ELSEVIER

European Journal of Pharmacology 345 (1998) 1–11

ejp

Review

Neuropeptide FF, pain and analgesia

Michel Roumy, Jean-Marie Zajac *

Institut de Pharmacologie et de Biologie Structurale, C.N.R.S. UPR 9062, 205 Route de Narbonne, 31077 Toulouse Cedex, France

Received 27 October 1997; revised 15 December 1997; accepted 19 December 1997

Abstract

Neuropeptide FF (Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) and the octadecapeptide neuropeptide AF (Ala–Gly–Glu–Gly–Leu–Ser–Ser–Pro–Phe–Trp–Ser–Leu–Ala–Ala–Pro–Gln–Arg–Phe–NH_2) were isolated from bovine brain, and were initially characterized as anti-opioid peptides. They can oppose the acute effects of opioids and an increase in their brain concentrations may be responsible for the development of tolerance and dependence to opioids. Numerous experiments suggest a possible neuromodulatory role for neuropeptide FF. A precursor protein has been identified, in particular in human brain. Neuropeptide FF immunoreactive neurons are present only in the medial hypothalamus, and the nucleus of the solitary tract, and in the spinal cord in the superficial layers of the dorsal horn and areas around the central canal. Depolarization induces a Ca^{2+}-dependent release of neuropeptide FF immunoreactivity from the spinal cord. Neuropeptide FF acts through stimulation of its own receptors and high densities of specific binding sites are found in regions related either to sensory input and visceral functions or to the processing of nociceptive messages. In both isolated dorsal root ganglion neurons and CA1 pyramidal neurons of the hippocampus, neuropeptide FF has little effect of its own but reverses the effects of μ-opioid receptor agonists. In agreement with the hypothesized anti-opioid role of neuropeptide FF, supraspinal injection lowers the nociceptive threshold and reverses morphine-induced analgesia in rats. Furthermore, immunoneutralization of neuropeptide FF increases endogenous and exogenous opioid-induced analgesia. Similarly, microinfusion of neuropeptide FF or neuropeptide FF analogs into the nucleus raphe dorsalis, the parafascicular nucleus, or the ventral tegmental area has no effect on the nociceptive threshold but inhibits the analgesia induced by co-injected morphine. Furthermore, infusion of neuropeptide FF into the parafascicular nucleus or the nucleus raphe dorsalis reverses the analgesic effect of morphine infused into the nucleus raphe dorsalis or the parafascicular nucleus, respectively, demonstrating remote interactions between neuropeptide FF and opioid systems. By contrast, intrathecal administration of neuropeptide FF analogs induces a long lasting, opioid-dependent analgesia and potentiates the analgesic effect of morphine. Analgesic effects of neuropeptide FF after supraspinal injection could also be observed, for example during nighttime. In young mice, (1DMe)Y8Famide (D.Tyr–Leu–(NMe)Phe–Gln–Pro–Gln–Arg–Phe–NH_2), a neuropeptide FF analog, increases δ-opioid receptor-mediated analgesia. These findings indicate that neuropeptide FF constitutes a neuromodulatory neuronal system interacting with opioid systems, and should be taken into account as a participant of the homeostatic process controlling the transmission of nociceptive information. © 1998 Elsevier Science B.V.

Keywords: Neuropeptide FF; Opioid; Analgesia

1. Introduction

Neuropeptide FF (NPFF, Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) and the octadecapeptide neuropeptide AF (Ala–Gly–Glu–Gly–Leu–Ser–Ser–Pro–Phe–Trp–Ser–Leu–Ala–Ala–Pro–Gln–Arg–Phe–NH_2) were isolated from bovine brain (Yang et al., 1985) using an antiserum directed against the molluscan peptide FMRFamide (Phe–Met–Arg–Phe–NH_2; Price and Greenberg, 1977). Neuropeptide FF has been variously referred to as mammalian FMRFamide-like peptide, F8Famide or morphine modulating neuropeptide since neuropeptide FF like FMRFamide decreases the nociceptive threshold of rats (Yang et al., 1985). Invertebrate FMRFamide and small cardioactive peptides with an N-terminal Arg–Met-amide amino acid sequence exist in almost all molluscan species where they might function as neuromodulators or neurotransmitters. The amino acid sequence similarity between FMRFamide and the opioid peptide [Met^5]enkephalin–Arg^6–Phe^7–amide pointed to possible structural and functional similarities in the two peptide families (Greenberg et al., 1983).

Neuropeptide FF together with cholecystokinin (CCK8-S, Asp–Tyr(SO_3H)–Met–Gly–Trp–Met–Asp–Phe–NH_2) or Tyr-MIF-1-like peptides (Tyr–Pro–Leu–Gly–NH_2) are termed anti-opioid substances indicating that the mor-

* Corresponding author. Tel.: +33-561175911; fax: +33-561175994.

phine-induced changes in neuronal responses involve various non-opioid neurotransmitter networks that participate in an homeostatic system which acts to damp the effects of opioids. Accordingly, in addition to pain inhibiting pathways, there are anti-analgesic neuronal peptides which act by blocking pain inhibition in the central nervous system. The triggering of these systems readily explains the development of tolerance and dependence induced by chronic administration of opioids.

An anti-opioid model of tolerance and dependence (Rothman, 1992) postulates the existence of endogenous compounds that are able to reduce some of the acute (in particular antinociceptive) opioid effects, and permanently mask the effects of exogenous and endogenous opioids. Accordingly, chronic treatment with morphine should increase the release of anti-opioid peptides which, by stimulating specific receptors and specific networks, attenuate the pharmacological effects of morphine. As more opioid is given, more anti-opioid is released inducing tolerance to opioid effects. Following cessation of opioid administration, residual excess of anti-opioid is partly responsible for the withdrawal syndrome.

This model implies that anti-opioid peptides such as neuropeptide FF should be considered as tonic modulators of opioid functions. They therefore present an important target for new pharmacological agents able to modulate endogenous opioid function and amplify analgesic opioid effects. Agonists of the anti-opioid system are anticipated to block the analgesia produced by administration of opioids, whereas antagonists or antisera against anti-opioid peptides would potentiate analgesia.

Our knowledge of the neuropeptide FF system is now sufficiently advanced to describe (in broad terms) the role of neuropeptide FF peptides in the central control of opioid function.

2. Neuropeptide FF as a neurotransmitter / neuromodulator

Neuropeptide FF and neuropeptide AF were first isolated from bovine brain, and neuropeptide FF-like immunoreactivity was later demonstrated in the central nervous system of several mammals including humans (Panula et al., 1987; Majane et al., 1988; Kivipelto et al., 1989). Currently known peptide sequences of the neuropeptide FF family in various species are summarized in Table 1. A gene encoding both neuropeptide FF and neuropeptide AF has been reported in bovine, rat, mouse and human (Vilim and Ziff, 1995). Recently the sequence of the human gene has been published revealing the amino acid sequences of two novel peptides (Perry et al., 1997; Table 1). A large body of experimental evidence summarized here, suggests a possible role for neuropeptide FF as a neurotransmitter.

Table 1
Known sequences of peptides of the neuropeptide FF family in various species

Species	Peptide sequence	Ref.
Bovine	FLFQPQRF–NH_2	
	AGEGLSSPFWSLAAPQRF–NH_2	Yang et al., 1985
Rat	FLFQPQRF–NH_2	
	AGEGLSSPFWSLAAPQRF–NH_2	Vilim and Ziff, 1995
	SLAAPQRF–NH_2	Yang and Martin, 1995
Man	SQAFLFQPQRF–NH_2	
	AGEGLNSQFWSLAAPQRF–NH_2	Perry et al., 1997

2.1. Distribution of neuropeptide FF

Neuropeptide FF is largely confined to the central nervous system (Lee et al., 1993). The anatomical distribution of neuropeptide FF immunoreactivity shows highest density in the spinal cord, hypothalamus and pons–medulla (Kivipelto et al., 1989; Majane et al., 1989; Allard et al., 1991) with low levels in the cortex and hippocampus (see review of Panula et al., 1996).

In rats, immunoreactive cell bodies are seen in only two types of brain nuclei following colchicine inhibition of axonal transport: the medial hypothalamus and the nucleus of the solitary tract (Panula et al., 1987). Combined retrograde tract tracing and immunohistochemical methods demonstrate that hypothalamic neuropeptide FF-containing neurons project bilaterally into the nucleus of the solitary tract, the lateral septal nucleus, the periaqueductal gray and some hypothalamic nuclei (Aarnisalo and Panula, 1995). Immunopositive neurons in the nucleus of the solitary tract project into the contralateral part of the nucleus, lateral parabrachial nuclei and ipsilateral periambigual regions (Kivipelto and Panula, 1991a).

In the rat spinal cord, neuropeptide FF immunoreactivity is primarily concentrated in the superficial laminae of the dorsal horn and in areas around the central canal (Panula et al., 1987; Allard et al., 1991). Immunoreactivity is restricted to large dense-cored vesicles in axonal and terminal profiles (Allard et al., 1991). Spinal cord transection induces a reduction of neuropeptide FF immunoreactivity caudal to the lesion (Majane et al., 1989), whereas dorsal rhizotomy does not alter the level of neuropeptide FF in the dorsal horn (Majane et al., 1989; Allard et al., 1991).

In control rats, no neuropeptide FF immunoreactive neuronal cell bodies are found in the spinal cord. In colchicine treated rats, immunoreactive cell bodies are detected in the dorsal horn and central region of the spinal cord (Kivipelto and Panula, 1991b). These results suggest that neuropeptide FF immunoreactivity derives both from local interneurons and descending nerve fibers.

However, species differences may exist since neuropeptide FF immunoreactivity is not found in the dorsal horn of

porcine spinal cord (Wasowicz and Panula, 1994). Similarly, during carrageenan inflammation of the hind paw, neuropeptide FF-neurons are seen in an area receiving innervation from the inflamed hind limb, but no neuropeptide FF immunoreactive neurons are found in rats pretreated with morphine (Kontinen et al., 1997). In these animals, morphine produces significant antinociception in thermal and mechanical nociceptive tests which is not modified by neuropeptide FF. This suggests that the role of neuropeptide FF in the modulation of nociception in the spinal cord may be markedly modified during acute inflammation (Kontinen et al., 1997).

2.2. Neuropeptide FF receptors

Allard and Simonnet first identified a single class of binding site to [^{125}I]Y8Famide ([^{125}I]–Tyr–Leu–Phe–Gln–Pro–Gln–Arg–Phe–Nh_2) in rat spinal cord (Allard et al., 1989). Neuropeptide FF binding sites have now been characterized using two different iodinated peptides: Y8Famide (Tyr–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) and (1DMe)Y8Famide (D.Tyr–Leu–(NMe)Phe–Gln–Pro–Gln–Arg–Phe–NH_2), the latter offering good resistance to enzymatic breakdown (Gicquel et al., 1992). Neuropeptide FF and related peptides bind with high affinity in rat spinal cord membranes or slices (K_i about 0.1 nM), while opioid ligands do not compete for these sites (Allard et al., 1989; Payza and Yang, 1993; Gouardères et al., 1997). Neuropeptide FF receptors in the rat spinal cord or mouse olfactory bulb may be coupled to a G-protein (Payza and Yang, 1993; Devillers et al., 1994b; Gherardi and Zajac, 1997) since neuropeptide FF binding is inhibited by guanine nucleotides that uncouple receptors from G-protein.

Autoradiographic studies using these radioligands reveal that neuropeptide FF binding sites are widely distributed in the central nervous system (Allard et al., 1989, 1992; Dupouy et al., 1996; Dupouy and Zajac, 1996). Some loci enriched in binding sites seem to be physiologically related to sensory input and visceral function. Neuropeptide FF binding sites are found in the spinal trigeminal tract, the nucleus tractus solitarius and the nucleus ambiguus (associated with the regulation of respiratory function). The presence of neuropeptide FF and opioid receptors in the nucleus tractus solitarius, is congruent with the ability of centrally administered neuropeptide FF and opioids to modulate heart rate and blood pressure (Barnard and Dockray, 1984; Wong et al., 1985; Chai et al., 1986).

Many neurons containing neuropeptide FF receptors are present in areas of the central nervous system involved in nociception and/or analgesia: periaqueductal gray, dorsal raphe, dorsal horn of the spinal cord. Of particular interest is the binding density observed in the vicinity of periaqueductal gray matter. The lateral and ventrolateral periaqueductal gray are both relatively enriched in binding sites, but mediate different types of analgesia and respond to topographically organized spinal inputs (Bandler and Shipley, 1994). This brain area, and the somatosensory pathways including the nucleus of the solitary tract, the parabrachial nucleus and the thalamus have also been shown to contain finite levels of neuropeptide FF-immunoreactivity (Panula et al., 1996).

The presence of neuropeptide FF receptors in periaqueductal gray matter, dorsal raphe nucleus and thalamus contrasted with the relatively limited distribution of binding sites in sensory cortex and limbic structures, suggesting that the pharmacological effects of neuropeptide FF agonists in nociception are not due to their influence on the affective components of pain (Dupouy and Zajac, 1995).

Neuropeptide FF is also localized in the vicinity of several neurochemical networks thought to be involved in pain modulation, such as septal nuclei, amygdala and hypothalamus, as spinal neurons providing nociceptive information were able to project directly onto a number of telencephalic targets (Burstein and Protrebic, 1993; Giesler et al., 1994). For example, the hypothalamus which contains neuropeptide FF and neuropeptide FF receptors, is believed to play an important role in several aspects of nociception (Giesler et al., 1994). Hypothalamus cells containing neuropeptide FF send extensive projections to the limbic system, in particular the septal area (Aarnisalo and Panula, 1995). Thus, the majority of neuropeptide FF receptors in the limbic system might be stimulated by neuropeptide FF released from these projections.

In the rat spinal cord, [^{125}I](1DMe)Y8Famide ([^{125}I] D.Tyr–Leu–(NMe)Phe–Gln–Pro–Gln–Arg–Phe–NH_2) binding sites are highly concentrated in laminae I–II of the dorsal horn (Allard et al., 1992, 1994; Dupouy and Zajac, 1996; Gouardères et al., 1997). An identical morphological layout was reported for rabbit, mouse (Dupouy et al., 1996) and human spinal cord (Allard et al., 1994). Although one study reports no significant change in dorsal horn of [^{125}I]Y8Famide binding in rhizotomized rats (Lombard et al., 1995), capsaicin-treated rats exhibited a bilateral decrease (47%) in [^{125}I](1DMe)Y8Famide binding in all spinal areas (Gouardères et al., 1996b). Unilateral sciatic nerve section and unilateral dorsal rhizotomy also induce a significant depletion, of 15 and 27%, respectively, in [^{125}I](1DMe)Y8Famide labeling in the ipsilateral relative to the contralateral dorsal horn (Gouardères et al., 1996b). These results suggest that some neuropeptide FF receptors are located on the primary afferent terminals of the dorsal horn, where they might play a primary role in the modulation of nociceptive transmission.

2.3. Cellular effects

The involvement of neuropeptide FF in pain modulation is further supported by electrophysiological studies. Neuropeptide FF produces excitatory effects on cultured mouse spinal cord neurons (Guzman et al., 1989) and attenuates

the inhibitory effect of μ-opioid receptor agonists on the C-fiber evoked firing of rat spinal cord neurons (Magnuson et al., 1990). α_2-adrenergic-induced inhibition of C-fiber-mediated activity is also attenuated (Sullivan et al., 1991).

μ-opioid receptor agonists reduce the conductance of three types of high threshold voltage-sensitive Ca^{2+} channels in acutely dissociated dorsal root ganglion neurons of young rats,: N, P and a presumptive Q channel (Moises et al., 1994). Thus, the selective μ-opioid receptor agonist DAGO (Tyr-D-Ala–Gly–(N–Me)Phe–Gly-ol) reduces the transient rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by high $[K^+]$ depolarization (Rebeyrolles et al., 1996). The neuropeptide FF analog (1DMe)Y8Famide (10 nM) has no effect on the resting or depolarization-induced rise in $[Ca^{2+}]_i$ but reverses the DAGO effect in half the neurons tested (Rebeyrolles et al., 1996). The effects of the specific δ-opioid receptor agonist [D-Ala^2]deltorphin-I are also reversed by (1DMe)Y8Famide (Roumy, unpublished observations). These results demonstrate the ability of neuropeptide FF to act as an anti-opioid on a single isolated dorsal root ganglion neuron.

In acutely dissociated dorsal root ganglion neurons of adult mice, that show little opioid sensitivity, (1DMe)Y8Famide increases the latency of the Ca^{2+} response to high $[K^+]$ depolarization, so that the Ca^{2+} response to short depolarizations (5–10 s) is, in fact, suppressed (Roumy and Zajac, 1996).

Morphine excites CA1 pyramidal neurons of the rat hippocampus through inhibition of γ-aminobutyric acid (GABA) release from interneurons. Pyramidal cell excitation is manifest as an increase in the extracellularly recorded population spikes and a reduction of GABA release results in an intracellularly recorded inhibitory postsynaptic potentials (IPSPs) of decreased magnitude. Neuropeptide FF does not affect the population spikes or amplitude of IPSPs, but reverses morphine effects on both parameters (Miller and Lupica, 1997). It has been suggested that neuropeptide FF acts as an anti-opioid in the CA1 region of the rat hippocampus, at a site presynaptic to the pyramidal neuron, possibly an inhibitory interneuron (Miller and Lupica, 1997)?

2.4. Neuropeptide FF release

High concentrations of K^+ (56 mM) and substance P both stimulate the Ca^{2+}-dependent release of neuropeptide FF immunoreactivity from in situ superfused rat spinal cord (Zhu et al., 1992). In dorsal half slices of rat spinal cord, neuropeptide FF immunoreactivity release is directly related to the extent of depolarization (increasing $[K^+]$ in the superfusing medium). Furthermore, the depolarizing agents veratridine and ouabain induce tetrodotoxin-sensitive release of neuropeptide FF. The depolarization-induced release of neuropeptide FF is Ca^{2+} dependent and is strongly reduced by ω-Agatoxin IVA, a specific antagonist of the high threshold voltage-sensitive Ca^{2+} channel of the P type (Devillers et al., 1995).

N-methyl-D-aspartate (NMDA) dose-dependently increases release of neuropeptide FF immunoreactivity, in the same preparation, under moderately depolarizing conditions (15 mM K^+). This release is suppressed by the NMDA receptor antagonists APV (2-amino-5-phosphonovalerate) and MK-801 ((5*R*,10s)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine), but is insensitive to the Na^+ channel blocker tetrodotoxin (Devillers et al., 1994a) indicating a direct effect of NMDA on neuropeptide FF-containing nerve endings.

Morphine infusion into the subarachnoid space stimulates the release of neuropeptide FF immunoreactivity from in situ superfused rat spinal cord which is suppressed by naloxone (Tang et al., 1984). Similarly, morphine induces a dose-dependent, naloxone-sensitive release of neuropeptide FF immunoreactivity from rat spinal cord dorsal half slices (Devillers et al., 1995).

This features suggest that neuropeptide FF could be physiologically released from nerve endings, in the spinal cord.

3. Anti-opioid effects of neuropeptide FF

The location of neuropeptide FF receptors in networks involved in pain modulation correlates well with the results of pharmacological experiments. Several studies have evidenced a role for neuropeptide FF in nociception and in the control of opioid functions and according to the anti-opioid model, neuropeptide FF produces hyperalgesia and inhibits opioid-induced analgesia.

3.1. Anti-analgesic effects of neuropeptide FF

The first indication of anti-opioid effects by neuropeptide FF peptides was the observation that FMRFamide immunoreactive material obtained from bovine brain attenuated morphine effects in the rat tail-flick test (Yang et al., 1985). The peptides contained in this immunoreactive material are neuropeptide FF and neuropeptide AF. When administered separately (i.c.v.), each peptide transiently lowered the nociceptive threshold in rats, neuropeptide FF being more active than the octadecapeptide. Consistent with this hyperalgesic action, neuropeptide FF (5 μg) also attenuated the morphine-induced increase in tail-flick latency (Yang et al., 1985).

Similarly, injections of neuropeptide FF in the third ventricle reverse morphine-induced analgesia in the tail-flick test in rats. The magnitude of the effect increases as a function of the magnitude of morphine-induced analgesia (Oberling et al., 1993).

Neuropeptide FF analogs, partially protected against enzymatic degradation, also produce a marked hyperalgic effect (Gicquel et al., 1992) and inhibit morphine analgesia

in the tail-flick test in mice at lower doses than neuropeptide FF does. The analgesic effect of 5.5 nmol morphine can be reduced by 8.8 nmol of (1DMe)Y8Famide, while a dose of 22 nmol neuropeptide FF is required to produce the same effect (Gicquel et al., 1992, 1994).

The physiological significance of these results is established by the fact that intrathecally administered G immunoglobulins (IgG) from an antiserum against neuropeptide FF is antinociceptive and augments both morphine- and stress-induced analgesia (Kavaliers and Yang, 1989; Kavaliers and Innes, 1992). Thus, the neutralization of endogenous neuropeptide FF increases endogenous and exogenous opioid-induced analgesia.

Neuropeptide FF decreases the nociceptive threshold in rats when given in the absence of exogenous opioids (Yang et al., 1985). This could be either due to the reversal of an endogenous opioid tone or to the activation of pain facilitatory pathways. Administration of naloxone 25 min after heroin abolishes the increase in tail-flick latency and indeed shortens it below the naloxone alone and basal values, implicating the existence of a pain facilitatory system. Morphine stimulates neuropeptide FF release from the rat spinal cord and spinal cord slices (Tang et al., 1984; Devillers et al., 1995). A single heroin injection (2.5 mg/kg) elicits a decrease in spinal neuropeptide FF content (Devillers et al., 1995). It may therefore be that neuropeptide FF networks themselves constitute such a pain facilitatory system, triggered by opioid receptor activation. However, a direct demonstration of this is still awaited.

The existence of an equilibrium between neuropeptide FF and opioids had been suggested by earlier observations showing that neuropeptide FF has complex effects on pain sensitivity. Day-time neuropeptide FF and neuropeptide AF injections (i.c.v., 0.1–10 μg) significantly reduce morphine (10 mg/kg) and restraint-induced analgesia after 30 min (Kavaliers, 1990). There is a day–night rhythm in nociceptive sensitivity, with control-handled and saline vehicle-injected mice displaying significantly greater thermal response latencies at night than during the day. During the night, neuropeptide FF (i.c.v.) and naloxone significantly reduce the elevated nocturnal latency of response to nociceptive thermal stimuli while the shorter day-time nociceptive responses are unaffected (Kavaliers, 1990). This indicates that neuropeptide FF may be associated with the expression of the day–night rhythm of opioid-mediated nociceptive sensitivity.

3.2. Local administration

When the i.c.v. route is used, neuropeptide FF is targeted to the cerebral aqueduct and fourth ventricle, areas of critical importance for the production of antinociception by morphine (Herz et al., 1970). However, a diffusion of the peptide could occur upon entry to the lateral or third ventricle and other brain sites may therefore be reached.

Microinfusion of morphine in the nucleus raphe dorsalis, identified by stimulation-produced analgesia studies as a 'pure analgesia region' (Fardin et al., 1984), causes analgesia in the tail-immersion test. This analgesia is reversed by co-infusion of (1DMe)Y8Famide into the nucleus raphe dorsalis (Dupouy and Zajac, 1995).

Another potential site of pain modulation in the forebrain is the parafascicular thalamic nucleus. Cells in the medial thalamic nuclei, responding exclusively to noxious stimuli are concentrated within the parafascicular nucleus (Andersen and Dafny, 1983). This region is particularly responsive to noxious stimuli (Peshanski et al., 1981) and receives serotonergic projections from the nucleus raphe dorsalis (Moore et al., 1978; Clements et al., 1985; Chen et al., 1992). Dorsal raphe stimulation modulates spontaneous activity and noxious-evoked responses in parafascicular nucleus neurons (Reyes-Vazquez et al., 1989). On the other hand, parafascicular nucleus stimulation activates periaqueductal gray neurons that are sensitive to peripheral nociceptive stimuli (Sakata et al., 1988). It has been proposed that nucleus raphe dorsalis-periaqueductal gray matter and the parafascicular nucleus area play a role in endogenous antinociceptive control mechanisms and pain modulation systems in rodents (Andersen and Dafny, 1983).

Infusion of (1DMe)Y8Famide (2.5 nmol) in the nucleus raphe dorsalis does not modify the animal response in the tail-immersion test but significantly reverses analgesia induced by co-injected morphine (27 nmol). Similarly, (1DMe)Y8Famide (5 nmol) inhibits morphine effects in the hot-plate test following co-injection into the parafascicular nucleus (Dupouy and Zajac, 1997). Furthermore, (1DMe)Y8Famide injected into the parafascicular nucleus attenuates analgesia induced by morphine injected into the nucleus raphe dorsalis. Likewise, a neuropeptide FF analog injected in the nucleus raphe dorsalis decreases the effect of 27 nmol morphine injected in the parafascicular nucleus (Dupouy and Zajac, 1997).

These data show that whilst neuropeptide FF exerts direct anti-opioid effects in both the nucleus raphe dorsalis and the parafascicular nucleus, it also acts remotely (Dupouy and Zajac, 1997). Direct anti-opioid effects of neuropeptide FF require functional serotonergic neurons: Serotonin depletion induced by systemic administration of *para*-chlorophenylalanine blocks the ability of (1DMe)Y8Famide to reverse morphine effects in both nuclei, although neuropeptide FF receptors are known not to be present on these neurons (Dupouy and Zajac, 1997).

Similarly, injection of (1DMe)Y8Famide (2 nmol) into the third ventricle in rats, produces brief hyperalgesia followed by dark phase analgesia. This antinociceptive effect is abolished by lesion of serotonin neurons (Delort-Laval et al., 1996).

Infusion of morphine into the ventral tegmental area induces analgesia in the formalin test in the rat. Infusion of neuropeptide FF (4.4 nmol) in the ventral tegmental area

has no effect on pain scores but blocks the analgesic effect of intra-ventral tegmental area morphine (6 nmol). It also blocks analgesia induced by footshock stress (Altier and Stewart, 1997). In the ventral tegmental area, neuropeptide FF receptors are localized on non-dopaminergic perikarya (Marco et al., 1995).

Interestingly, as previously mentioned (Dupouy and Zajac, 1995), morphine infused into the nucleus raphe dorsalis, ventral tegmental area or the parafascicular nucleus produces an analgesic effect as efficiently as it does when injected by an i.c.v. route. Similarly, efficacious doses of neuropeptide FF or (1DMe)Y8Famide are in the same range after local and i.c.v. administration (Table 2), indicating the existence of a multiplicity of brain sites controlling nociception that are equally sensitive to neuropeptide FF and morphine. However, in the rat dorsal raphe, a very low concentration of (1DMe)Y8Famide (about 1/10 that of morphine) is sufficient to reduce morphine analgesia, whereas after i.c.v. injection in mice, a two-fold excess of (1DMe)Y8Famide is required to inhibit opioid analgesia (Table 2). This difference can be explained either by lower peptide degradation rates in the dorsal raphe, or by a very high efficiency of neuropeptide FF analogs in this brain area.

3.3. Neuropeptide FF involvement in tolerance and dependence to opioid-analgesia

Several observations indicate that neuropeptide FF may be involved in opioid tolerance and dependence as suggested by the anti-opioid model. Intrathecal injection of IgG from FMRFamide antiserum attenuates acute morphine tolerance in rats (Tang et al., 1984). In morphine-tolerant rats, injection (i.c.v.) of IgG from neuropeptide FF antiserum restores the analgesic response to morphine (i.c.v.) (Lake et al., 1991). In accordance with the anti-opioid model, acute administration of morphine releases neuropeptide FF immunoreactivity from rat spinal cord (Tang et al., 1984), and chronic administration of morphine increases the level of neuropeptide FF in the cerebrospinal fluid (Malin et al., 1990b).

A variety of evidence also suggests that neuropeptide FF plays a role in opiate dependence and abstinence. Injection (i.c.v.) of IgG from neuropeptide FF antiserum reverses morphine dependence as indicated by the subsequent prevention of naloxone-precipitated abstinence syndrome (Malin et al., 1990b). A low dose (2 μg) of neuropeptide FF precipitates an opiate abstinence syndrome when injected into the third ventricle of morphine-dependent rats, whilst a higher dose (15 μg) is required to induce a morphine-reversible quasi-morphine abstinence syndrome in naive rats (Malin et al., 1990a). Analgesia is greatest three hours following implantation of a morphine pellet and then decreases rapidly over a 12 h period. A significant reduction of neuropeptide FF immunoreactivity is observed 1 h after morphine pellet implantation (-25 to -45%) followed by a dramatic increase 3–6 h later (Stinus et al., 1995).

Nitric oxide synthesis inhibition attenuates neuropeptide FF-induced abstinence-like signs (Malin et al., 1996). Nitric oxide synthase activation has been reported to attenuate opiate analgesia (Przewlocki et al., 1993).

4. Pro-opioid effects of neuropeptide FF

In contrast to pharmacological effects observed in rats and mice following supraspinal injection, other evidence has accumulated indicating that neuropeptide FF may display opioid-like effects dependent upon the animal model, injection route and pharmacological tests used. Thus, i.t. injections of neuropeptide FF or its analog (1DMe)Y8Famide produce a long-lasting analgesia in rats (Gouardères et al., 1993, 1996a). This analgesia is, however, opioid systems-dependent, since it was markedly reduced by both specific μ- and δ-opioid receptor antagonists (Gouardères et al., 1996a). Furthermore sub-effective doses of both neuropeptide FF and (1DMe)Y8Famide enhance and prolong the antinociceptive effects of both morphine and [D-Ala^2]deltorphin-I (Gouardères et al., 1993, 1996a).

A sub-antinociceptive dose of FMRFamide attenuates the action of morphine in the early phase (10–60 min; Gouardères et al., 1993) as previously reported by others authors describing anti-opioid effects after i.t. FMRFamide in rat (Tang et al., 1984). This suggests that both the anti-opioid effect and analgesia can co-exist after i.t. administration.

In another study, although neuropeptide FF (0.05–10 nmol) did not produce antinociceptive effects in the rat tail flick test after intrathecal injection it clearly potentiated the antinociceptive effect of morphine (Kontinen and Kalso, 1995). Potentiation is prevented by the selective δ-opioid receptor antagonist, naltrindole (28 nmol, i.t.) indicating that δ-opioid receptors are appropriately stimulated. In contrast dexmedetomidine, a specific α_2-adrenergic receptor agonist, produces a dose-dependent antinociceptive effect which is not potentiated by neuropeptide FF (Kontinen and Kalso, 1995). However, i.t. (1DMe)Y8Famide enhances and prolongs the analgesic effect of dexmedetomidine, particularly in the paw pressure test in rat (Gouardères et al., 1994).

The reduction of neuropeptide FF-induced antinociception, after i.t. administration, by opioid antagonists suggests that neuropeptide FF releases endogenous opioids. This however, has not yet been demonstrated experimentally.

In rats with mechanical hyperalgesia following sciatic nerve ligature (mononeuropathic rats), i.t. injection of (1DMe)Y8Famide (0.086–0.64 nmol) exerted a more marked, dose-dependent antinociceptive effect than in normal rats (Coudoré et al., 1997).

Table 2
Effects of neuropeptide FF and neuropeptide FF analogs in tests of nociceptive threshold

Species	Route of drug administration	Analgesic test	Opioid	NPFF	Response observed	Ref.
Mice	icv	TF	morphine 5.5 nmol	NPFF 22 nmol	reversal of morphine analgesia	Gicquel et al., 1992
Mice	icv	TF	morphine 5.5 nmol	1DMe 8.8 nmol	reversal of morphine analgesia	Gicquel et al., 1992
Mice	icv	TI	morphine 2.75 nmol	1DMe 4–30 nmol	reversal of morphine analgesia	Desprat and Zajac, 1997
Young mice	icv	TI	morphine 2.75 nmol	1DMe 4–30 nmol	anti-analgesia and increase opioid effect	Desprat and Zajac, 1997
			[D.Ala^2]deltorphin-I 3 nmol	1DMe 4–30 nmol	anti-analgesia and increase opioid effect	
Mice	icv	HP	morphine 10 mg/kg	NPFF 8.7 nmol	reversal of morphine analgesia	Kavaliers, 1990
Rat	icv	TF		NPFF 8.75 nmol	hyperesthesic analgesia, the night	Oberling et al., 1993
Rat	icv	TF		NPFF 8.75 nmol	reversal of morphine analgesia, the day	Oberling et al., 1993
Rat	icv	TI	morphine 27 nmol	1DMe 2.5 nmol	reversal of morphine analgesia	Dupouy and Zajac, 1995
Rat	DR	TI, HP	morphine 27 nmol	1DMe 2.5 nmol	reversal of morphine analgesia	Dupouy and Zajac, 1995
Rat	PF	HP	morphine 27 nmol	1DMe 5 nmol	reversal of morphine analgesia	Dupouy and Zajac, 1997
Rat	VTA	formalin test	morphine 6 nmol	NPFF 4.4 nmol	reversal of morphine analgesia	Altier and Stewart, 1997
Rat	it	TF, paw pressure		NPFF ED50 12 nmol	analgesia	Gouardères et al., 1993
		TF	morphine 6.6 nmol	NPFF 0.05 nmol	potentiation of opioid effects	
Rat	it	TF, paw pressure		1DMe ED 50 1 nmol	analgesia	Gouardères et al., 1996a,b
		TF	morphine 13 nmol	1DMe 0.009 nmol	potentiation of opioid effects	
			[D.Ala^2]deltorphin-I 20 nmol	1DMe 0.009 nmol	potentiation of opioid effects	
Rat	it	TF	morphine 7.8 nmol	NPFF 10 nmol	potentiation of opioid effects	Kontinen and Kalso, 1995
Rat	it	TF	morphine 53 nmol	NPFF 4.4 nmol	hyperalgesia anti-opioid	Yang et al., 1985
Rat mononeuropathic	it	vocalization thresholds		NPFF 0.086–0.64 nmol	analgesia	Coudoré et al., 1997
Rat inflammation	it	paw flick test	morphine 1.6 nmol	NPFF 10 nmol	no effect	Kontinen et al., 1997

Doses (in nmol) were calculated, when possible, by using molecular weights of 1140 g for neuropeptide FF, 375 g for morphine hydrochloride and 504 g for morphine sulphate. icv, intracerebroventricular; it, intrathecal; TF, tail flick test; HP, hot plate test; TI, tail immersion test; NPFF, neuropeptide FF; 1DMe, (1DMe)Y8Famide.

Similarly, i.t. injection of SLAAPQRFamide (11 and 22 nmol), a neuropeptide FF-like peptide isolated recently from rat brain and spinal cord (Yang and Martin, 1995), produces weak antinociceptive action in the tail-flick and paw pressure tests. At subeffective doses (0.06–0.6 nmol) the peptide increased the duration of antinociception produced by intrathecal morphine (Jhamandas et al., 1996).

Several studies have also demonstrated an analgesic effect after i.c.v. injection of neuropeptide FF. I.c.v. administration of neuropeptide FF in rats induces a rapid and short-lasting hyperesthesic effect during the day or night, followed by a long-lasting analgesic effect during nighttime. Its magnitude is related to the size of the hyperesthesic effect (Oberling et al., 1993). This type of analgesia has been previously observed following supraspinal administration of [D.Met^2]FMRFamide, which produced a dose-dependent, naloxone reversible reduction of thermal nociception in rat (Raffa and Connelly, 1992).

The potentiation of morphine-induced antinociception by i.t. neuropeptide FF is relatively long lasting. Degradation of the peptide by peptidases within the cerebrospinal fluid is relatively rapid and the half-life of neuropeptide FF-receptor complex ($t_{1/2} = 80$ min) is too short to explain long-lasting effects (Gouardères et al., 1997).

Biochemical studies in which neuropeptide FF is injected i.c.v. bilaterally, do not show altered concentrations of noradrenaline, serotonin or dopamine in either the limbic area, hypothalamus or lower brain stem of male Wistar rats (Attila et al., 1995). Morphine (10 mg/kg s.c.) elevates 3,4-dihydroxyphenylacetic acid and/or homovanillic acid in the limbic area and hypothalamus. Combination of morphine and neuropeptide FF decreases noradrenaline in the limbic area. It elevates both 3-methoxy,4-hydroxyphenylethylene glycol (in hypothalamus and lower brainstem) and 5-hydroxyindoleacetic acid (in the limbic area and hypothalamus). These effects are typical of morphine at doses larger than 10 mg/kg and suggest a synergistic interaction between morphine and neuropeptide FF. Neuropeptide FF does not attenuate but rather enhances the changes induced by acute morphine in cerebral monoamines (Kivipelto et al., 1992).

Neuropeptide FF analogs, in common with opiates, inhibit intestinal transit (Millon et al., 1993) and potentiate opioid effects. In summary the pharmacological data indicate that an increase or a potentiation of opioid effects can be mediated by neuropeptide FF and its analogs.

While different laboratories agree upon that neuropeptide FF potentiates analgesia induced by co-injection of morphine or μ-opioid receptor agonists, there is disagreement concerning the direct analgesic effect of neuropeptide FF after intrathecal injection. Kontinen and Kalso (1995) did not observe the same analgesic effect of neuropeptide FF as Gouardères et al. (1993, 1996a). Given the differences in animals used, pharmacological tests and nociceptive stimulations, the basal level of enkephalin release can nevertheless explain such discrepancies if NPFF potentiates opioid effects. Differences in the level of opioid release, and hence in the basal sensitivities of the animals, may allow neuropeptide FF to cause sufficient opioid stimulation to produce analgesic effects commensurate with those observed by potentiation of exogenous opioid effects.

As suggested by Kontinen and Kalso (1995), the potentiation of μ-opioid-induced antinociception can be elicited either by activation of enkephalinergic neurons or inhibition of neuronal activities inhibiting opioid effects. Neuropeptide FF may exert its anti-opioid effect at δ-opioid autoreceptors leading to decreased negative feedback control of the endogenous opioid release, and potentiation of effects mediated by postsynaptic μ-opioid-receptors.

5. Anti-opioid model and analgesia

The apparent contradiction between anti-opioid and analgesic effects of neuropeptide FF relates not only to spinal and supraspinal injection sites since, during the dark phase, neuropeptide FF can induce analgesia after i.c.v. injection (Oberling et al., 1993).

After i.c.v. injection in rats, the hyperesthesic effect of neuropeptide FF was followed, during nighttime, by a clear analgesic effect. The magnitude of the analgesic effect was observed to be dependent upon that of preceding hyperesthesia. Oberling et al. (1993) have proposed that the analgesic rebound could result from the triggering of endogenous opiate systems which are more active during the night in rodents (Wesche and Frederickson, 1979). Thus, supraspinal and spinal analgesia induced by neuropeptide FF or neuropeptide FF analogs could derive from the same phenomenon. Such complex pharmacological behavior elicited by neuropeptide FF can be explained if it modulates μ- and δ-opioid antinociception differentially or if its ability to block opioid-induced activity occurs at dose levels different from those that induce opioid-like effects.

In adult mice, opioid-induced analgesia is predominantly mediated by μ-receptors whereas both μ- and δ-receptors are involved in 14 day-old mice (Desprat and Zajac, 1997). (1DMe)Y8Famide at low dose levels reduces the analgesic effect of DAGO and [D.Ala^2]deltorphin-I. However, a high dose of (1DMe)Y8Famide (22 nmol) increases both morphine and [D-Ala^2]deltorphin-I induced analgesia. Following blockade of δ-opioid receptors with naltrindole, residual antinociceptive effects of [D-Ala^2]deltorphin-I are decreased by (1DMe)Y8Fa in 14 day-old mice (Desprat and Zajac, 1997).

Dose–response curves for (1DMe)Y8Famide in the presence of respective δ- and selective μ-opioid receptor antagonists, naltrindole or naltrexone, indicate that (1DMe)Y8Famide preferentially reverses μ-receptor-mediated but increases δ-receptor-mediated analgesia. These findings demonstrate differential control of μ- and δ-in-

duced analgesia by neuropeptide FF receptors (Desprat and Zajac, 1997).

The view that the analgesic effects of neuropeptide FF indirectly require the stimulation of δ-receptors is suggested by the lack of an analgesic effect by 22 nmol (1DMe)Y8Famide in the presence of naltrindole, a selective δ-opioid receptor antagonist. According to this model (1DMe)Y8Famide reverses only opioid analgesia induced by μ-receptors. Alternatively, the similarity of (1DMe) Y8Famide effects to morphine and [D-Ala^2]deltorphin-I analgesia suggests that the efficacy of neuropeptide FF receptors stimulation is related rather to the level of opioid system stimulation. In this case, the intrinsic efficacy of the agonists used is more relevant than their selectivity.

6. Conclusion

Neuropeptide FF constitutes a neuromodulatory neuronal system that is involved in the control of the transmission of nociceptive information. More precisely, neuropeptide FF appears to be a modulator of opioid antinociceptive function.

Neuropeptide FF has clear antiopioid effects, mostly at the supraspinal level. These have been demonstrated after i.c.v. injection, after microinfusion into particular brain nuclei and even at the level of the single isolated neuron. By reason of its antiopioid activity neuropeptide FF may play a significant role in opioid tolerance and dependence.

However, other experimental evidence shows that neuropeptide FF has opioid-like effects. It causes opioid-dependent analgesia and potentiates the antinociceptive action of morphine at the spinal level. Furthermore, supraspinal antinociceptive activity has also been established. The exact nature of cellular and molecular mechanisms underlying the anti- and pro-opioid effects of neuropeptide FF are presently unknown.

The need for a simple classification system has originally placed neuropeptide FF in the anti-opioid category. However, it now seems clear that the effects of this peptide on opioid analgesia are complex, and the term 'anti-opioid' no longer adequately describes its physiological role.

Acknowledgements

We are particularly indebted to Dr J.C. Meunier for his critical comments and to Dr. C. Topham for correcting English style. Our research is supported by the Centre National de la Recherche Scientifique, the Ministère de l'Education Nationale, de l'Enseignement Superieur et de la Recherche (97H004).

References

Aarnisalo, A.A., Panula, P., 1995. Neuropeptide FF-containing efferent projections from the medial hypothalamus of rat: A *phaseolus vulgaris* leucoagglutinin study. Neuroscience 65, 175–192.

Allard, M., Geoffre, S., Legendre, P., Vincent, J.D., Simonnet, G., 1989. Characterization of rat spinal cord receptors to FLFQPQRFamide, a mammalian morphine modulating peptide: A binding study. Brain Res. 500, 169–176.

Allard, M., Theodosis, D.T., Rousselot, P., Lombard, M.C., Simonnet, G., 1991. Characterization and localization of a putative morphine-modulating peptide, FLFQPQRFamide, in the rat spinal cord: Biochemical and immunocytochemical studies. Neuroscience 40, 81–92.

Allard, M., Zajac, J.-M., Simonnet, G., 1992. Autoradiographic distribution of receptors to FLFQPQRFamide, a morphine-modulating peptide, in rat central nervous system. Neuroscience 49, 101–116.

Allard, M., Jordan, D., Zajac, J.-M., Ries, C., Martin, D., Monkouanga, D., Kopp, N., Simonnet, G., 1994. Autoradiographic localization of receptors for neuropeptide FF, FLFQPQRFamide, in human spinal sensory system. Brain Res. 633, 132–137.

Altier, N., Stewart, J., 1997. Neuropeptide FF in the VTA blocks the analgesic effects of both intra-VTA morphine and exposure to stress. Brain Res. 758, 250–254.

Andersen, E., Dafny, N., 1983. Dorsal raphe stimulation reduces responses of parafascicular neurons to noxious stimulation. Pain 15, 323–331.

Attila, M., Kurkijarvi, U., Kivipelto, L., Panula, P., Ahtee, L., 1995. The antiopioid peptide, neuropeptide FF, enhances the effects of acute morphine on the cerebral monoamines in rats. Pharmacol. Res. 32, 293–298.

Bandler, R., Shipley, M.T., 1994. Columnar organization in the midbrain periaqueductal gray: Modules for emotional expression?. Trends Neurosci. 17, 379–389.

Barnard, C.S., Dockray, G.J., 1984. Increases in arterial blood pressure in the rat in response to a new vertebrate neuropeptide, LPLRFamide, and a related molluscan peptide FMRFamide. Regul. Pept. 8, 209–215.

Burstein, R., Protrebic, S., 1993. Retrograde labeling of neurons in the spinal cord that project directly to the amygdala or the orbital cortex in the rat. J. Comp. Neurol. 335, 469–485.

Chai, S.H., Tang, J., Han, J., 1986. Antiopioid activity of the cardioexcitatory peptide in central modulation of cardiovascular functions. Eur. J. Pharmacol. 130, 315–318.

Chen, J., Zeng, S.-L., Rao, Z.-R., Shi, J.-W., 1992. Serotoninergic projections from the midbrain periaqueductal gray and nucleus raphe dorsalis to the nucleus parafascicularis of the thalamus. Brain Res. 584, 294–298.

Clements, J.R., Beitz, A.J., Fletcher, T.F., Mullett, M.A., 1985. Immunocytochemical localization of serotonin in the rat periaqueductal gray: A quantitative light and electron microscopic study. J. Comp. Neurol. 236, 60–70.

Coudoré, M.A., Courteix, C., Eschalier, A., Zajac, J.-M., Wilcox, G.L., Fialip, J., 1997. Evidence for the involvement of tachykinins, excitatory amino acids and neuropeptide FF in two animal models of neurogenic pain. In: Résumés de la 1ère Réunion de la Société Française de Pharmacologie (Marseilles, France), vol. 17, p. 23.

Delort-Laval, S., Allard, M., Rivot J.P., Stinus, L., 1996. Intracerebroventricular administration of NPFF induced antinociceptive effect: Involvement of serotoninergic neurons, neuropharmacological approach. In: Abstracts of the 8th World Congress on Pain, Vancouver, Canada, vol. 102, p. 360.

Desprat, C., Zajac, J.M., 1997. Differential modulation of μ- and δ-opioid antinociception by neuropeptide FF receptors in young mice. Neuropeptides 31, 1–7.

Devillers, J.-P., Labrouche, S.A., Castes, E., Simonnet, G., 1994a. Glutamate via NMDA receptors promotes the release of NPFF, an anti-opioid peptide, from rat spinal cord slices. Regul. Pept. 54, 85–86.

Devillers, J.-P., Mazarguil, H., Allard, M., Dickenson, A.H., Zajac, J.-M., Simonnet, G., 1994b. Characterization of a potent agonist for NPFF receptors: Binding study in rat spinal cord membranes. Neuropharmacology 33, 661–669.

Devillers, J.-P., Labrouche, S.A., Castes, E., Simonnet, G., 1995. Release of neuropeptide FF, an anti-opioid peptide, in rat spinal cord slices is

voltage- and Ca^{2+}-sensitive: Possible involvement of P-type Ca^{2+} channels. J. Neurochem. 64, 1567–1575.

Dupouy, V., Zajac, J.-M., 1995. Effects of neuropeptide FF analogs on morphine analgesia in the nucleus raphe dorsalis. Regul. Pept. 59, 349–356.

Dupouy, V., Zajac, J.-M., 1996. Neuropeptide FF receptors in rat brain: A quantitative light microscopic autoradiographic study using [^{125}I][D.Tyr1, (NMe)Phe3] NPFF. Synapse 24, 282–296.

Dupouy, V., Zajac, J.-M., 1997. Neuropeptide FF receptors control morphine-induced analgesia in the parafascicular nucleus and the nucleus raphe dorsalis. Eur. J. Pharmacol. 330, 129–137.

Dupouy, V., Puget, A., Eschalier, A., Zajac, J.-M., 1996. Species differences in the localization of neuropeptide FF receptors in rodents and lagomorph brain and spinal cord. Peptides 17, 399–405.

Fardin, V., Oliveras, J.-L., Besson, J.-M., 1984. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat. I. The production of behavioral side effects together with analgesia. Brain Res. 306, 105–123.

Gherardi, N., Zajac, J.-M., 1997. Neuropeptide FF receptors of mouse olfactory bulb: Binding properties and stimulation of adenylate cyclase activity. Peptides 18, 577–583.

Gicquel, S., Mazarguil, H., Allard, M., Simonnet, G., Zajac, J.-M., 1992. Analogues of F8Famide resistant to degradation, with high affinity and in situ effects. Eur. J. Pharmacol. 222, 61–67.

Gicquel, S., Mazarguil, H., Desprat, C., Allard, M., Devillers, J.-P., Simonnet, G., Zajac, J.-M., 1994. Structure–activity study of neuropeptide FF: Contribution of N-terminal regions to affinity and activity. J. Med. Chem. 37, 3477–3481.

Giesler, G.J., Katter, J.J.T., Dado, R.J., 1994. Direct spinal pathways to the limbic system for nociceptive information. Trends Neurosci. 17, 244–250.

Gouardères, C., Sutak, M., Zajac, J.-M., Jhamandas, K., 1993. Antinociceptive effects of intrathecally administered F8Famide and FMRFamide in the rat. Eur. J. Pharmacol. 237, 73–81.

Gouardères, C., Jhamandas, K., Sutak, M., Zajac, J.-M., 1994. Analogs of neuropeptide FF modulate spinal nociception in the rat. Regul. Pept. Suppl. 1, 113–114.

Gouardères, C., Jhamandas, K., Sutak, M., Zajac, J.-M., 1996a. Role of opioid receptors in the spinal antinociceptive effects of neuropeptide FF analogues. Br. J. Pharmacol. 117, 493–501.

Gouardères, C., Kar, S., Zajac, J.-M., 1996b. Presence of neuropeptide FF receptors on primary afferent fibres of the rat spinal cord. Neuroscience 74, 21–27.

Gouardères, C., Tafani, J.A.M., Mazarguil, H., Zajac, J.-M., 1997. Autoradiographic characterization of rat spinal neuropeptide FF receptors by using [^{125}I][D.Tyr^{1}, (NMe)Phe^{3}]NPFF. Brain Res. Bull. 42, 231–238.

Greenberg, M.J., Painter, S.D., Doble, K.E., Nagle, G.T., Price, D.A., Lheman, H.K., 1983. The molluscan neurosecretory peptide FMRFamide: Comparative pharmacology and relationship to the enkephalins. Fed. Proc. 42, 82–86.

Guzman, A., Legendre, P., Allard, M., Geoffre, S., Vincent, J.D., Simonnet, G., 1989. Electrophysiological effects of FLFQPQRFamide, an endogenous brain morphine modulating peptide, on cultured mouse spinal-cord neurons. Neuropeptides 14, 253–261.

Herz, A., Albus, K., Metys, J., Schubert, P., Teschemacher, H.J., 1970. On the central sites for the antinociceptive action of morphine and fentanyl. Neuropharmacology 9, 539–551.

Jhamandas, K., Sutak, M., Yang, H.-Y.T., 1996. Modulation of spinal opioid antinociception by neuropeptide FF-like endogenous peptide. Soc. Neurosci. 22, 1313.

Kavaliers, M., 1990. Inhibitory influences of mammalian FMRFamide (Phe–Met–Arg–Phe-amide)-related peptides on nociception and morphine- and stress-induced analgesia in mice. Neurosci. Lett. 115, 307–312.

Kavaliers, M., Innes, D., 1992. Sex differences in the effects of neuropeptide FF and IgG from neuropeptide FF on morphine- and stress-induced analgesia. Peptides 13, 603–607.

Kavaliers, M., Yang, H.-Y.T., 1989. IgG from antiserum against endogenous mammalian FMRF-NH_2-related peptides augments morphine- and stress-induced analgesia in mice. Peptides 10, 741–745.

Kivipelto, L., Panula, P., 1991a. Central neuronal pathways containing FLFQPQRFamide-like (morphine-modulating) peptides in the rat brain. Neuroscience 41, 137–148.

Kivipelto, L., Panula, P., 1991b. Origin and distribution of neuropeptide-FF-like immunoreactivity in the spinal cord of rats. J. Comp. Neurol. 307, 107–119.

Kivipelto, L., Majane, E.A., Yang, H.-Y.T., Panula, P., 1989. Immunohistochemical distribution and partial characterization of FLFQPQRF-amide-like peptides in the central nervous system of rats. J. Comp. Neurol. 286, 269–287.

Kivipelto, L., Aarnisalo, A., Panula, P., 1992. Neuropeptide FF is colocalized with catecholamine-synthesizing enzymes in neurons of the nucleus of the solitary tract. Neurosci. Lett. 143, 190–194.

Kontinen, V.K., Kalso, E.A., 1995. Differential modulation of α_2-adrenergic and μ-opioid spinal antinociception by neuropeptide FF. Peptides 16, 973–977.

Kontinen, V.K., Aarnisalo, A.A., Idänpään-Heikkilä, J.J., Panula, P., Kalso, E., 1997. Neuropeptide FF in the rat spinal cord during carrageenan inflammation. Peptides 18, 287–292.

Lake, J.R., Hammond, M.V., Shaddox, R.C., Hunsicker, L.M., Yang, H.-Y.T., Malin, D.H., 1991. IgG from neuropeptide FF antiserum reverses morphine tolerance in the rat. Neurosci. Lett. 132, 29–32.

Lee, C.-H., Wasowicz, D.H., Brown, R., Majane, E.A., Yang, H.-Y.T., Panula, P., 1993. Distribution and characterization of neuropeptide FF-like immunoreactivity in the rat nervous system with a monoclonal antibody. Eur. J. Neurosci. 5, 1339–1348.

Lombard, M.-C., Simonnet, G., Zajac, J.-M., Besson, J.-M., Allard, M., 1995. Distribution of neuropeptide FF (FLFQPQRFamide) receptors in the adult rat spinal cord: Effects of dorsal rhizotomy and neonatal capsaicin. Neuroscience 68, 1229–1235.

Magnuson, D.S.K., Sullivan, A.F., Simonnet, G., Roques, B.P., Dickenson, A.H., 1990. Differential interactions of cholecystokinin and FLFQPQRF-NH_2 with μ and δ opioid antinociception in the rat spinal cord. Neuropeptides 16, 213–218.

Majane, E.A., Casanova, M.F., Yang, H.-Y.T., 1988. Biochemical characterization of FMRFNH_2-like peptide in spinal cord of various mammalian species using specific radioimmunoassays. Peptides 9, 1137–1144.

Majane, E.A., Panula, P., Yang, H.-Y.T., 1989. Rat brain distribution and spinal cord neuronal pathway of FLFQPQRF-NH_2, a mammalian FMRF-NH_2-like peptide. Brain Res. 494, 1–12.

Malin, D.H., Lake, J.R., Fowler, D.E., Hammond, M.V., Brown, S.L., Leyva, J.E., Prasco, P.E., Dougherty, T.M., 1990a. FMRF-NH2-like mammalian peptide precipitates opiate-withdrawal syndrome in the rat. Peptides 11, 277–280.

Malin, D.H., Lake, J.R., Hammond, M.V., Fowler, D.E., Rogillio, R.B., Brown, S.L., Sims, J.L., Leecraft, B.M., Yang, H.-Y.T., 1990b. FMRF-NH_2-like mammalian octapeptide: Possible role in opiate dependence and abstinence. Peptides 11, 969–972.

Malin, D.H., Lake, J.R., Jones, J.A., Morel, J.A., Moon, W.D., Corbit, B.P., Smith, D.A., Claunch, A.E., Kacher, D., Stevens, P.A.L., Webb, S., 1996. Nitric oxide synthesis inhibition attenuates behavioral actions of neuropeptide FF. Peptides 17, 615–618.

Marco, N., Stinus, L., Allard, M., LeMoal, M., Simonnet, G., 1995. Neuropeptide FLFQPQRFamide receptors within the ventral mesencephalon and dopaminergic terminal areas: Localization and functional antiopioid involvement. Neuroscience 64, 1035–1044.

Miller, K., Lupica, C., 1997. Neuropeptide FF inhibition of morphine effects in the rat hippocampus. Brain Res. 750, 81–86.

Millon, M., Fioramonti, J., Gicquel, S., Zajac, J.-M., Bueno, L., 1993. Comparative action of Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2

analogs on intestinal motility and nociception in rats. J. Pharmacol. Exp. Ther. 265, 96–102.

Moises, H.C., Rusin, K.I., Macdonald, R.L., 1994. μ- and κ-opioid receptors selectively reduce the same transient components of high-threshold calcium current in rat dorsal root ganglion sensory neurons. J. Neurosci. 14, 5903–59016.

Moore, R.Y., Halaris, A.E., Jones, B.E., 1978. Serotonin neurons of the midbrain raphe: Ascending projections. J. Comp. Neurol. 180, 417–438.

Oberling, P., Stinus, L., LeMoal, M., Simonnet, G., 1993. Biphasic effect on nociception and antiopiate activity of neuropeptide FF (FLFQPQRFamide) in the rat. Peptides 14, 919–924.

Panula, P., Kivipelto, L., Nieminen, O., Majane, E.A., Yang, H.-Y.T., 1987. Neuroanatomy of morphine-modulating peptides. Med. Biol. 65, 127–135.

Panula, P., Aarnisalo, A.A., Wasowicz, K., 1996. Neuropeptide FF, a mammalian neuropeptide with multiple functions. Prog. Neurobiol. 48, 461–487.

Payza, K., Yang, H.-Y.T., 1993. Modulating of neuropeptide FF receptors by guanine nucleotides and cations in membranes of rat brain and spinal cord. J. Neurochem. 60, 1894–1899.

Perry, S.J., Huang, E.Y., Cronk, D., Bagust, J., Sharma, R., Walker, R.J., Wilson, S., Burke, J.F., 1997. A human gene encoding morphine modulating peptides related to NPFF and FMRFamide. FEBS Lett. 409, 426–430.

Peshanski, M., Guilbaud, G.G., Gautron, M., 1981. Posterior intralaminar nuclei in the rat neuronal responses to noxious and non-noxious cutaneous stimuli. Exp. Neurol. 72, 226–238.

Price, D.A., Greenberg, M.J., 1977. Structure of a molluscan cardioexcitatory neuropeptide. Science 197, 670–671.

Przewlocki, R., Greenberg, H., Przewlocka, B., 1993. Inhibition of nitric oxide synthase enhances morphine antinociception in the rat spinal cord. Life Sci. 53, 1–5.

Raffa, R.B., Connelly, C.D., 1992. Supraspinal antinociception produced by [D-Met^2]-FMRFamide in mice. Neuropeptides 22, 195–203.

Rebeyrolles, S., Zajac, J.-M., Roumy, M., 1996. Neuropeptide FF reverses μ-opioid effect on Ca^{2+} channels in rat spinal ganglion neurons. NeuroReport 7, 2979–2981.

Reyes-Vazquez, C., Qiao, J.-T., Dafny, N., 1989. Nociceptive responses in nucleus parafascicularis thalami are modulated by dorsal raphe stimulation and microiontophoretic application of morphine and serotonin. Brain Res. Bull. 23, 405–411.

Rothman, R.B., 1992. A review of the role of anti-opioid peptides in morphine tolerance and dependence. Synapse 12, 129–138.

Roumy, M., Zajac, J.-M., 1996. Effects of neuropeptide FF on intracellular Ca^{2+} in mouse spinal ganglion neurons. Eur. J. Pharmacol. 306, 291–295.

Sakata, S., Shima, F., Kato, M., Fukui, M., 1988. Effects of thalamic parafascicular stimulation on the periaqueductal gray and adjacent reticular formation neurons. A possible contribution to pain control mechanisms. Brain Res. 451, 85–96.

Stinus, L., Allard, M., Gold, L., Simonnet, G., 1995. Change in CNS Neuropeptide FF-like material, pain sensitivity, and opiate dependence following chronic morphine treatment. Peptides 16, 1235–1241.

Sullivan, A.F., Kalso, E.A., McQuay, H.J., Dickenson, A.H., 1991. FLFQPQRFamide modulates α_2-adrenergic antinociception in the rat dorsal horn in vivo. Brain Res. 562, 327–328.

Tang, J., Yang, H.-Y.T., Costa, E., 1984. Inhibition of spontaneous and opiate-modified nociception by an endogenous neuropeptide with Phe–Met–Arg–Phe–NH_2-like immunoreactivity. Proc. Natl. Acad. Sci. USA 81, 5002–5005.

Vilim, E.S., Ziff, E., 1995. Cloning of the neuropeptide NPFF and NPAF precursor from bovine, rat, mouse and human. Soc. Neurosci. 21, 760.

Wasowicz, K., Panula, P., 1994. Distribution of neuropeptide FF in porcine spinal cord in comparison with other neuropeptides and serotonin. J. Comp. Neurol. 346, 530–540.

Wesche, D.L., Frederickson, R.C., 1979. Diurnal differences in opioid peptide levels correlated with nociceptive sensitivity. Life Sci. 24, 1861–1868.

Wong, T.M., Greenberg, M.J., Tse, S.Y.H., 1985. Cardiovascular effects of intraventricular injection of FMRFamide, met-enkephalin and their common analogs in the rat. Comp. Biochem. Physiol. 81C, 175–179.

Yang, H.-Y.T., Martin, B.M., 1995. Isolation and characterization of a neuropeptide FF-like peptide from brain and spinal cord of rat. Soc. Neurosci. 21, 760.

Yang, H.-Y.T., Fratta, W., Majane, E.A., Costa, E., 1985. Isolation, sequencing, synthesis, and pharmacological characterization of the two brain neuropeptides that modulate the action of morphine. Proc. Natl. Acad. Sci. USA 82, 7757–7761.

Zhu, J., Jhamandas, K., Yang, H.-Y.T., 1992. Release of neuropeptide FF (FLFQPQRF-NH_2) from rat spinal cord. Brain Res. 592, 326–332.