1984). Concerning the digestive effects of endotoxin, it has been shown that naloxone pretreatment prevents the bloody diarrhoea induced by endotoxic shock in dogs (Ganes et al., 1987). Moreover, endotoxin administration induces in sheep and rabbits changes in intestinal motility that involve opioid receptors located in the central nervous system (Duranton and Buéno, 1984; Fioramonti et al., 1984).

The numerous mediators found to be involved in the effects of endotoxins include platelet-activating factor (PAF) which exerts cardiovascular and gastrointestinal alterations similar to those observed following endotoxin administration. Like endotoxins, PAF induces systemic hypotension and increases vascular permeability (Bessin et al., 1983), necrosis and ulceration of the gastrointestinal tract (Wallace et al., 1987) and intestinal hypermotility (Pons et al., 1991).

Despite the marked effect of opioids on gastrointestinal function and the modulation of opioid effects by neuropeptide FF, most of the work on neuropeptide FF has focussed on its role in nociception. The role of neuropeptide FF on gastrointestinal function in healthy states has been little studied. We thus aimed to evaluate in rats the central action of a neuropeptide FF enzyme resistant analog, (1DME)Y8Fa (D-Tyr-D-Leu[N–Me]–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) (Gicquel et al., 1992), on intestinal motor alterations and body temperature variations in disease states induced by *E. coli* endotoxin and PAF, and also to gain an insight into its mechanism of action by comparing its effects to those of naloxone.

2. Materials and methods

2.1. Animal preparation

Male Wistar rats (250–300 g), individually housed and fed a pelleted rat diet, were used for these experiments. The animals were prepared for long-term electromyographic recordings of intestinal motility using a previously described technique (Ruckebusch and Fioramonti, 1975). Briefly, under acepromazine (0.6 mg/kg i.p.) and ketamine (120 mg/kg i.p.) anesthesia, nichrome wire electrodes (Driver Harris, Mantes La Jolie), 80 μm in diameter and 60 cm in length, were implanted in the duodenum and the jejunum 5 and 20 cm from the pylorus, respectively. To measure body temperature, a thermistor probe (NTC type, code 10K3A1, Farnell, Villefranche Sur Saône) was placed in the peritoneal cavity. For intravenous (i.v.) injections, the rats were fitted with a small silicone catheter (0.9 mm in diameter) inserted into the right jugular vein. The free ends of the electrodes, the thermistor probe wires and the catheter were exteriorized on the back of the neck and protected by a glass tube attached to the skin. In addition, the rats were fitted with a small polyethylene catheter (ID, 0.3 mm; OD, 0.7 mm) inserted into a lateral ventricle of the brain (Stewart et al., 1978) for intracerebroventricular (i.c.v.) administrations. All protocols were approved by the Local Animal Care and Use Committee of INRA.

2.2. Recordings

Electromyographic and temperature recordings began 5 days after surgery. Intestinal electrical activity was recorded using an electroencephalograph machine (Mini-huit, Alvar, Paris) with a paper speed of 5 cm/min and a short time constant (0.03 s) to record spike bursts selectively. Spiking activity was summed every 20 s by an integrator circuit and automatically plotted on the *y*-axis of a potentiometric recorder (L6514, Linseis, Selb) with a paper speed of 5 cm/h. This integrated record permitted a clear determination of the intestinal pattern of myoelectrical activity. Intraperitoneal temperature was measured by connecting the thermistor probe to an electronic thermometer developed in our laboratory. It was calibrated to give an initial output of 0 V at 35°C with a sensitivity of 200 mV/°C. The temperature was recorded on one channel of the potentiometric recorder.

2.3. Experimental procedure

Experiments were performed in 15 h fasted rats. After two hours of intestinal motility and temperature recording, *E. coli* endotoxin (100 μg/kg, i.v.) or PAF (25 μg/kg, i.p.) was administered. Endotoxin and PAF injections were preceded (10 min) by the administration of saline (5 μl i.c.v., 0.2 ml s.c.), (1DME)Y8Fa (40 and 100 μg/kg, i.c.v.) or naloxone (1 mg/kg, s.c.). The doses of endotoxin and PAF were chosen based on their similar effects on intestinal motility (Pons et al., 1991). The doses of (1DME)Y8Fa and naloxone were taken from previous experiments (Million et al., 1993).

Endotoxins are known to induce tolerance and a previous study had shown that changes in intestinal motility are significantly reduced over the sixth administration of endotoxin given at four-day intervals (Pons et al., 1991). Consequently, animals did not receive more than three injections of endotoxin and were divided into three groups of eight animals. Group 1 received endotoxin preceded randomly by saline and (1DME)Y8Fa, 40 and 100 μg/kg; group 2 received endotoxin preceded by saline and naloxone; group 3 received PAF preceded by saline, (1DME)Y8Fa, 40 and 100 μg/kg, and naloxone. There was an interval of at least 4 days between two consecutive administrations of endotoxin or PAF to the same animal.

2.4. Chemicals

The following chemicals and reagents were used: acepromazine from Vetoquinol (Lure), ketamine from Rhone-Merieux (Lyon), lipopolysaccharide (endotoxin) from *E. coli* serotype 0111:B4, PAF (L-α-phosphati-

dylcholine, β-acetyl-γ-*O*-alkyl) and naloxone hydrochloride from Sigma Chemical (St. Louis, MO) and (1DME)Y8Fa, synthetized as previously described (Gicquel et al., 1992). (1DME)Y8Fa was dissolved in 0.1 N HCl, brought to neutral pH by subsequent addition of 0.1 N NaOH and saline to reach the required concentration. The other compounds were dissolved in saline.

2.5. Data analysis

The effects of endotoxin and PAF on intestinal motility were assessed from the duration of the disruption of migrating myoelectric complexes and changes in body temperature were expressed as the difference from the mean control period. Values are expressed as means ± S.D. Statistical analysis was performed using the analysis of variance and Student's *t* test for paired data. Differences were considered significant for P values < 0.05.

3. Results

3.1. Effects of endotoxin and PAF on intestinal motility

The myoelectric activity of the small intestine in fasted rats was organized into migrating myoelectric complexes recurring every 12–15 min as described previously (Ruckebusch and Fioramonti, 1975). Each migrating myoelectric complex consisted of irregular spiking activity (phase 2) followed by a short (4–5 min) period of intense and regular activity (phase 3). These phases of activity were separated by a 2 to 3 min quiescent period (phase 1) (Figs. 1 and 2). The migrating myoelectric complexes were propagated from the duodenum to the jejunum.

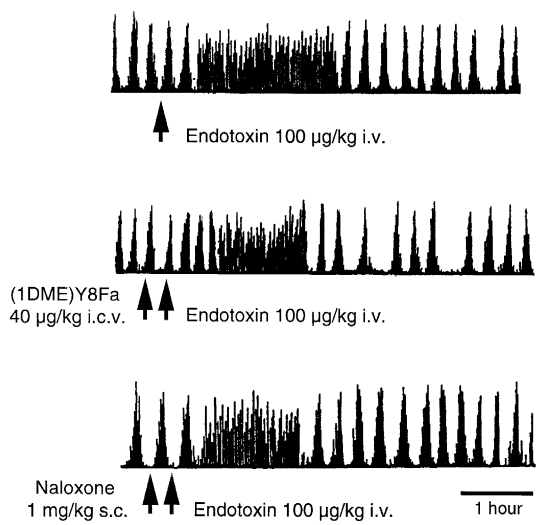

Fig. 1. Integrated records of jejunal electromyograms showing the disruption of the cyclic migrating myoelectric complex profile induced by endotoxin after a period of latency, the reduction of the period of migrating myoelectric complex disruption by (1DME)Y8Fa and naloxone, and the increase in latency duration by (1DME)Y8Fa.

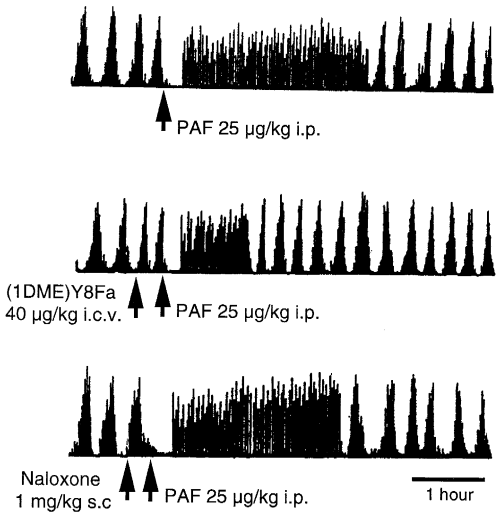

Fig. 2. Integrated records of jejunal electromyograms showing that PAF induced disruption of migrating myoelectric complexes, preceded by a transient inhibition. (1DME)Y8Fa, but not naloxone, reduced the duration of migrating myoelectric complex disruption induced by PAF.

Endotoxin (100 µg/kg, i.v.) disrupted the cyclic pattern of migrating myoelectric complexes for 145.7 ± 14.2 min in the jejunum after a latency period of 20.2 ± 9.6 min (Figs. 1 and 3). During this period of migrating myoelectric complex disruption, the myoelectrical activity was characterized by the presence of repetitive clusters of spike bursts occurring at intervals of 1–2 min and lasting 0.4–0.5 min, as previously described (Pons et al., 1991).

PAF (25 µg/kg, i.p.) also disrupted the migrating myoelectric complexes and induced clusters of spike bursts similar to those observed after endotoxin. Migrating myoelectric complex disruption lasted 149.3 ± 10.5 min and was preceded by a period of total inhibition of spiking activity which lasted 10.2 ± 2.1 min (Figs. 2 and 4). No significant differences were observed between the effects of endotoxin or PAF on the duodenum and the jejunum.

Naloxone or (1DME)Y8Fa at the doses utilized did not have a significant effect on either basal intestinal motor pattern or body temperature.

3.2. Action of (1DME)Y8Fa and naloxone on the effects of endotoxin and PAF on intestinal motility

Prior (10 min) i.c.v. administration of (1DME)Y8Fa, 40 and 100 µg/kg, reduced significantly ($P < 0.05$) (to 75.3 ± 15.1 and 49.8 ± 11.3 min, respectively) the duration of the MMC disruption induced by endotoxin. It also significantly prolonged the latency between endotoxin administration and migrating myoelectric complex disruption (Figs. 1 and 3).

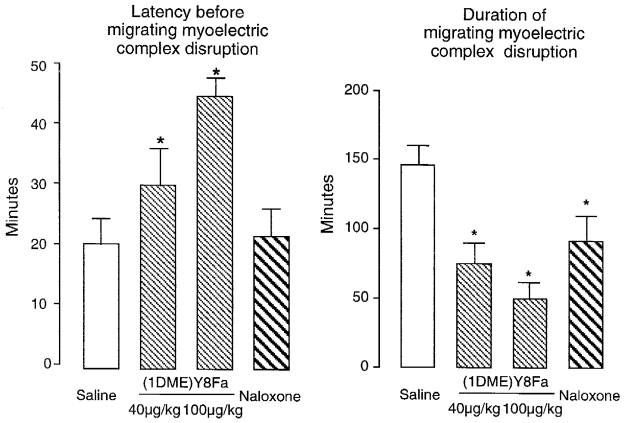

Fig. 3. Latency and duration of jejunal migrating myoelectric complex disruption induced by endotoxin (100 μg/kg, i.v.) and their modifications induced by (1DME)Y8Fa and naloxone. Values are means ± S.D. ($n = 8$ animals). * $P < 0.05$ compared with saline.

At the doses used, (1DME)Y8Fa had no effect on PAF-induced transient inhibition of intestinal motility. However, it significantly reduced ($P < 0.05$) the duration of migrating myoelectric complex disruption to 81.7 ± 12.2 and 53.5 ± 13.1 min at the doses of 40 and 100 μg, respectively (Figs. 2 and 4).

Naloxone (1 mg/kg, s.c.) significantly reduced the duration of migrating myoelectric complex disruption induced by endotoxin to 91.2 ± 16.4 min but did not significantly modify ($P > 0.05$) the duration of the latency period (Figs. 1 and 3). Naloxone had no effect on inhibition and on the migrating myoelectric complex disruption induced by PAF (Figs. 2 and 4).

3.3. Effects of endotoxin and PAF on body temperature

The basal core temperature of fasted rats was 37.8 ± 0.2°C. Endotoxin (100 μg/kg, i.v.) increased body temperature for more than 6 h. The average increase during the first 6 h post-injection was 1.9 ± 0.4°C (Table 1). The body temperature increase was characterized by the occurrence of two peak elevations at 1.5–2 and 4–5 h post-endotoxin administration (Fig. 5).

In contrast, intraperitoneal administration of PAF at 25 μg/kg induced an immediate reduction of core body temperature which lasted about 2 h. The average decrease during the first 2 h post-injection was −1.0 ± 0.6°C (Table 1).

3.4. Action of (1DME)Y8Fa and naloxone on the effects of endotoxin and PAF on body temperature

At the dose of 40 μg/kg i.c.v., (1DME)Y8Fa did not modify the endotoxin-induced increase in body temperature. At the dose of 100 μg/kg i.c.v., it changed the endotoxin-induced biphasic temperature profile into a long-lasting monophasic type of increase (Fig. 5). How-

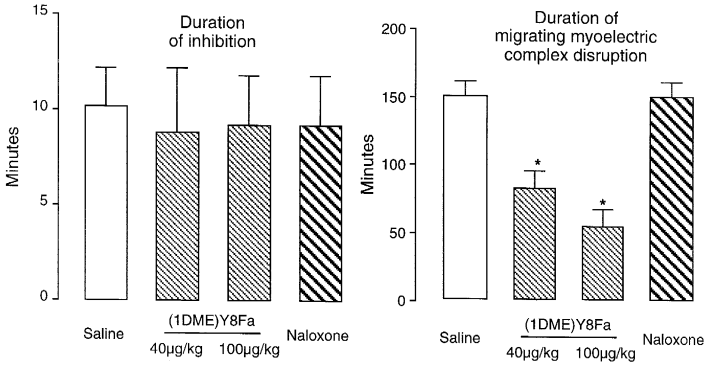

Fig. 4. Duration of jejunal transient inhibition and of the migrating myoelectric complex disruption induced by PAF (25 μg/kg, i.p.). (1DME)Y8Fa, but not naloxone, reduced the duration of migrating myoelectric complex disruption. Values are means ± S.D. ($n = 8$ animals). * $P < 0.05$ compared with saline.

Table 1
Effects of (1DME)Y8Fa and naloxone on the changes in body temperature (°C) induced by endotoxin and PAF

	Saline	(1DME)Y8Fa, i.c.v.		Naloxone
		40 μg/kg	100 μg/kg	1 mg/kg, s.c.
Endotoxin 100 μg/kg, i.v.	1.9 ± 0.4	1.9 ± 0.5	2.1 ± 0.6	1.9 ± 0.5
PAF 25 μg/kg, i.p.	−1.0 ± 0.6	−0.03 ± 0.2 [a]	0.2 ± 0.3 [a]	−0.9 ± 0.7

Temperature was measured at 12 min intervals for 6 h after endotoxin and 2 h after PAF. Values are means ± S.D. ($n = 8$ animals).
[a] $P < 0.01$ compared with saline.

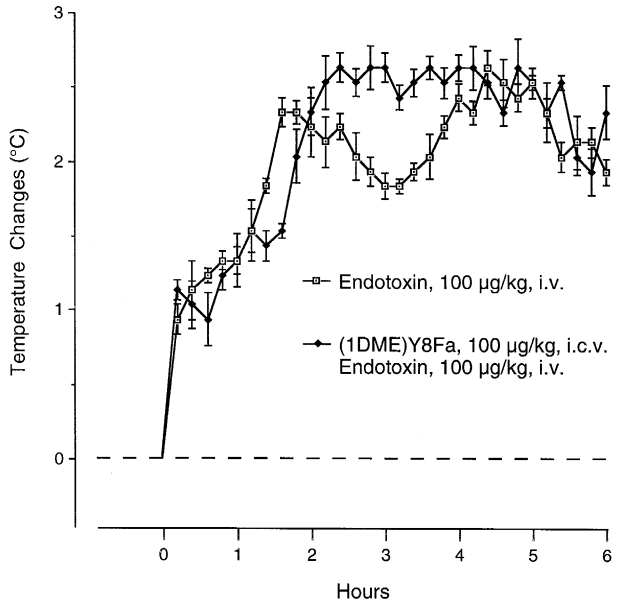

Fig. 5. Changes in body temperature after endotoxin preceded or not by (1DME)Y8Fa. The biphasic profile of the increase in body temperature induced by endotoxin was changed to a monophasic increase by (1DME)Y8Fa. Values are means ± S.D. ($n = 8$ animals).

ever, the average increase in temperature determined over 6 h was not significantly different ($P > 0.05$) from that observed after endotoxin preceded by saline (Table 1). On the other hand, (1DME)Y8Fa at both doses utilized, abolished the decrease in body temperature triggered by PAF (Fig. 6, Table 1). Naloxone (1 mg/kg) had no effect on the fever induced by endotoxin or on PAF-induced hypothermia (Table 1).

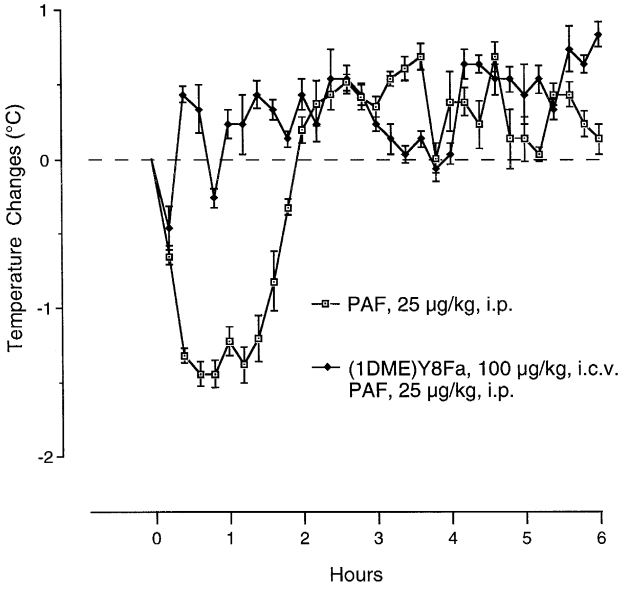

Fig. 6. Changes in body temperature after administration of PAF, preceded or not by (1DME)Y8Fa. The PAF-induced hypothermia was suppressed by (1DME)Y8Fa. Values are means ± S.D. ($n = 8$ animals).

4. Discussion

The present results showed that central administration of the neuropeptide FF analog, (1DME)Y8Fa, attenuates intestinal motor disturbances induced by *E. coli* endotoxin and PAF in fasted rats, while naloxone attenuates only disturbances induced by endotoxin. The results also show that neuropeptide FF, but not naloxone, antagonizes the PAF-induced hypothermia.

Both endotoxin and PAF disrupted the cyclic occurrence of migrating myoelectric complexes that were replaced by a pattern of clusters of spike bursts, also described as minute-rhythm (Fleckenstein et al., 1982). This pattern has been observed after endotoxin in ruminants (Duranton and Buéno, 1984) and rabbits (Fioramonti et al., 1984), and after endotoxin or PAF in rats (Pons et al., 1991). The intestinal motor disturbances as well as the bloody diarrhoea induced by endotoxin are blocked by naloxone (Duranton and Buéno, 1984; Fioramonti et al., 1984; Ganes et al., 1987). Since naloxone attenuated the effects of endotoxin on intestinal motility, our results support the involvement of endogenous opioids in the digestive effects of endotoxin in rats.

Likewise, since both neuropeptide FF and naloxone attenuate the migrating myoelectric complex disruption induced by endotoxin in a similar fashion, it is possible that neuropeptide FF acts by modulating the opioid system. Indeed, previous in vitro (Demichel et al., 1993) and in vivo (Raffa and Jacoby, 1989; Million et al., 1993) studies have indicated that neuropeptide FF exerts its effect on digestive motility through this system. It also appears that opioids are included in a cascade of reactions mediating the effects of endotoxin. Among the mediators involved in this cascade are opioids (Holaday and Faden, 1978), PAF (Wallace et al., 1987), free radicals (Yoshikawa et al., 1983), eicosanoids (Lefer, 1985) and cytokines (Dinarello, 1991). The site of action of opioids is not known but may be associated with free radicals since opioid peptides are able to stimulate superoxide production by polymorphonuclear leukocytes (Sharp et al., 1987) and naloxone inhibits superoxide release from neutrophils (Simpkins et al., 1985). It can also be associated with cytokines since interleukin-1 is known to stimulate the release of β-endorphin by T lymphocytes (Heijnen et al., 1991).

However, since the action of endotoxin on motility involves several mediators, the similarity between naloxone and (1DME)Y8Fa could be simply circumstantial. The failure of naloxone and (1DME)Y8Fa to totally block the action of endotoxin indicates the involvement of several pathways. In line with this, the prolongation by (1DME)Y8Fa, but not by naloxone, of the delay of appearance of MMC disruption after endotoxin administration indicates the presence of a non-opioid component in the neuropeptide FF action. The possibility of a non-opioid component is supported by the blockade of PAF-induced MMC disruption by (1DME)Y8Fa but not by naloxone. In

the present work, PAF induced a biphasic effect on intestinal motility, a transient total inhibition followed by a long period of irregular activity, suggesting the involvement of different mediators and pathways in its action. Several mediators have been proposed for the various actions of PAF. Among them, are eicosanoids (Wallace and Whittle, 1986) histamine and serotonin (Esplugues and Whittle, 1989), and free radicals (Pons et al., 1991). Local opioid-sensitive afferent neurons have been found to be involved in the gastric mucosal damage induced by PAF (Esplugues et al., 1989) but our results indicate that opioids are not involved in the PAF-induced disruption of migrating myoelectric complexes. It is to be noted that intestinal motility alterations induced by endotoxin (100 μg/kg) and PAF (25 μg/kg) were similar in profile and duration. It is thus unlikely that the differences observed between naloxone and (1DME)Y8Fa arise from the doses of endotoxin and PAF used. The dose of naloxone used in the present study was that currently found to antagonize several opioid actions and it is very likely that the lack of effect of naloxone against the actions of PAF was not due to use of a too low dose of naloxone.

Specific receptors of neuropeptide FF have been identified, mainly in the central nervous system (Allard et al., 1989), and the reduction of the intestinal motor effects of endotoxin and PAF was observed in our study after intracerebroventricular administration of (1DME)Y8Fa, suggesting a central pathway for the effects of endotoxin and PAF. Opioids in the central nervous system must be involved in the effects of endotoxin since intravenous endotoxin increases the concentration of opioid peptides in the cerebrospinal fluid (Carr et al., 1982) and since intracerebroventricular administration of naloxone, at a dose without effect by the i.v. route, blocks the digestive motor action of endotoxin (Duranton and Buéno, 1984; Fioramonti et al., 1984).

Endotoxin administration induced a biphasic increase in body temperature as previously described (Kluger, 1991). It is generally agreed that endotoxin-induced fever involves mediators such as interleukins-1 and -6, prostaglandins E and tumor necrosis factor (Kluger, 1991).

The lack of involvement of endogenous opioids in endotoxin-induced fever, indicated in our study by the lack of action of naloxone, has already been reported (Yirmiya et al., 1994). At the dose of 100 μg/kg, (1DME)Y8Fa did not antagonize the endotoxin-induced fever but changed the biphasic profile of temperature into a monophasic one. This action of (1DME)Y8Fa probably does not depend on its anti-opiate properties since it is not mimicked by naloxone, but the mechanisms involved remain to be elucidated.

In contrast, PAF induced severe hypothermia. To our knowledge whether PAF acts as a cryogen or induces the production of endogenous cryogens such as vasopressin or α-MSH (Kluger, 1991) which do act at the hypothalamic level to lower the thermoregulatory set point is not known. Nevertheless, (1DME)Y8Fa antagonized the PAF-induced hypothermia and this effect very likely does not involve an anti-opiate action since it was not reproduced by naloxone. However, PAF is a potent hypovolemic and hypotensive agent (Vargaftig et al., 1980) and hypothermia could have occurred as a result of PAF effect on the cardiovascular system. In line with this, neuropeptide FF is a mammalian peptide which belongs to a family of molluscan cardioactive peptides (Raffa, 1988) and it has been shown that the molluscan peptide, FMRF, administered intracerebroventricularly increases arterial pressure in rats (Barnard and Dockray, 1984). This cardiovascular response has been confirmed with neuropeptide FF administered i.v. in rats (Roth et al., 1987; Allard et al., 1995). It is indeed likely that (1DME)Y8Fa antagonized the PAF-induced hypothermia by correcting the cardiovascular action of PAF. This would mean a physiological antagonism rather than an action of neuropeptide FF on the pathway inducing the hypothermia triggered by PAF.

Finally, evidence shows that central neuropeptide FF or its analogs modulate intestinal motility in physiological states through pro-opioid and anti-opioid like actions (Raffa and Jacoby, 1989; Gicquel et al., 1993; Million et al., 1993). The present data provide the first evidence that central (1DME)Y8Fa, a neuropeptide FF analog, modulates in a dose-dependent manner intestinal motility and body temperature variations in diseases states induced by endotoxin and PAF. The attenuating effect of (1DME)Y8Fa on intestinal motility alterations triggered by endotoxin may involve an opioid pathway. However, its action against PAF-induced intestinal motor disturbances and its effect on body temperature variation induced by both endotoxin and PAF does not involve the opioid system.

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